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平成 14 年 6 月

最高のPCR特異性を!

AccuPrime™ Taq DNA Polymerase

AccuPrime™ Taq DNA Polymerase	12339-016	200 反応分
AccuPrime™ SuperMix I	12342-010	200 反応分
AccuPrime™ SuperMix II	12341-012	200 反応分

フィデリティと収量とのバランスのとれたPCRには

Platinum* Taq High Fidelity Elongase* Enzyme Mix

Elongase* Enzyme Mix	10480-010	100 反応分
Platinum* Taq DNA Polymerase High Fidelity	11304-011	100 units

問題のあるテンプレートのPCRには

PCRx Enhancer System

PCRx Enhancer System	11495-017	250 反応分
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最高の酵素で完全長cDNAを!

SuperScript™ II RT ThermoScript™ RT

SuperScript™ II RT	18064-014	10,000 units
ThermoScript™ RT	12236-014	25 反応分

単一のRNA標本から複数のメッセージを検出するには 優れた柔軟性のあるツーステップRT-PCRを!

SuperScript™ First-Strand Synthesis System for RT-PCR	11904-018	50 反応分
ThermoScript™ RT-PCR System plus Platinum* Taq DNA Polymerase	11146-057	25 反応分
ThermoScript™ RT-PCR System plus Platinum* Taq DNA Polymerase High Fidelity	11146-040	100 反応分

多くの試料を簡単に処理するには 高感度なワンステップRT-PCRを!

SuperScript™ One-Step RT-PCR System with Platinum* Taq DNA Polymerase	10928-034	25 反応分
SuperScript™ One-Step RT-PCR System for Long Templates	11922-010	25 反応分

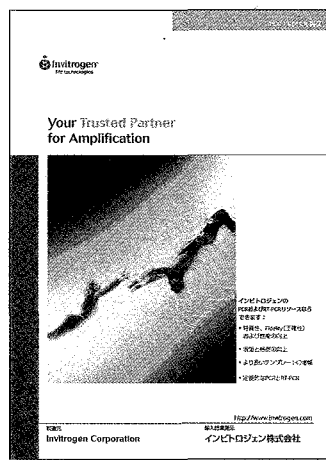
フィデリティの高いPCRを!

Platinum® Pfx

Platinum® Pfx DNA Polymerase	11708-013	100 反応分
------------------------------	-----------	---------

PCR/RT-PCR日本語パンフレット インビトロジェンのPCR/RT-PCR製品なら...

- ★特異性、Fidelity(正確性)および性能の向上
- ★収量と感度の向上
- ★定量的なPCRとRT-PCR



各製品の性能、特長等を記載しております。
当社ホームページからもダウンロードできます。

⇒ <http://www.invitrogen.co.jp>

高性能定量的PCR/RT-PCRには

Platinum® Quantitative PCR Platinum® Quantitative RT-PCR

Platinum® Quantitative RT-PCR ThermoScript™ One-Step System	11731-015	100 反応分
Platinum® Quantitative PCR SuperMix-UDG	11730-017	100 反応分
ROX Reference Dye	12223-012	500 µl

<http://www.invitrogen.com>

製造元

Invitrogen Corporation

輸入総発売元

インビトロジェン株式会社
(旧 ライフテックオリエンタル株式会社)

〒103-0007 東京都中央区日本橋浜町2-35-4 日本橋浜町パークビル
TEL (03) 3663-8143 FAX (03) 3663-8242



第45回日本神経化学会 抄 録 集



● 会 期 平成14年7月17日（水）～19日（金）

● 会 場 かでる2.7

〒060-0002 札幌市中央区北2条西7丁目

TEL: 011-231-4111

ホテル札幌ガーデンパレス

〒060-0001 札幌市中央区北1条西6丁目

TEL: 011-261-5311

● 大会長 野村 靖幸

（北海道大学大学院薬学研究科薬理学分野）

● 事務局 第45回日本神経化学会大会事務局

〒060-0812 札幌市北区北12条西6丁目

TEL: 011-706-3247 FAX: 011-706-4987

E-mail: 45jsn@pharm.hokudai.ac.jp

URL: <http://www.hokudai.ac.jp/pharma/yakuri/45JSN.htm>

目 次

■ 第45回日本神経化学会大会 大会長、組織委員、プログラム委員	159
■ 学会会場案内	160
■ 学会への参加受付	164
■ 発表要領	166
■ 大会日程概要	169
■ 日本神経化学会からのお知らせ	172
■ プログラム (英文)	
Plenary Lectures (PL-1~PL-4)	173
Invited Lectures (IL-1~IL-4)	174
2002 ISN Symposium (ISN-1~ISN-5)	175
Open Symposium (OS-1~OS-6)	176
Symposium (S1~S11)	177
Mini Symposium (MS1~MS6)	188
Poster	192
■ プログラム (和文)	
特別講演 (PL-1~PL-4)	225
招待講演 (IL-1~IL-4)	226
国際神経化学会シンポジウム (ISN-1~ISN-5)	227
市民公開シンポジウム (OS-1~OS-6)	228
シンポジウム (S1~S11)	229
ミニシンポジウム (MS1~MS6)	237
ポスター	241
■ 発表抄録	
Plenary Lectures (PL-1~PL-4)	269
Invited Lectures (IL-1~IL-4)	273
2002 ISN Symposium (ISN-1~ISN-5)	277
Open Symposium (OS-1~OS-6)	282
Symposium (S1~S11)	285
Poster	313
■ 謝辞	428
■ ランチョンセミナー	429
■ 奨励賞選考シンポジウム	430
■ Author Index	431
■ Keyword Index	437
■ 編集後記	443

第 45 回日本神経化学会大会 会長

野村 靖幸 (北海道大学大学院薬学研究科)

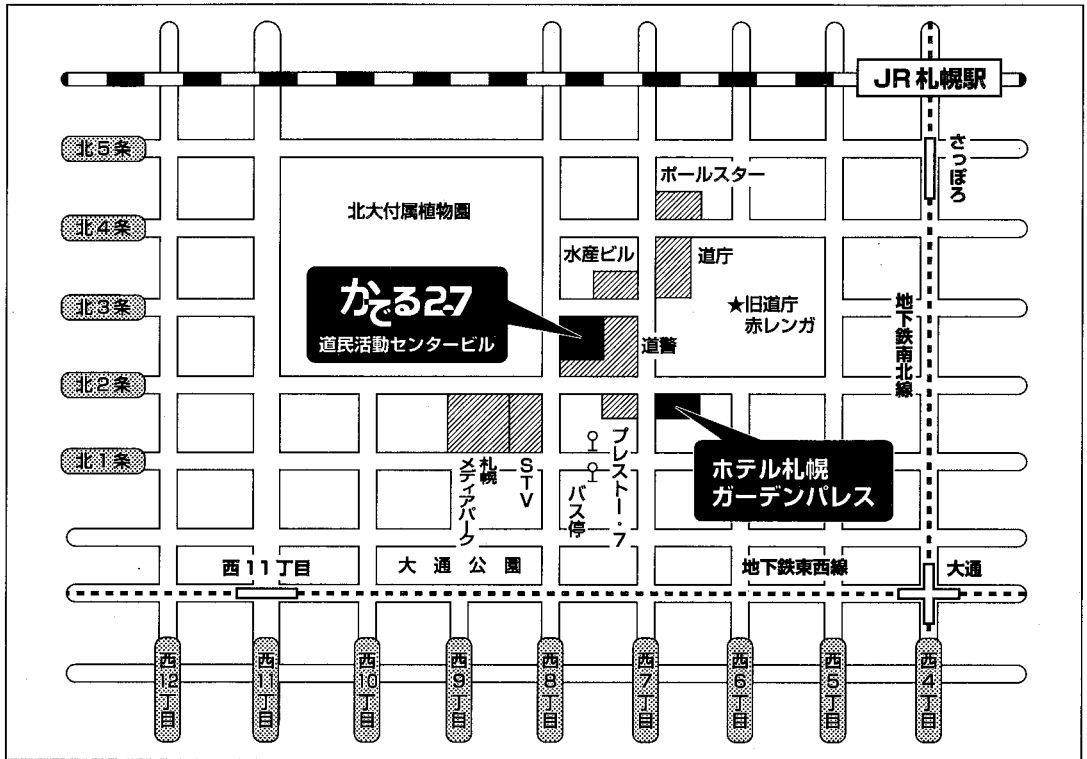
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三輪 聡一 (北海道大学大学院医学研究科)	金子 雅幸 (北海道大学大学院薬学研究科)
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平 敬宏 (北海道大学大学院薬学研究科)	

会場へのアクセス



●新千歳空港 — 札幌

●連絡バス

新千歳空港 — 札幌 約90分 (820円)

●鉄 道

新千歳空港 — 札幌 JR快速エアポート 約40分 (1,040円)

各方面から航空機での新千歳空港までの所要時間

東 京 (約90分)

大 阪 (約105分)

名古屋 (約95分)

仙 台 (約75分)

広 島 (約110分)

福 岡 (約130分)

沖 縄 (約180分)

●かでの2.7 (A・E・F会場)

JR札幌駅から徒歩 約7分

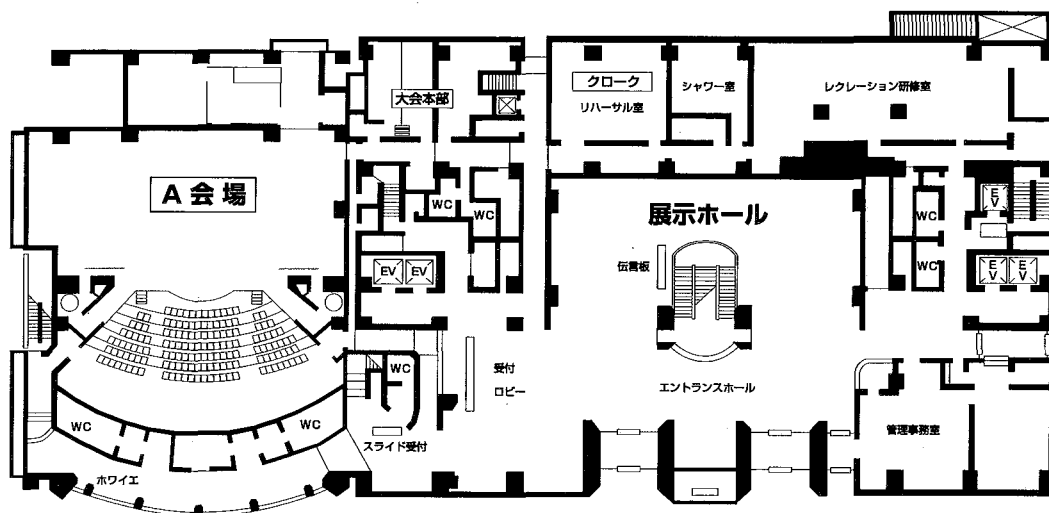
●ホテル札幌ガーデンパレス (B・C・D会場)

JR札幌駅から徒歩 約8分

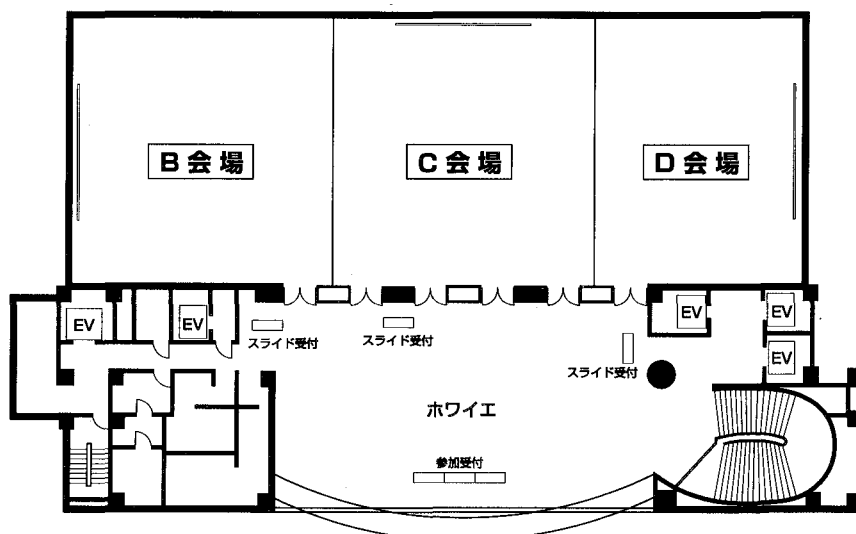
地下鉄大通駅から徒歩 約5分

学会場案内

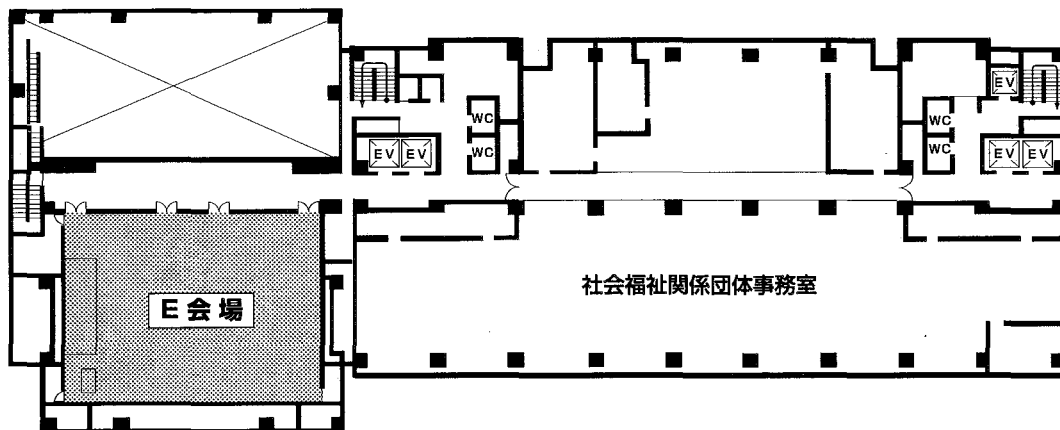
●かでの2.7 1階〔A会場, 展示会場, 総合受付, 大会本部〕



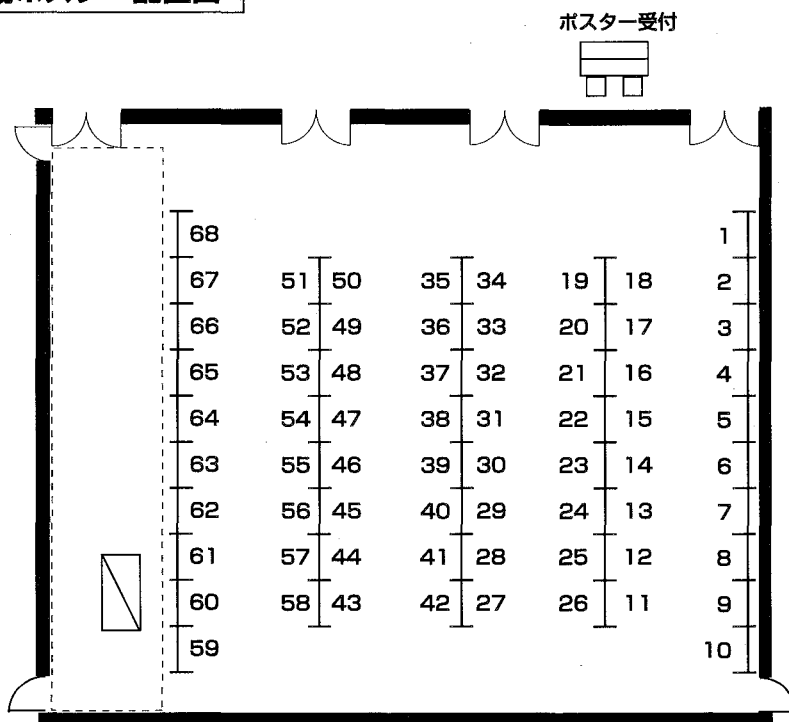
●ホテル札幌ガーデンパレス 2階〔B会場, C会場, D会場〕



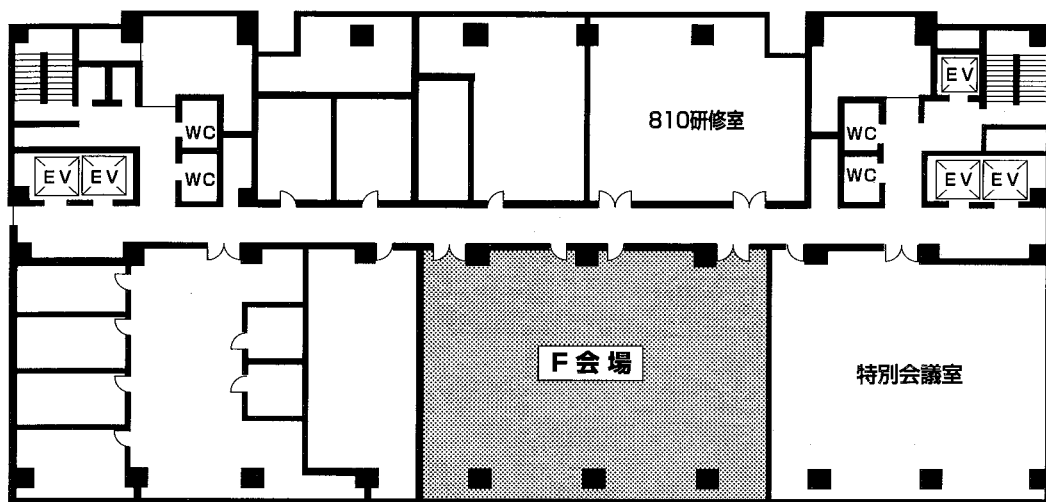
●かでのる2.7 4階 (E会場)



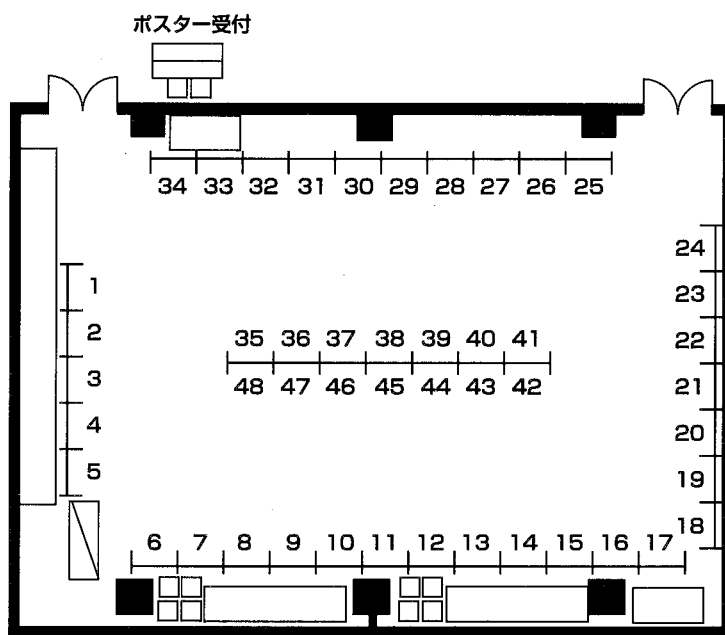
E 会場ポスター配置図



●かでの2.7 8階 (F会場)



F 会場ポスター配置図



学会への参加受付

1. 参加者の皆様へ

●参加登録をお済ませの方へ

学会当日は大会事務局からお送り致しました参加登録証（名札）を必ずご持参ください。また会期中は名札を胸にお付けくださいますようお願い申し上げます。万一お忘れになられた場合には総合受付までお申し出ください。公開シンポジウムを除き、名札のない方の入場はお断り致します。

●当日参加登録される方へ

受付はかでの2.7の1階受付において午前8時30分より開始します。お渡しした名札を胸にお付けのうえご入場ください。参加費は以下の通りです。学生の方はその旨お申し出ください。お渡しする名札には学生であることを明記させていただきます。

当日受付参加費

会 員	11,000円
非 会 員	12,000円
学 生 会 員	4,000円（学生証をご提示ください）
学 生 非 会 員	4,000円（学生証をご提示ください）
懇 親 会	8,000円

●非会員の方へ

本学会の筆頭発表者は本学会会員に限らせていただいております。非会員の筆頭発表者（代表発表者）は速やかに正会員としての入会手続きをお取りください。詳細は下記日本学会事務センターにお問い合わせください。「神経化学」誌のプログラム号（本誌）、抄録集は正会員の方のみ送付されます。ただし参加登録を既にお済ませの非会員の方へも、プログラム号だけは札幌大会事務局から直接送らせていただきます。抄録集は学会当日、総合受付でも販売いたしますので、ご希望の方はご購入求めください（6,000円）。

〒113-8622

東京都文京区本駒込5-16-9

（財）日本学会事務センター

日本神経化学会事務局

TEL:03-5814-5810 FAX:03-5814-5825

ホームページ <http://wwwsoc.nii.ac.jp/jsn/>

●取得単位について

本学会への参加は、日本神経学会、日本小児神経学会、および日本良導絡自律神経学会の認定医研修単位の対象となります。詳細はそれぞれの学会に直接ご照会ください。

2. お知らせ

●会場の入場について

会場は午前8時30分にならないと入場できません。ご注意ください。

●クローク

会期中は、かでの2.7内にクロークを設ける予定ですが、スペースに限りがございます。出来るだけホテル等のクロークをご利用いただくようお願い致します。

●呼び出し、伝言

放送での参加者の呼び出し、お電話等の取り次ぎは一切いたしません。かでの2.7 1階ロビーに伝言板を設置しておりますのでご利用ください。

●宿泊・交通・観光案内

かでの2.7の1階ロビーに近畿日本ツーリストの案内デスクを設置します。また会期前のお問い合わせは下記までお願いします。

近畿日本ツーリスト株式会社 札幌駅前支店

「第45回日本神経化学会札幌大会」デスク

〒060-0807

札幌市北区北7条西4丁目 新北海道ビルディング11階

電話：011-716-5522 FAX：011-716-5503

担当：山下 恵二

●学会本部

会期中は、かでの2.7の1階の大ホールの楽屋を本部に致します。連絡先等の詳細は第45回日本神経化学会（札幌）ホームページにてお知らせいたします。ホームページにてご確認ください。

URL：<http://www.hokudai.ac.jp/pharma/yakuri/45JSN.htm>

●懇親会

大会2日目（18日）の19:00よりホテル札幌ガーデンパレスB会場にて行いますので、是非ご参加ください（参加費：8,000円）

講演発表要領

1. 会場

A、B、C、D各会場において同時進行で行われます。

2. 発表・討論、進行

- 1) 各演者の発表・討論の時間はプログラムに記載されていますので、時間厳守をお願いいたします。
- 2) 進行は座長の責任において行ってください。シンポジウム終了時間を厳守願います。
- 3) 演者は講演開始10分前までに、次演者席にお着きください。
- 4) 座長はご担当のシンポジウムの開始15分前までに、各シンポジウム会場の受付までお越しください。
- 5) 討論者は、あらかじめ会場内の討論用マイクの前でお待ちください。
- 6) 討論においては、簡潔にご発言ください。
- 7) ミニシンポジウムの講演時間は発表13分、討論5分です。時間の厳守をお願い致します。

3. 発表機材

- 1) シンポジウムでは、4.および5.に記載の発表機材をご使用いただけます。ご使用にあたっては、4.および5.の各項にある使用上の注意をよくお読みください。ミニシンポジウムに関しては時間の関係上スライド発表でのみ行ってください。
- 2) スクリーンは1面です。スライド並写や複数の機材による並写はできません。

4. スライド映写機

- 1) スライドは35mm判とします。
- 2) 紙マウントのスライドは避け、できる限りプラスチックマウントのスライドをご用意ください。
- 3) スライド枚数に制限はありません（ただし、予定の発表時間はお守りください）。
- 4) 同じスライドを重複して使用される場合は、必要枚数分をご用意ください。
- 5) スライド映写機の操作は演者の口頭の指示により係員が行います。

5. 液晶プロジェクター（PC）

- 1) パソコンは演者が持参してください。
- 2) ミニDサブ15pinの出力端子が、本体またはアダプターにあることが必要です。
- 3) ソフトウエアは「PowerPoint」の使用を強く推奨いたします。「PowerPoint」以外のソフトウエアをご使用の方は、予め準備事務局（E-mail：45jsn@pharm.hokudai.ac.jp, cw@c-work.co.jp, tel. 011-706-3247, fax. 011-706-4987）にご相談ください。
- 4) パソコン持参ではなく、MO、CD-Rなどでご持参予定の方は予め準備事務局にご相談ください。
- 5) パソコンからの映写ができることを、実際にお使いになる会場で、事前にご確認くださるようお願いいたします。午前の講演予定の方は講演前あるいは前日等、午後のシンポジウムの方は当日のポスター発表討論時間等、いずれもスタッフを配置していますので、会場にお越しいただき試写をお願いいたします。
- 6) プロジェクター、パソコンなどの不具合により映写不能となる事態も予想されますので、講演の主旨を聴衆に伝えることができる程度に、バックアップのスライドを必ずご用意ください。
- 7) パソコンの操作は演者が演台で行うことになります。

6. スライド等の受付および返却

- 1) スライド等は講演開始30分前までに各会場の受付にご提出ください。スライド等引換証をお渡しします。
- 2) スライドをご使用の場合は、ご自分で所定のスライドホルダーにスライドを入れ、試写により確認の上、ご提出ください。
- 3) 講演終了後は速やかに、各会場受付にてスライド等をお受け取りください。引換証と交換に返却いたします。

7. 司会者、座長の方へ

- 1) 担当される各セッション開始20分前までには各会場入りし、次座長席にお着きください。
- 2) 時間厳守に留意していただき運営してください。
- 3) シンポジウムおよびミニシンポジウムでは最後に必ずまとめの時間をとって、フロアーを交えて討論を行ってください。

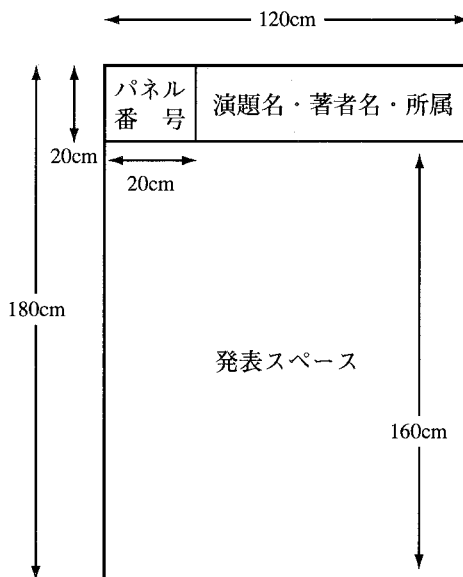
ポスター発表（一般演題）要領

1. 会場

ポスター発表は、E会場（かでの2.7, 4F大会議室）およびF会場（かでの2.7, 8F 820研修室）にて行われます。

2. 発表要領

- 1) 右図のように、縦20cm×横100cmのサイズに演題、著者名、所属を和英文にて記入したものをご用意ください。
- 2) 発表内容は右図発表スペース（縦160cm×横120cm）に収まるようにしてください。
- 3) パネル番号は大会事務局にて用意します。
- 4) 展示内容については英語により行うことを推奨します。
- 5) 演題番号で示される会場、発表日、パネル番号に従って、下記の掲示時間の間、ポスターを掲示してください。
- 6) プッシュピンはポスター受付でお受け取りください。のり、セロハンテープ等は使用しないでください。
- 7) 掲示時間が終了しましたら、ポスターは各自で撤去してください。撤去されなかった掲示物は大会本部にて処分しますのでご了承ください。



3. 掲示時間

7月17日（水） 9：00—17：00

7月18日（木） 9：00—17：00

4. 討論

- 1) 発表者の方には、ポスター受付でリボンをお渡しいたしますので、討論の際には胸につけて、各ポスターパネルの前で参加者の質疑に応じてください。
- 2) 討論は、演題番号の奇数・偶数で2つのグループに分け、2交替制で行います。下記の討論時間には発表者はポスターの前で待機してください。

7月17日（水）	演題番号 奇数	11：30—12：10
	演題番号 偶数	13：10—13：50
7月18日（木）	演題番号 奇数	11：30—12：10
	演題番号 偶数	13：10—13：50

第1日目 7月17日(水) 日程表

	かでの2.7 1F	ホテル札幌ガーデンパレス 2F			かでの2.7 4F & 8F
	A 会場	B 会場	C 会場	D 会場	E, F会場 (ポスター)
9:00	招待講演 IL-1 本間研一 座長：辻 省次	ミニシンポジウム MS1 「細胞形態および運動の 制御と神経系形成」 座長：福島伸之 五嶋良郎 田代朋子	ミニシンポジウム MS2 「神経細胞死」 座長：和中明生 木山博資	奨励賞選考 シンポジウム 座長：池中一裕	ポスター掲示
10:00	招待講演 IL-2 桐野 豊 座長：馬場明道		ミニシンポジウム MS3 「神経栄養因子」 座長：高松 研 古川昭栄		
11:00					
11:30					討 論 (奇数番号)
11:45					
12:10	評議員会	ランチョンセミナー-1 杉本八郎 座長：小山 司			
13:00					討 論 (偶数番号)
13:10					
14:00					ポスター撤去
14:30	国際神経化学会 (ISN) シンポジウム 座長：御子柴克彦 P. R. Dunkley	シンポジウム S1 「中枢神経系再生のストラ テジー」 座長：岡野栄之	シンポジウム S2 「PET・MRIを用いたヒト 脳機能イメージング：現 状と展望」 座長：定藤規弘	シンポジウム S3 「神経機能分子の構造的 理解」 座長：芳賀達也	
17:00	市民公開シンポジウム OS 「心と行動を分子で読む」 座長：武田雅俊 西川 徹				
20:00					

第2日目 7月18日(木) 日程表

	かでの2.7 1F	ホテル札幌ガーデンパレス 2F			かでの2.7 4F & 8F
	A 会場	B 会場	C 会場	D 会場	E, F会場 (ポスター)
9:00	特別講演 PL-1 廣川信隆 座長：三木直正	ミニシンポジウム MS4 「神経変性疾患」 座長：柳澤勝彦 遠山正彌	ミニシンポジウム MS5 「神経伝達物質」 座長：熊倉鴻之助 野村正彦	シンポジウム S4 「脳の器官培養系の技術と 応用を探る」 座長：田中正彦 坂口卓也	ポスター掲示
10:00	特別講演 PL-2 辻本賢英 座長：高坂新一		ミニシンポジウム MS6 「受容体シグナリング」 座長：村山俊彦 山脇成人		
11:00					
11:30					
11:45					
12:10	総 会	ランチョンセミナー-2 服部信孝 座長：田代邦雄			討 論 (奇数番号)
13:00					
13:10					
14:00					討 論 (偶数番号)
14:30	特別講演 PL-3 David S. Bredt 座長：野村靖幸	シンポジウム S6 「脳神経系細胞系譜決定と その可塑的变化の分子メカ ニズム」 座長：池前一裕	シンポジウム S7 「チロシンリン酸化による シナプス機能の制御」 座長：武井延之 高橋正身	シンポジウム S8 「グリア細胞の機能と病態 ：神経疾患の治療に向けて」 座長：下濱 俊, 北村佳久, 馬場広子	ポスター撤去
15:00	特別講演 PL-4 Peter R. Dunkley 座長：宮本英七				
16:00	シンポジウム S5 「協調的分子配置による シナプスの機能発現」 座長：阿部輝雄 白尾智明				
17:00					
18:30					
19:00					
20:30		懇 親 会			

第3日目 7月19日(金) 日程表

	かでの2.7 1F	ホテル札幌ガーデンパレス 2F			
	A 会場	B 会場	C 会場	D 会場	
9:00	招待講演 IL-3 西本毅治 座長：東田陽博	シンポジウム S9 「mRNA制御と神経疾患」 座長：今泉和則 塩見春彦	シンポジウム S10 「プロテオグリカンによる 神経機能調節機構」 座長：前田信明 大平敦彦	シンポジウム S11 「神経・内分泌系と免疫系 間におけるクロストーク」 座長：川島紘一郎 大熊康修	
10:00	招待講演 IL-4 大隅良典 座長：小宮義璋				
11:00					
11:30					

学会からのお知らせ

1. 総 会

日時：7月18日（木） 11：45 - 13：00

会場：A会場（かでの2.7 大ホール）

平成14年度総会議題（案）

1. 理事長報告
2. 庶務報告
3. 出版・広報報告
4. プログラム編成報告
5. 委員会報告
6. 平成14年度奨励賞選考結果報告
7. 平成13年度決算の件
同 監査報告
8. 会費改定の件
9. 平成15年度予算の件
10. 評議員選任の件
11. 名誉会員推薦の件
12. 理事改選の件
13. 次期大会の件
14. その他

2. 評議員会

日時：7月17日（水） 11：45 - 13：00

会場：A会場（かでの2.7 大ホール）

評議員会議題（案）

「日本神経化学会の国際化および学会のあり方について」

Plenary Lectures

July 18 (Thu.) 9 : 00 - 10 : 00 Room A

Chairperson : Naomasa Miki (Osaka Univ. Grad. Sch. Med.)

- PL-1 Neuronal function and intracellular transport: gene, structure, dynamics, function and diseases of kinesin superfamily motor proteins, KIFs.

Nobutaka Hirokawa

(University of Tokyo, Graduate School of Medicine)

July 18 (Thu.) 10 : 00 - 11 : 00 Room A

Chairperson : Shinichi Kohsaka (Natl. Inst. Neurosci.)

- PL-2 Molecular basis of apoptosis: Bcl-2 family proteins as a life-or-death switch

Yoshihide Tsujimoto

(Osaka University Medical School, Dept. of Post-Genomics & Diseases, CREST of JST)

July 18 (Thu.) 14 : 00 - 15 : 00 Room A

Chairperson : Yasuyuki Nomura (Hokkaido Univ., Grad. Sch. Pharm. Sci.)

- PL-3 Synaptic plasticity regulated by stargazin and PSD-95

David S. Bredt

(Department of Physiology, University of California at San Francisco)

July 18 (Thu.) 15 : 00 - 16 : 00 Room A

Chairperson : Eishichi Miyamoto (Kumamoto Univ. Sch. Med.)

- PL-4 Tyrosine hydroxylase phosphorylation

Peter R. Dunkley

(School of Biomedical Sciences, Faculty of Health, The University of Newcastle)

Invited Lectures

July 17 (Wed.) 9 : 00 – 10 : 00 Room A _____

Chairperson : Shoji Tsuji (Niigata Univ. Brain Res. Insc.)

IL-1 Molecular mechanism and hierarchical structure of circadian clock

Ken-ichi Honma

(Hokkaido University Graduate School of Medicine, Department of Physiology)

July 17 (Wed.) 10 : 00 – 11 : 00 Room A _____

Chairperson : Akemichi Baba (Osaka Univ. Grad. Sch. Pharmaceutical Sci.)

IL-2 Molecular and neural mechanisms of eyeblink conditioning studied with mutant mice

Yutaka Kirino

(Laboratory of Neurobiophysics, School of Pharmaceutical Sciences, The University of Tokyo)

July 19 (Fri.) 9 : 00 – 10 : 00 Room A _____

Chairperson : Haruhiro Higashida (Kanazawa Univ. Grad. Sch. Med. Sci.)

IL-3 Regulation of nucleocytoplasmic transport and cell proliferation by Ran GTPase.

Takeharu Nishimoto

(Department of Molecular Biology, Graduate School of Medical science, Kyushu University)

July 19 (Fri.) 10 : 00 – 11 : 00 Room A _____

Chairperson : Yoshiaki Komiya (Gunma Univ. Faculty Med.)

IL-4 Bulk protein degradation - Molecular dissection of autophagy -

Yoshinori Ohsumi

(National Institute for Basic Biology)

July 17 (Wed.)

14 : 00 - 17 : 00

Room A

2002 ISN Symposium

Molecular Basis of Neuronal Signaling

Chairpersons : Katsuhiko Mikoshiba (Univ. Tokyo, Japan)
Peter R. Dunkley (Univ. Newcastle, Australia)

ISN-1 AMPA receptor trafficking during plasticity

Robert Malinow

(Cold Spring Harbor Laboratory, USA)

ISN-2 Molecular genetics of the synapse formation and function

Masayoshi Mishina

(Department of Molecular Neurobiology and Pharmacology, Graduate School of Medicine, University of Tokyo)

ISN-3 Cdk5: A new player at the neuromuscular synapse

Nancy Y. Ip

(Department of Biochemistry, Molecular Neuroscience Center, and Biotechnology Research Institute, Hong Kong University of Science and Technology, China)

ISN-4 Structure, regulation and function of PKB/Akt—a pleiotropic protein kinase involved in multiple signaling pathways

Brian A. Hemmings

(Friedrich Miescher-Institute, Switzerland)

ISN-5 Excitatory amino acid transporters: New insights into structure, function and regulation

Susan G. Amara

(Vollum Institute and Howard Hughes Medical Institute, Oregon Health & Science University, USA)

July 17 (Wed.)

17 : 00 - 20 : 00

Room A

Open Symposium

Reading Human Brain and Behavior on Molecular Level

Chairpersons : Masatoshi Takeda (Osaka Univ. Grad. Sch. Med.)

Toru Nishikawa (Tokyo Med. Dent. Univ. Grad. Sch.)

OS-1 How important model animals are in research for psychiatric disorders!

○ Keiji Wada¹, Yuko Santo-Yamada¹, Etsuko Wada^{1,2}, Hiroshi Maeno¹, Kazuyuki Yamada^{1,3}

(¹Natl. Inst. Neurosci. NCNP, ²JSPS, ³BSI, RIKEN)

OS-2 Mental and behavioral activities in relation to brain signal transduction

○ Toshikazu Saito

(Dept. Neuropsychiatry, Sapporo Med. Univ. Sch. Med.)

OS-3 PET imaging on developmental disorder and learning formation

○ Yasuyoshi Watanabe

(Dept. Physiology, Osaka City Univ. Grad. Sch. Med.)

OS-4 How do genes and environment generate human behaviors? The perspectives of evolutionary and developmental biology

○ Shigenobu Kanba

(Dept. Neuropsychiatry, Yamanashi Med. Univ.)

OS-5 A novel pharmacological approach to the molecular basis of schizophrenia

○ Toru Nishikawa¹, Yasushi Kajii^{1,2}, Shin-ichiro Muraoka¹, Asami Umino¹, Yasukazu Kuroda¹

(¹Section of Psychiatry and Behavioral Sci., ²Tokyo Med. Dent. Univ. Grad. Sch., Mitsubishi Pharma Co.)

OS-6 Antidepressants: New pharmacological strategies

○ Tsukasa Koyama

(Dept. Psychiatry, Hokkaido Univ. Grad. Sch. Med.)

July 17 (Wed.)

9 : 00 - 11 : 00

Room D

Young Scientists Lectureship Award

Chairperson : Kazuhiro Ikenaka (Natl. Inst. Physiol. Sci.)

Symposium

July 17 (Wed.)

14 : 30 - 17 : 00

Room B

S1 Neural Regeneration

Chairperson : Hideyuki Okano (Keio Univ., Sch. Med.)

- S1-1** **New strategies using adult neural stem cells in defined media and the absence of gene transfer**
 ○Tetsuro Shingo, Isao Date, Takeshi Ohmoto, Samuel Weiss
 (Dept. Neurol. Surg., Okayama Univ. Med. Sch., Genes Develop. Res. Group., Univ. Calgary Fac. Med.)
- S1-2** **Recent advance in the adenovirus mediated gene transfer system for neuronal regeneration**
 ○Hiroshi Kiyama, Kazuhiko Namikawa
 (Dept. Anatomy, Grad. Sch. Med., Osaka City Univ.)
- S1-3** **AAV vectors and gene therapy for Parkinson's disease**
 ○Keiya Ozawa¹, Shin-ichi Muramatsu², Lijun Wang^{1,2}, Kunihiro Ikeguchi², Ken-ichi Fujimoto², Takashi Okada¹, Hiroaki Mizukami¹, Keiji Terao³, Imaharu Nakano²
 (¹Div. Genet. Therapeut., Ctr. Mol. Med., Jichi Med. Sch., ²Dpt. Neurol., Jichi Med. Sch., ³Tsukuba Primate Ctr., Nat. Inst. Infect. Dis.)
- S1-4** **Transdifferentiation of bone marrow stromal cells into neurons and Schwann cells, and application to the CNS regeneration**
 ○Mari Dezawa
 (Dept. Anat., Yokohama City Univ. Sch. Med.)
- S1-5** **Strategies toward their potential therapeutic application in to spinal cord injury model**
 ○Hideyuki Okano
 (Dept. Physiol., Keio Univ. Sch. Med., CREST, JST)

July 17 (Wed.)

14 : 30 - 17 : 00

Room C

S2 Functional neuroimaging studies with PET and MRI: present status and future perspective

Chairperson : Norihiro Sadato (Natl. Inst. Physiol. Sci.)

- S2-1** Cross-modal plasticity in the deaf and blind: studies with non-invasive human neuroimaging techniques
○Norihiro Sadato
(Natl. Inst. Physiol. Sci.)
- S2-2** Quantification and Alteration of Dopamine Receptors in Living Human Brain
○Tetsuya Suhara
(Natl. Inst. Radiol. Sci., CREST)
- S2-3** Imaging of cholinergic system of the brain
○Hiroki Namba
(Dept. Neurosurg., Hamamatsu Univ. School of Med.)
- S2-4** The application of PET imaging to clinical researches on the histaminergic neuron system: From neurochemical studies to drug development.
○Kazuhiko Yanai
(Dept. Med., Grad. Sch. Pharmacopathol. Sci., Tohoku Univ.)
- S2-5** PET imaging of neural substrates for fatigue sensation
○Yasuyoshi Watanabe
(Dept. Physiol., Osaka City Univ. Grad. Sch. Med.)

July 17 (Wed.)

14 : 30 - 17 : 00

Room D

S3 Introduction to Structural Biology of the Neuron

Chairperson : Tatsuya Haga (Gakushuin Univ., Dept. Chem.)

- S3-1** **The voltage-sensitive sodium channel has a bell-shaped structure with multiple cavities; Structure revealed by single particle analysis**
 ○Chikara Sato¹, Yutaka Ueno¹, Toshihiko Ogura¹, Masahiko Sato², Yoshinori Fujiyoshi³
 (¹N.I.A.I.S.T., ²Itoham Foods Inc., ³Dept. Biophy., Fac. Sci., Kyoto Univ.)
- S3-2** **Conformation of a peptide bound to a G-protein coupled receptor**
 ○Masahiro Shirakawa
 (Grad. Sch. Integ. Sci., Yokohama City Univ.)
- S3-3** **Molecular mechanism of signal transduction mediated by Grb2**
 ○Fuyuhiko Inagaki
 (Grad. Sch. Pharmaceut. Sci., Hokkaido Univ., CREST)
- S3-4** **Structure and function of signaling proteins controlling cell adhesion and cytoskeleton**
 ○Toshio Hakoshima
 (Nara Institute of Science and Technology/CREST)
- S3-5** **Mechanism of processive movement of monomeric KIF1A motor revealed with biophysics, cryo EM and X ray crystallography.**
 ○Nobutaka Hirokawa
 (Dept. Cell Biol. and Anat., Grad. Sch. Med., Univ. of Tokyo)

July 18 (Thu.)

9 : 00 - 11 : 30

Room D

S4 Technology-based advances in applications of organotypic cultures from the central nervous system

Chairpersons : Masahiko Tanaka (Fujita Health Univ., Inst. Comprehensive)
Takuya Sakaguchi (Shionogi Co., Ltd., Discovery Res. Lab.)

- S4-1 Aspects and applications of organotypic brain slice cultures**
○Takuya Sakaguchi
(Development. Res. Lab., Shionogi & Co., Ltd.)
- S4-2 Development of hippocampal mossy fibers in organotypic cocultures**
○Yuji Ikegaya, Norio Matsuki
(Lab. Chem. Pharmacol., Grad. Sch. Pharmaceut. Sci., Univ. Tokyo)
- S4-3 Activity-dependent branch formation of cortical axons: A morphological and electrophysiological study using organotypic slice cultures**
○Nobuhiko Yamamoto, Naofumi Uesaka, Satoshi Hirai, Soichiro Ohnami
(Lab. Neurosci., Grad. Sch. Frontier Biosci., Osaka Univ.)
- S4-4 Analysis of neuron-glia interaction involved in the dendritic morphogenesis of cerebellar Purkinje cells using an organotypic slice culture system**
○Masahiko Tanaka
(Div. Cell Biol., Inst. Comprehens. Med. Sci., Fujita Hlth. Univ.)
- S4-5 The role of Pax6 in the mammalian olfactory bulb development**
○Tadashi Nomura, Noriko Osumi
(Dep. Dev. Neurobiol., Tohoku. Univ. Grad. Sch. Med.)

July 18 (Thu.)

16 : 00 - 18 : 30

Room A

S5 Expression of synaptic functions by cooperative molecular localization

Chairpersons : Teruo Abe (Niigata Univ. Brain Res. Inst.)
Tomoaki Shirao (Gunma Univ. Sch. Med.)

- S5-1** **A Possible mechanism for attaching synaptic vesicles to microtubules**
○Teruo Abe
(Dept. Cell. Neurobiol., Brain Res. Inst., Niigata Univ.)
- S5-2** **Diversity of Ca²⁺channel subtypes in presynaptic terminals**
○Hiromu Yawo
(Dept. Develop. Biol. Neurosci., Tohoku Univ. Grad. Sch. Life Sci., CREST, JST)
- S5-3** **Synaptic targeting of PSD scaffold proteins containing leucine-zipper motifs; PSD-Zip45 and PSD-Zip70**
○Kenji Sobue, Daijiro Konno, Shinichi Usui, Kei Hori, Hisato Maruoka
(Dept. Neuroscience (D13), Osaka Univ. Grad. Sch. Med.)
- S5-4** **Differential dynamic behavior of postsynaptic density proteins and its regulation by neuronal activity**
○Shigeo Okabe
(Dept. Cell Biol., Grad. Sch. Med., Tokyo Medical and Dental Univ.)
- S5-5** **Role of drebrin in signal transmission within the dendritic spine**
○Tomoaki Shirao, Satoshi Tanaka, Hiroyuki Yamazaki, Yuko Sekino
(Dept. Neurobiol. & Behav., Gunma Univ. Sch. Med.)

July 18 (Thu.)

14 : 30 - 17 : 00

Room B

S6 Molecular mechanism underlying neural cell lineage determination and its plastic changes

Chairperson : Kazuhiro Ikenaka (Natl. Inst. Physiol. Sci.)

S6-1 Molecular control of neural stem cell fate

○Masato Nakafuku, Motoshi Nagao

(Dept. Neurobiol., Grad. Sch. Med., Univ. Tokyo)

S6-2 Neurogenin 1 as a positive bHLH regulator of neuronal differentiation

So-Yeon Kim¹, Yong-Soo Yoon¹, Yong-Jin Jeon¹, Mi-Young Jung¹, Seong-Soo Kim¹, Jiwon Kim¹, Seung-Up Kim², Young-Don Lee¹, ○Haeyoung Suh-Kim^{1,2}

(¹Dept. Anat., Ajou Univ., Sch. Med., ²BDRC, Ajou Univ., Sch. Med.)

S6-3 Cell-external cues and cell-intrinsic programs regulating lineage-determination in the developing brain.

○Tetsuya Taga, Takumi Takizawa, Kinichi Nakashima

(Dept. Cell Fate Modulation, Inst. Mol. Embryol. Genet., Kumamoto Univ.)

S6-4 Molecular mechanisms underlying oligodendrocyte development

○Tetsushi Kagawa, Kazuhiro Ikenaka

(Neuralinfo. Lab., Natl. Inst. Physiol. Sci.)

S6-5 Genetic dissection of differentiation and outgrowth of the cranial motor and sensory neurons in zebrafish

○Hitoshi Okamoto

(Lab. Dev. Gene Reg., BSI, RIKEN, CREST, JST)

July 18 (Thu.)

14 : 30 - 17 : 00

Room C

S7 Regulation of synaptic functions by protein tyrosine phosphorylation

Chairpersons : Nobuyuki Takei (Niigata Univ., Brain Res. Inst.)
Masami Takahashi (Kitasato Univ. Sch. Med.)

S7-1 Function and regulation of the Src family tyrosine kinases

○Masato Okada

(Dept. Oncogene Res., Res. Inst. Microbial Dis., Osaka Univ.)

S7-2 Regulatory roles of protein tyrosine phosphatases in Ca^{2+} -dependent neurotransmitter release from neuronal cells.

○Makoto Itakura¹, Saori Yamamori¹, Hiroshi Ohnishi², Masami Takahashi^{1,3}

(¹Dept. Biochem. Sch. Med., Kitasato Univ., ²Biosignal Research Center Inst. Molecular and Cellular Regulation, Gunma Univ., ³CREST, JST)

S7-3 Modulation of the NMDA receptor by a protein tyrosine kinase and PSD-95

○Makoto Inui

(Dept. Pharmacol., Yamaguchi Univ. Sch. Med.)

S7-4 Positive and negative regulations of synaptic function by receptor tyrosine kinases.

○Nobuyuki Takei, Hisaaki Namba, Mako Narisawa-Saito, Tadasato Nagano, Hiroyuki Nawa

(Dept. Mol. Neurobiol., Brain Res. Inst., Niigata Univ.)

S7-5 Roles of tyrosine kinase subfamily ErbB receptor on synapses

○Miwako Ozaki, Tsutomu Hashikawa

(Lab. Neural Archi., BSI, RIKEN)

July 18 (Thu.)

14 : 30 - 17 : 00

Room D

S8 Glial function and neurodegeneration: Possible therapeutic targets of neurodegenerative disorders

Chairpersons : Shun Shimohama (Kyoto Univ. Grad. Sch Med.)
Yoshihisa Kitamura (Kyoto Pharmaceutical Univ.)
Hiroko Baba (Tokyo Univ. Pharmacy)

- S8-1** Glia in development, function, degeneration and regeneration of the nervous system
○Shun Shimohama
(Dept. Neurol., Grad. Sch. Med., Kyoto Univ.)
- S8-2** Unique responses of astrocytes to Alzheimer's amyloid β protein
○Kazuho Abe, Miwa Misawa
(Dept. Pharmacol., Sch. Pharm., Hoshi Univ.)
- S8-3** Regulation of amyloid- β clearance in microglia
○Yoshihisa Kitamura, Jun-ichi Kakimura, Takashi Taniguchi
(Dept. Neurobiol., Kyoto Pharm. Univ.)
- S8-4** Therapeutic application of glia-neuron interaction on neurodegeneration
○Keiji Wada¹, Takayuki Harada¹, Chikako Harada¹, Yu-Lai Wang¹, Mami Noda², Hitoshi Osaka^{1,3}
(¹Dept. Degene. Neurol. Dis., Natl. Inst. Neurosci. NCNP, ²Lab. Pathophysiol. Grad. Sch. Pharm. Sci., Kyushu Univ., ³PRESTO, JST)
- S8-5** Reg family; new members of neuron-Schwann cell mediator during nerve regeneration.
○Kazuhiko Namikawa, Munehisa Fukushima, Koichi Murakami, Hiroshi Kiyama
(Dept. Anatomy, Osaka City Univ., Grad. Sch. Med.)
- S8-6** Changes of axonal protein organization under abnormal myelin conditions
○Hiroko Baba
(Dept. Mol. Neurobiol., Tokyo Univ. Pharm. Life Sci.)

July 19 (Fri.)

9 : 00 - 11 : 30

Room B

S9 Disturbance of mRNA factory in neurological diseases

Chairpersons : Kazunori Imaizumi (Nara Inst. Sci. Technology)
Haruhiko Shiomi (Univ. Tokushima Inst. Genome Res.)

- S9-1 A circadian output in *Drosophila* mediated by fragile X mental retardation gene**
Shunsuke Ben Inoue¹, Masami Shimoda², Izumi Nishinokubi³, Mikiko Chihara Siomi¹, Norio Ishida^{3,4,5}, ○Haruhiko Shiomi¹
(¹Inst. Genome Res., Univ. Tokushima, ²Nat. Inst. Agrobiol. Sci., ³Clock Cell Biol., Nat. Inst. AIST., ⁴Dept. Biomole. Engineering., Tokyo Inst. Tech., ⁵Cent. Interdis. Res.)
- S9-2 The mutations in the neural sex-determinant gene *fruitless* in *Drosophila* enhances male-to-male courtship**
○Daisuke Yamamoto
(Sch. Human Sci., Waseda Univ.)
- S9-3 Ribosomal protein L38 is essential for early embryogenesis and neural tube formation of mice**
○Toshihiko Shiroishi
(Mammalian Genet. Lab., Genet. Strains Res. Center, Natl. Inst. Genet.)
- S9-4 The RNA-binding protein causes aberrant splicing of presenilin-2 pre-mRNA in sporadic Alzheimer's disease**
○Taiichi Katayama^{1,3}, Takayuki Manabe^{1,3}, Shinsuke Matsuzaki^{1,3}, Kazunori Imaizumi^{2,3}, Masaya Tohyama^{1,3}
(¹Dept. Anat. Neurosci., Osaka Univ. Grad. Sch. Med., ²Div. Structural Cell Biol., Nara Inst. Sci. Tech.(NAIST), ³CREST/JST)
- S9-5 Aberrant mRNA splicing in neurodegenerative disorders**
○Kazunori Imaizumi
(Div. Structural Cell Biol., Nara Inst. Sci.&Tech. (NAIST))

July 19 (Fri.)

9 : 00 - 11 : 30

Room C

S10 Proteoglycans and Neural Function

Chairpersons : Nobuaki Maeda (Tokyo Metropolitan Inst., Neuroscience)
Oohira Atsuhiko (Aichi Pref. Colony)

- S10-1** Expression and possible function of versican/Bral1 complex at the nodes of Ranvier in the CNS
○Toshitaka Oohashi
(Dept. Mol. Biol.&Biochem., Grad. Sch. Med. Dent., Okayama Univ.)
- S10-2** Signal transduction of PTP ζ and morphogenesis of Purkinje cells
○Nobuaki Maeda¹, Masahiko Tanaka²
(¹Dept. Dev. Neurosci., Tokyo. Metro. Inst. Neurosci., ²Inst. Compre. Med. Sci., Fujita Health Univ.)
- S10-3** Signal Transduction of Phosphacan via TAG-1/Lyn in Lipid Rafts
○Kohji Kasahara, Yutaka Sanai
(Tokyo Metro., Inst., Med., Sci.)
- S10-4** Structure and expression pattern of neuroglycan C, a brain-specific transmembrane chondroitin sulfate proteoglycan, in the developing brain
○Sachiko Aono, Yoshihito Tokita, Shinobu Yamauchi, Takuya Shuo, Atsuhiko Oohira
(Dept. Perinatol., Inst. Dev. Res., Aichi Human Service Ctr.)
- S10-5** Specific heparan sulfate structures involved in retinal axon targeting
○Atsushi Irie
(Dep. Biochem. Cell Res., Tokyo Metropolitan Inst. Med. Sci.)

July 19 (Fri.)

9 : 00 - 11 : 30

Room D

S11 Neurohumoral-Immune Crosstalk

Chairperson : Kouichirou Kawashima (Kyoritsu College Pharmacy)

Yasunobu Okuma (Hokkaido Univ., Grad. Sch. Pharm. Sci.)

S11-1 Brain cytokine expression mediated by the vagus nerve and leptin

○Yasunobu Okuma, Toru Hosoi, Yasuyuki Nomura

(Dept. Pharmacol., Grad. Sch. Pharm. Sci., Hokkaido Univ.)

S11-2 Functional regulation of primary afferent neurons by interleukin-1 β

○Norimitsu Morioka¹, Atsuko Inoue², Yoshihiro Nakata²

(¹Dept. Dental Pharmacol., Grad. Sch. Biomed. Sci., Hiroshima Univ., ²Dept. Pharmacol., Grad. Sch. Biomed. Sci., Hiroshima Univ.)

S11-3 Mechanism by which endogenous glucocorticoid controls lymphocyte trafficking

○Ryoichi Nagatomi

(Dept. Med. Sci. Sports & Exerc., Tohoku Univ. Grad. Sch. Med.)

S11-4 Presence and involvement of non-neuronal lymphoid cholinergic system in regulation of immune function

○Takeshi Fujii, Koichiro Kawashima

(Dept. Pharmacol., Kyoritsu Col. Pharmacy)

S11-5 The effects of nicotine on Th1/Th2 balance in murine model of both Th1- and Th2-mediated inflammatory bowel disease

○Yuhji Murata¹, Jugoh Itoh²

(¹Dept. Gastroenterol., Aomori Gen. Hosp., ²First Dept. Int. Med., Hirosaki Univ.)

Mini Symposium

July 17 (Wed.)

9:00 - 11:30

Room B

MS1 From Cell Morphology and Polarity to Neural Morphogenesis

Chairpersons : Nobuyuki Fukushima (Hokkaido Univ. Grad. Sch. Med)
Yoshio Goshima (Yokohama City Univ. Sch. Med.)
Tomoko Tashiro (Aoyama-gakuin Univ.)

- MS1-1** Reelin signaling and Cdk5 in the control of neuronal positioning
(P02-E-68) ○Toshio Ohshima¹, Masaharu Ogawa², Katsuhiko Mikoshiba¹
(¹Lab. for Developmental Neurobiol., ²Lab. for Cell Culture Development, BSI, RIKEN)
- MS1-2** CRMP-2 and axon formation
(P02-F-32) ○Yuko Fukata, Kozo Kaibuchi
(Dept. Cell Pharmacol. Grad. Sch. Med., Nagoya Univ.)
- MS1-3** Dynamic morphological changes of migrating neurons during the cortical development
(P02-F-29) ○Hidenori Tabata¹, Kazunori Nakajima^{1,2}
(¹Dept. Mol. Neurobiol., Inst. DNA Med., Jike Univ. Sch. Med., ²PRESTO, JST)
- MS1-4** Lysophospholipid signaling in the regulation of neuronal morphology and motility
(P02-E-47) ○Nobuyuki Fukushima
(Dept. Biochem. Grad. Sch. Med., Hokkaido Univ.)
- MS1-5** A role of semaphorin in newronal network formation in cerebral cortex
(P02-E-46) ○Yoshio Goshima, Yukio Sasaki
(Dept. Pharmacol. Yokohama City Univ. Sch. of Med.)
- MS1-6** The roles of WAVes in filopodia formation at NG108 growth cones
(P02-E-45) ○Shigeaki Miyamoto¹, Motohiro Nozumi¹, Hiroyuki Nakagawa¹, Hiroaki Miki², Tadaomi Takenawa²
(¹Dept. Biochem. Eng., Kyushu Inst. Tech., ²Dept. Biochem., Inst. Med. Sci., Univ. of Tokyo)
- MS1-7** Neurite outgrowth effects of withanolide A, withanoside IV and withanoside VI in A β 25-35
(P02-F-27) induced atrophy of neurites
○Tomoharu Kuboyama, Chihiro Tohda, Katsuko Komatsu
(Res. Ctr. Ethnomed., Inst. of Natural Med., Toyama Med. & Pharm. Univ.)
- MS1-8** Expression of tau isoforms containing exon2 and 3 during synaptogenesis *in vivo* and *in vitro*
(P02-F-28) ○Chikara Kitamura, Yuta Wakayama, Masaki Masuno, Miyuki Noro, Tomoko Tashiro
(Dept. Biochemistry, Coll. Science and Engineering, Aoyama-gakuin Univ.)

July 17 (Wed.)

9:00 - 10:15

Room C

MS2 Neuronal Cell Death

Chairpersons : Akio Wanaka (Nara Med. Univ.)
Hiroschi Kiyama (Osaka City Univ., Sch. Med.)

- MS2-1** Transcription factors induced in cultured cortical neurons by beta amyloid peptide
(P01-F-30) ○Yoko Uchida, Fujiya Gomi
(Dept. Neuropathol., TMIG)
- MS2-2** Activity-dependent expression of *alivin 1* and its involvement in the survival of cerebellar granule
(P01-F-32) neurons
○Tomio Ono^{1,2}, Naoko Suzuki¹, Yoshiaki Kikkawa², Hiromichi Yonekawa², Seiichi Kawashima¹
(¹Dept. Mol. Biol., Tokyo Metro. Inst. Med. Sci., ²Dept. Lab. Animal Sci., Tokyo Metro. Inst. Med. Sci.)

- MS2-3 (P01-F-4)** Induction of differential responses in neurons and astrocytes by A β protein and non-A β component (NAC) of Alzheimer's disease amyloid through generation of reactive oxygen species and activation of NF- κ B
 ○Seigo Tanaka, Masanori Takehashi, Kunihiro Ueda
 (Lab. Mol. Clin. Chem., Instit. Chem. Res., Kyoto Univ.)
- MS2-4 (P01-F-2)** Niemann-Pick C1 deficiency reduces the levels of cholesterol in detergent insoluble, low-density membrane domains leading to activation of MAP kinase pathway and increased phosphorylation of tau
 ○Naoya Sawamura^{1,2}, Jian-Sheng Gong¹, Ta-Yuan Chang³, Katsuhiko Yanagisawa¹, Makoto Michikawa¹
 (¹Dept. Dementia Res., NILS, ²JSPS, ³Dept. Biochemistry, Dartmouth Med. Sch.)

July 17 (Wed.)

10:15 - 11:30

Room C

MS3 Neurotrophic Factor

**Chairpersons : Ken Takamatsu (Toho Univ. Sch. Med.)
 Shoei Furukawa (Gifu Pharm. Univ.)**

- MS3-1 (P02-F-14)** Mechanisms of riluzole-induced brain-derived neurotrophic factor synthesis in the rat hippocampus
 ○Ritsuko Katoh-Semba, Kanefusa Kato
 (Inst. Dev. Res. Aichi Human Service Center)
- MS3-2 (P02-F-15)** bFGF evokes a rapid glutamate release through activation of the MAPK pathway in cultured cortical neurons.
 ○Tadahiro Numakawa^{1,2,3}, Daisaku Yokomaku^{1,2}, Kazuyuki Kiyosue², Naoki Adachi¹, Tomoya Matsumoto^{1,2}, Yumiko Numakawa^{1,2}, Takahisa Taguchi², Hiroshi Hatanaka¹, Masashi Yamada¹
 (¹Div. Protein Biosynthesis, Inst. Protein Res., Osaka Univ., ²Neuronics R.G. Div. Human Life Tech. Natl. Inst. Advanced Industrial Sci. Tech. (AIST), ³a JSPS Postdoctoral Fellow)
- MS3-3 (P02-F-16)** Change of the expression of glial cell line-derived neurotrophic factor (GDNF) in the rat sciatic nerve after its transection
 ○Yoshihisa Yamada¹, Atsumi Nitta², Katsuji Shimizu¹, Shoei Furukawa²
 (¹Dept. of Orthop. Surg., Gifu Univ. Sch. Med., ²Lab. of Mol. biol., Gifu Pharm. Univ.)
- MS3-4 (P02-F-2)** Interactions between necln and E2F1 in terminal differentiation and survival of sensory neurons
 ○Risa Takazaki, Taichi Uetsuki, Hideo Taniura, Kazuaki Yosikawa
 (Div. Regulation of Macromolecular Functions Inst. Protein Res. Osaka Univ.)

July 18 (Thu.)

9:00 - 11:30

Room B

MS4 Neurodegenerative Diseases

**Chairpersons : Katsuhiko Yanagisawa (Natl. Inst. Longevity Sci.)
 Masaya Tohyama (Osaka Univ. Grad. Sch. Med.)**

- MS4-1 (P01-F-20)** Behavioral abnormality in tail suspension test in MPTP-treated mice model of Parkinson's disease
 ○Atsushi Mori^{1,2}, Masami Nakai¹, Yasuhide Mitsumoto¹, Tetsuji Moriizumi²
 (¹Second Institute of New Drug Discovery, Otsuka Pharmaceutical Co., Ltd., ²2nd Dep. of Anatomy, Shinshu University School of Medicine)

- MS4-2 Role of oxidative protein in neuronal cell death of Parkinson's disease.**
(P01-F-16) ○Wakako Maruyama¹, Masayo Shamoto-Nagai¹, Masashi Tanaka², Makoto Naoi³
 (¹Lab. Biochem. Metabol. Dept. Basic Gerontol. NILS, ²Gifu International Biotechnol., ³Dept. Brain Sci. Instit. Appl. Biochem.)
- MS4-3 Mechanism of neuroprotection by propargylamines**
(P01-F-17) ○Makoto Naoi¹, Wakako Maruyama², Yukihiro Akao³, Hong Yi³, Yoshihide Tsujimoto⁴
 (¹Dept. Brain Sci. Instit. Appl. Biochem., ²Lab. Biochem. Metabol. Dept. Basic Gerontol. NILS, ³Gifu International Biotechnol., ⁴Lab. Mol. Genetics and CREST of JST, Osaka Univ. Grad. Sch. Med.)
- MS4-4 Activation of CREB-dependent transcription system suppresses expanded polyglutamine induced cytotoxicity.**
(P01-F-28) ○Mitsuteru Shimohata, Takayoshi Shimohata, Shuichi Igarashi, Satoshi Naruse, Shoji Tsuji
 (Dept. Neurol, Brain Res. Ins, Niigata Univ.)
- MS4-5 Functional analysis of the putative transcription factors for the human Huntington's disease gene**
(P01-F-19) ○Kazunori Tanaka^{1,2}, Junko Shouguchi-Miyata², Natsuki Miyamoto¹, Joh-E Ikeda^{1,2}
 (¹SORST, Japan Science and Technology Corporation (JST), ²Dept. Mol. Neurosci., Inst. Med. Sci., Tokai Univ. Sch. Med.)
- MS4-6 ALS2 gene accounting for amyotrophic lateral sclerosis 2 encodes multi guanine exchanging factor (GEF) domains acting for small GTPases**
(P01-F-18) ○Joh-E Ikeda, Asako Otomo, Ryota Kunita, Eri Yoshida, Shinji Hadano, Hitoshi Osuga
 (SORST/JST, Dept. Mol. Neurosci., Inst. Med. Sci., Tokai Univ. Sch. Med.)
- MS4-7 Short-term memory impairment in juvenile stroke-prone spontaneously hypertensive rats, an animal model of attention-deficit/hyperactivity disorder, is improved by cholinergic intervention.**
(P01-E-60) ○Hiroko Togashi¹, Ken-ichi Ueno¹, Machiko Matsumoto¹, Hideya Saito², Mitsuhiro Yoshioka¹
 (¹Dept. Pharmacol., Hokkaido Univ. Grad. Sch. Med., ²Dept. Basic Sci. Japan Red Cross Hokkaido College Nursing)
- MS4-8 Activation of Forkhead transcription factor following transient forebrain ischemia in gerbil hippocampus**
(P01-F-39) ○Kohji Fukunaga¹, Takayuki Kawano^{1,2}, Yukitaka Ushio², Eishichi Miyamoto¹
 (¹Dept. Pharmacol. Kumamoto Univ. Sch. Med., ²Dept. Neurosurgery Kumamoto Univ. Sch. Med.)

July 18 (Thu.)

9:00 - 10:15

Room C

MS5 Neurotransmitters

Chairpersons : Kounosuke Kumakura (Sophia Univ., Life Sci. Inst.)
Masahiko Nomura (Saitama Med. Sch.)

- MS5-1 Characterization of D-serine-regulated genes in rat cerebral cortex**
(P01-E-33) ○Naoki Yamamoto¹, Hideto Tsuchida¹, Asami Umino¹, Dai Shimazu^{1,2}, Shinichiro Sakurai^{1,2}, Go Taniguchi^{1,2}, Toru Nishikawa^{1,2}
 (¹Dept. Mental Disorder Res., Natl. Inst. Neurosci., NCNP, ²Section of Psychiatry and Behavioral Sci., Tokyo Med. Dent. Univ. Grad. Sch.)
- MS5-2 Biochemical properties and partial purification of pChAT, a novel splice variant of choline acetyltransferase.**
(P01-E-34) ○Jean-Pierre Bellier, Yoshinari Aimi, Masami Park, Tadashi Hisano, Petra Minnasch, Akinori Matsuo, Osamu Yasuhara, Ikuo Tooyama, Hiroshi Kimura
 (Mol. Neurosci. Res. Center, Shiga Univ. Med. Sci.)
- MS5-3 Behavioral and neurochemical changes by repetitive transcranial magnetic stimulation: focused on anxiolytic properties during exposure to the elevated plus-maze test**
(P01-E-42) ○Manabu Kanno¹, Machiko Matsumoto², Hiroko Togashi², Mitsuhiro Yoshioka², Yukio Mano¹
 (¹Dept. Rehabilitation Med. Grad. Sch. Med., Hokkaido Univ., ²Dept. Pharmacol. Grad. Sch. Med., Hokkaido Univ.)

- MS5-4 Stimulation of phospholipase A₂ activity by anandamide in PC12 cells**
(P01-E-41) ○Akiyoshi Someya, Syunji Horie, Toshihiko Murayama
 (Lab. Chem. Pharmacol., Grad. Sch. Pharm. Sci., Chiba Univ.)

July 18 (Thu.)

10:15 - 11:30

Room C

MS6 Signal Transduction

Chairpersons : Toshihiko Murayama (Chiba Univ., Faculty Pharmaceutical Sci.)
Shigeto Yamawaki (Hiroshima Univ. Faculty Med.)

- MS6-1 Neurospheres isolated from hippocampus of adult mice**
(P01-E-2) ○Tomoya Kitayama, Masanori Yoneyama, Yukio Yoneda
 (Dept. Mol. Pharmacol., Kanazawa Univ. Facul. Pharm. Sci.)
- MS6-2 Functional characterization of gabapentin transport and its interaction with voltage-gated Ca²⁺ channel in human primarily cultured astrocytes**
(P01-E-1) ○Takuya Fujita¹, Satoru Yamanaka¹, Kenji Shinomiya¹, Miyuki Wada¹, Miyuki Fukui¹, Akira Yamamoto¹, Michiko Oka²
 (¹Dept. Biochem. Pharmacol., Kyoto Pharmaceutical Univ., ²Nippon Shinyaku Co., Ltd.)
- MS6-3 M5 muscarinic receptors mediate the vasodilator actions of acetylcholine in cerebral arterioles and arteries.**
(P01-E-4) ○Yamada Masahisa¹, Noguchi Takanori², Lamping G Kathryn³, Duttaroy Alokesh⁴, Zhang Weilie⁴, Faraci M Frank³, Ogawa Masaharu¹, Wess Jugen⁴
 (¹Lab. Cell Culture Develop., RIKEN Brain Sci. Inst., ²Lab. Behavioral Genetics, RIKEN Brain Sci. Inst., ³Dept. Internal Med. Pharmacol., Cardiovascular Center, Univ. Iowa, ⁴Lab. Bioorganic Chem., Natl. Inst. Diabetes and Digestive and Kidney Diseases)
- MS6-4 A novel human HRD1 involved in ER-associated degradation is induced by unfolded protein response and protects from ER stress-induced cell death via its ubiquitin ligase activity**
(P02-E-26) ○Masayuki Kaneko, Masataro Ishiguro, Yoshifumi Niinuma, Yasuyuki Nomura
 (Dept. Pharmacol., Grad. Sch. Pharm. Sci., Hokkaido Univ.)

Poster

Receptors and Transporters

- P01-E-1 Functional characterization of gabapentin transport and its interaction with voltage-gated Ca^{2+} channel in human primarily cultured astrocytes**
(MS6-2) ○Takuya Fujita¹, Satoru Yamanaka¹, Kenji Shinomiya¹, Miyuki Wada¹, Miyuki Fukui¹, Akira Yamamoto¹, Michiko Oka²
(¹Dept. Biochem. Pharmacol., Kyoto Pharmaceutical Univ., ²Nippon Shinyaku Co., Ltd.)
- P01-E-2 Neurospheres isolated from hippocampus of adult mice**
(MS6-1) ○Tomoya Kitayama, Masanori Yoneyama, Yukio Yoneda
(Dept. Mol. Pharmacol., Kanazawa Univ. Facul. Pharm. Sci.)
- P01-E-3 Functional expression of GABA_B receptors in cultured calvarial osteoblasts**
○Sayumi Fujimori, Eiichi Hinoi, Yukio Yoneda
(Dept. Mol. Pharmacol., Kanazawa Univ. Pharm. Sci.)
- P01-E-4 M5 muscarinic receptors mediate the vasodilator actions of acetylcholine in cerebral arterioles and arteries.**
(MS6-3) ○Yamada Masahisa¹, Noguchi Takanori², Lamping G Kathryn³, Duttaroy Alokesh⁴, Zhang Weilie⁴, Faraci M Frank³, Ogawa Masaharu¹, Wess Jugen⁴
(¹Lab. Cell Culture Develop., RIKEN Brain Sci. Inst., ²Lab. Behavioral Genet., RIKEN Brain Sci. Inst., ³Dept. Internal Med. Pharmacol., Cardiovascular Cent., Univ. Iowa, ⁴Lab. Bioorganic Chem., Natl. Inst. Diabetes and Digestive and Kidney Diseases)
- P01-E-5 Expression pattern of oncostatin M receptor β in the murine central nervous system**
○Shinobu Tamura¹, Yoshihiro Morikawa¹, Minoru Tanaka², Atsushi Miyajima², Emiko Senba¹
(¹Dept. Anat. & Neurobiol., Wakayama Med. Sch., ²Lab. Cell. Biosyn., Inst. Mol. Cell. Biosci., Tokyo Univ.)
- P01-E-6 Involvement of mitochondria in Ca^{2+} response to ATP in PC12 cells**
Kouji Maruyama, Toshio Ohta, ○Shigeo Ito
(Lab. Pharmacol., Grad. Sch. Vet. Med., Hokkaido Univ.)
- P01-E-7 Comparison of the effects of PSD-95 and MALS-2 on the channel activity of the NR1/NR2B-subtype NMDA receptor**
○Takashi Iwamoto^{1,2}, Yasue Yamada¹, Takamasa Oda¹, Yoshifumi Watanabe², Kenji Sobue³, Makoto Inui¹
(¹Dept. Pharmacol. Sch. Med., Yamaguchi Univ., ²Dept. Neuropsychiat. Sch. Med., Yamaguchi Univ., ³Dept. Neurochem. & Neuropharmacol. Grad. Sch., Med., Osaka Univ.)

P01-E-8 Accumulation of [³H]L-serine in rat brain synaptosomal fractions

○Takeshi Takarada, Vladimir J. Balcar, Katsuhiko Baba, Akiko Takamoto, Yukio Yoneda
(Dept. Mol. Pharmacol., Kanazawa Univ. Pharm. Sci.)

P01-E-9 Functional heteromeric NMDA receptors expressed in cultured rat calvarial osteoblasts

○Yukio Yoneda, Eiichi Hinoi, Sayumi Fujimori
(Dept. Mol. pharmacol., Kanazawa Univ. Pharm. Sci.)

P01-E-10 Functional metabotropic glutamate receptor group III subtype expressed in primary cultures of rat calvarial osteoblasts

○Maki Inoue, Eiichi Hinoi, Sayumi Fujimori, Akihiro Takemori, Yukio Yoneda
(Dept. Mol. Pharmacol., Kanazawa Univ. Pharm. Sci.)

P01-E-11 Regulation of dopamine D2-receptor mediated endocytosis by endophilin-3 in the olfactory glomeruli.

○Hiroko Sugiura¹, Ken Iwata², Masato Matsuoka³, Masao Norita³, Takashi Yamauchi⁴, Masumi Ichikawa⁵, Tatsuya Haga², Kanato Yamagata¹
(¹Dept. Neuropharm., Tokyo Metropol. Inst. Neurosci., ²Inst. Biomolecul. Sci., Facul. Sci., Gakushuin Univ., ³Neurobiol. & Anat., Niigata Univ. Grad. Sch. Med. & Den., ⁴Dept. Biochem., Grad. Sch. Pharm., Tokushima Univ., ⁵Dept. Develop. Morphol., Tokyo Metropol. Inst. Neurosci.)

P01-E-12 Expression of dominant negative isoform of rat norepinephrine transporter

○Shigeo Kitayama¹, Kei Kumagai², Katsuya Morita², Toshihiro Dohi²
(¹Dept. Dent. Pharmacol., Okayama Univ. Grad. Sch. Med. Dent., ²Dept. Dent. Pharmacol., Hiroshima Univ. Grad. Sch. Biomed. Sci.)

P01-E-13 Effects of atypical antipsychotics on 5-HT_{1A} receptor-mediated [³⁵S]GTP γ S binding in native brain membranes

○Yuji Odagaki, Toshio Yamauchi
(Dept. Neuro-psychiat., Saitama Med. Sch.)

P01-E-14 Delphilin: a novel PDZ protein that synaptically colocalizes and interacts with glutamate receptor δ 2

○Susumu Kawamoto¹, Yohei Miyagi², Tetsuji Yamashita¹, Masahiro Fukaya³, Tomoko Sonoda¹, Toshiaki Okuno⁴, Kazuyuki Yamada³, Masahiko Watanabe³, Kenji Okuda¹, Masayoshi Mishina⁴
(¹Yokohama City Univ., ²Kanagawa Cancer Center, ³Hokkaido Univ., ⁴Univ. Tokyo/CREST)

P01-E-15 Molecular cloning of human p40BBP and its distribution in human brain

○Tokiko Hama, Yukiko Honda
(Mitsubishi Kagaku Inst. of Life Sci.)

P01-E-16 Expression of ASCT2 neutral amino acid transporter in cultured astrocytes and neurons

○Shigeki Furuya¹, Yoko Shinoda¹, Syogo Endo², Thomas Launey², Kazuyuki Yoshida^{1,3}, Masahiko Watanabe⁴, Jyunya Mitoma⁵, Yoshio Hirabayashi¹

(¹Neuronal Circuit Mech. Res. G., RIKEN BSI, ²Lab. for Memory and Learning, RIKEN BSI, ³United Grad. Sch. Agri. Sci., Tokyo Univ. Agri. Tech., ⁴Dept. Anatomy, Hokkaido Univ. Med. Sch., ⁵Glycobiology Program, The Burnham Inst.)

P01-E-17 Expression of dopamine transporter in striatal astrocytes of parkinsonian rats

○Masato Asanuma, Ikuko Miyazaki, Youichirou Higashi, Norio Ogawa
(Dept. Brain Sci., Grad. Sch. Med. & Dent., Okayama Univ.)

P01-E-18 Differential regulation of aquaporin 5 and 9 expression in astrocytes by protein kinase A

○Kazuya Sobue¹, Naoki Yamamoto², Masaaki Inagaki¹, Hiroaki Itho¹, Yoshihito Fujita¹, Hirotada Katsuya¹, Kiyofumi Asai²

(¹Dept. Anesthesiol. Med. Crisis Management, Nagoya City Univ. Grad. Sch. Med. Sci., ²Dept. Mol. Neurobiol., Nagoya City Univ. Grad. Sch. Med. Sci.)

P01-E-19 Dopamine-uptaking property of astrocytes via dopamine transporter

○Ikuko Miyazaki, Masato Asanuma, Youichirou Higashi, Norio Ogawa
(Dept. Brain Sci., Grad. Sch. Med. & Dent., Okayama Univ.)

P01-E-20 Critical regions for inositol 1,4,5-trisphosphate receptor gating

○Keiko Uchida^{1,2,3}, Hiroshi Miyauchi¹, Teiichi Furuichi⁴, Takayuki Michikawa^{1,5}, Katsuhiko Mikoshiba^{1,2,5}

(¹Dept. Basic Med. Sci., Div. Mol. Neurobiol., Inst. Med. Sci., Univ. Tokyo, ²Lab. Dev. Neurobiol., BSI, RIKEN, ³Dept. Pediatrics, KEIO Univ. Sch. Med., ⁴Lab. Mol. Neurogenesis, BSI, RIKEN, ⁵Calcium Oscillation Project, ICORP, JST)

P01-E-21 Conformational changes in inositol 1,4,5-trisphosphate receptor regulated by calcium

○Kozo Hamada¹, Katsuhiko Mikoshiba^{1,2,3}

(¹Lab. Dev. Neurobiol., BSI, RIKEN, ²Div. Mol. Neurobiol., Inst. Med. Sci., Tokyo Univ., ³ICORP, JST)

P01-E-22 Agonist-specific translocation of μ -opioid receptor and protein kinase C in living cells

○Akira Yoshida¹, Makoto Inoue¹, Naoaki Saito², Hiroshi Ueda¹

(¹Dept. Mol. Pharmacol. Nagasaki Uni. Sch. Pharmac. Sci., ²Lab. Mol. Pharmacol. Biosignal Res. Cent., Kobe Univ.)

P01-E-23 Tissue expression and identification of coding single nucleotide polymorphisms (cSNP) in human T2R receptors.

○Takashi Ueda¹, Shinya Ugawa¹, Yusuke Ishida², Yasuhiro Shibata¹, Shingo Murakami², Shoichi Shimada¹

(¹Dept. Mol. Morphol. Grad. Sch. Med. Sci., Nagoya City Univ., ²Dept. Otolaryngol., Nagoya City Univ.)

P01-E-24 Developmental expression pattern of TROY in mouse central nervous system

○Tomoko Hisaoka¹, Yoshihiro Morikawa¹, Toshio Kitamura², Emiko Senba¹
(¹Dept. Anat. & Neurobiol., Wakayama Med. Sch., ²Div. Cell. Thera., Advan. Clin. Res. Cent., Inst. Med. Sci., Tokyo Univ.)

P01-E-25 Identification and characterization of molecules that interact with melanin-concentrating hormone (MCH) receptor 1

○Yumiko Saito, Yuuki Kawamura, Mitsue Tetsuka, Li Yue, Kei Maruyama
(Dept. Pharmacol. Saitama Med. Sch.)

P01-E-26 Coupling with ADP-ribosyl cyclase of group III metabotropic glutamate receptors in retina

○Haruhiro Higashida¹, Xiao-Liang Chen¹, Jia-Sheng Zhang¹, Mami Noda², Naoto Hoshi¹, Minako Hashii¹, Shigeru Yokoyama¹
(¹Depart. Biophys. Kanazawa Univ Grad. Sch. Med., ²Depart. Pathophysiol. Kyushu Univ. Grad. Sch. Pharm. Sci.)

P01-E-27 Hippocampal α 2A -adrenergic receptors are involved in methamphetamine-induced ambulatory activity in mouse

○Masahiro Nishio, Yasunari Kanda, Katsushige Mizuno, Yasuhiro Wawanabe
(Dept. Pharmacol., National Defense Med. Col.)

P01-E-28 Regulation of P2X receptor by ubiquitin C-terminal hydrolase L1

○Mami Noda¹, Yoshiko Kanahori¹, Aki Shimada¹, Shunsuke Aoki^{2,4}, Yu-Lai Wang², Hitoshi Osaka^{2,3}, Keiji Wada²
(¹Lab. Pathophysiol. Grad. Sch. Pharm. Sic. Kyushu Univ., ²Dept. Degene. Neurol. Dis., Natl. Inst. Neurosci., NCNP, ³PRESTO, JST, ⁴JSPS)

P01-E-29 Protein kinase C regulates aquaporin expression in astrocytes

○Naoki Yamamoto¹, Kazuya Sobue², Yoko Kawai³, Yutaka Miura¹, Hirotada Katsuya², Kiyofumi Asai¹
(¹Dept. Mol. Neurobiol., Nagoya City Univ. Grad. Sch. Med. Sci., ²Anesthesiol. Med. Crisis Management, Nagoya City Univ. Grad. Sch. Med. Sci., ³Nagoya City Univ. Sch. Nursing)

P01-E-30 cyclic AMP-induced up-regulation of Na⁺-dependent vitamin C transporter in C6 glioma

○Satoru Yamanaka¹, Kohei Mori¹, Akira Yamanaka¹, Michiko Oka², Takuya Fujita¹
(¹Dept. Biochem. Pharmacol., Kyoto Pharmaceutical Univ., ²Nippon Shinyaku Co., Ltd.)

P01-E-31 Functional characterization of MPP⁺ uptake mediated by norepinephrine transporter in PC-12 cells

○Kenji Shinomiya, Satoru Yamanaka, Akira Yamamoto, Takuya Fujita
(Dept. Biochem. Pharmacol., Kyoto Pharmaceut. Univ.)

P01-E-32 Intracellular dynamics of IP3 receptor as revealed by tagging with GFP

○Yoko Tateishi¹, Mitsuharu Hattori¹, Tomohiro Nakayama^{1,2}, Takeshi Nakamura⁴, Hiroko Bannai^{1,3}, Miwako Iwai¹, Keiko Uchida^{1,5}, Takayuki Michikawa^{1,4}, Takafumi Inoue^{1,4}, Katsuhiko Mikoshiba^{1,3,4}

(¹Div. Molecular Neuroscience, Inst. Med. Sci., Univ. Tokyo., ²Dept. Pediatrics, Tokyo Women's Med. Univ., ³Lab. Dev. Neurosci., BSI, RIKEN, ⁴Calcium Oscillation project, ICORP, JST, ⁵Dept. Pediatrics, Keio Univ. Sch. Med.)

Neurotransmitters and Modulators (Function, Release, Uptake and Metabolism)

P01-E-33 Characterization of D-serine-regulated genes in rat cerebral cortex

(MS5-1) ○Naoki Yamamoto¹, Hideto Tsuchida¹, Asami Umino¹, Dai Shimazu^{1,2}, Shinichiro Sakurai^{1,2}, Go Taniguchi^{1,2}, Toru Nishikawa^{1,2}

(¹Dept. Mental Disorder Res., Natl. Inst. Neurosci., NCNP, ²Sec. Psychiatry Behavioral Sci., Tokyo Med. Dent. Univ. Grad. Sch.)

P01-E-34 Biochemical properties and partial purification of pChAT, a novel splice variant of (MS5-2) choline acetyltransferase.

○Jean-Pierre Bellier, Yoshinari Aimi, Masami Park, Tadashi Hisano, Petra Minnasch, Akinori Matsuo, Osamu Yasuhara, Ikuo Tooyama, Hiroshi Kimura
(Mol. Neurosci. Res. Cent., Shiga Univ. Med. Sci.)

P01-E-35 Suppressive effects of trihexyphenidyl on methamphetamine-induced dopamine release in the nucleus accumbens: Relevance to pharmacotherapy

○Kazuaki Shimosato¹, Satoru Watanabe¹, Shigeo Kitayama²

(¹Dept. Pharmacol., Kawasaki Med. Sch., ²Dept. Dent. Pharmacol., Okayama Univ. Grad. Sch. Med. Dentist.)

P01-E-36 Serotonergic signaling inhibits hyperalgesia induced by spinal cord damage

○Hideki Horiuchi, Tadanori Ogata, Tadao Morino, Haruyasu Yamamoto
(Dept. Orthop. Surg., Ehime Univ)

P01-E-37 Nicotine inhibits ERE binding via MAPK cascade in the hippocampus.

○Shozo KITO, Akiko S. SHINGO
(Hyogo Univ. Sch. Health Sci.)

P01-E-38 Functional interaction between GABAergic and DAergic system through the corticostriatal pathways

○Machiko Matsumoto¹, Hiroko Togashi¹, Manabu Kanno², Satoshi Ohashi¹, Ken-ichi Ueno¹, Mitsuhiro Yoshioka¹

(¹Dept. Pharmacol., Hokkaido Univ. Grad. Sch. Med., ²Dept. Rehabilitation Med., Hokkaido Univ. Grad. Sch. Med.)

P01-E-39 Effect of different species of aluminum on glutamate-induced overexpression of PLC- δ 1 protein level in primary cultured rat cortical neurons

○Sadaki Fujimoto¹, Kentaro Nishida¹, Shun Shimohama², Kazuki Nagasawa¹
(¹Dept. Environ. Biochem., Kyoto Pharm. Univ., ²Dept. Neurol. Grad. Sch. Med., Kyoto Univ.)

P01-E-40 5HT-1 and 5HT-3 serotonin receptor subtypes regulate pain sensation in the spinal cord level: Normal and hyperalgesic animal model

○Tadanori Ogata, Hideki Horiuchi, Tadao Morino, Haruyasu Yamamoto
(Dept. Orthopaedic Surg., Ehime Univ. Sch. Med.)

P01-E-41 Stimulation of phospholipase A₂ activity by anandamide in PC12 cells

(MS5-4) ○Akiyoshi Someya, Syunji Horie, Toshihiko Murayama
(Lab. Chem. Pharmacol., Grad. Sch. Pharm. Sci., Chiba Univ.)

P01-E-42 Behavioral and neurochemical changes by repetitive transcranial magnetic stimulation: focused on anxiolytic properties during exposure to the elevated plus-maze test

○Manabu Kanno¹, Machiko Matsumoto², Hiroko Togashi², Mitsuhiro Yoshioka², Yukio Mano¹
(¹Dept. Rehabilitation Med., Grad. Sch. Med., Hokkaido Univ., ²Dept. Pharmacol. Grad. Sch. Med., Hokkaido Univ.)

P01-E-43 The mutation of two amino acid residues of tyrosine hydroxylase dramatically enhances the ability of neuroendocrine AtT-20 cells to synthesize L-DOPA

○Akira Nakashima¹, Yoko S Kaneko¹, Keiji Mori¹, Kentaro Fujiwara², Toshimitsu Tsugu¹, Takahiro Suzuki³, Toshiharu Nagatsu³, Akira Ota¹
(¹Dept. Physiology, Sch. Med., Fujita Health Univ., ²Dept. Internal Med., Sch. Med., Fujita Health Univ., ³Div. Mol. Genet., Inst. Compr. Med. Sci., Fujita Health Univ.)

P01-E-44 The expression of GTP cyclohydrolase I mRNA in murine locus coeruleus is enhanced by peripheral administration of lipopolysaccharide

○Yoko S. Kaneko¹, Keiji Mori¹, Akira Nakashima¹, Ikuko Nagatsu², Akira Ota¹
(¹Dept. Physiol., Sch. Med., Fujita Health Univ., ²Dept. Anat., Sch. Med., Fujita Health Univ.)

P01-E-45 The effect of ophiobolin A, a novel calmodulin antagonist, on neurotransmitter release from rat cerebellar granule cells and PC12 cells

○Kosuke Ando¹, Yoshihisa Kudo¹, Masami Takahashi^{2,3}
(¹Tokyo Univ. Pharmacy and Life Sci., ²Dept. Biochem., Kitasato Univ. Sch. Med., ³CREST/JST)

P01-E-46 Effect of peripheral lipopolysaccharide administration on dopamine content in murine anterior olfactory nucleus

○Keiji Mori¹, Yoko S kaneko¹, Akira Nakashima¹, Kentaro Fujiwara², Ikuko Nagatsu³, Akira Ota¹

(¹Dept. Physiol., Fujita Health Univ. Sch. Med., ²Dept. Internal Med., Fujita Health Univ. Hospital, ³Fujita Health Univ. Sch. Med.)

P01-E-47 Changes in substance P and its processing enzyme activity in rat dorsal root ganglia following sciatic nerve ligation

○Ryoko Nakajima¹, Ken Tsuchida¹, Toshiyuki Chikuma², Takeshi Kato¹

(¹Grad. Sch. Int. Sci., Yokohama City Univ., ²Dept. Hygien. Chem., Showa Pharm. Univ.)

P01-E-48 Prenatal and lactational exposure of Bisphenol A to mice effects on monoaminergic neurons

○Toyoko Hiroi, Kazushi Okada, Takashi Yoneda, Yoshihiko Funae

(Dept. Chem. Biol., Osaka City Univ. Grad. Med. Sch.)

Animal Models

P01-E-49 Functional abnormalities of the brain serotonergic system in PACAP-deficient mice

○Norihito Shintani¹, Kazuhiro Tanaka¹, Chihiro Kawaguchi¹, Hitoshi Hashimoto¹, Toshio Matsuda², Akemichi Baba^{1,3}

(¹Dept. Mol. Neuropharmacol., Grad. Sch. Pharm. Sci., Osaka Univ., ²Dept. Med. Pharmacol. Grad. Sch. Pharm. Sci., Osaka Univ., ³Dept. Mol. Pharmacol. Grad. Sch. Med., Osaka Univ.)

P01-E-50 Selective deficit of serotonin_{1A} receptor-mediated regulation of cortical dopamine release in isolated mice

○Toshio Matsuda¹, Yukio Ago¹, Masaki Sakaue², Yutaka Koyama¹, Akemichi Baba²

(¹Lab. Med. Pharmacol., Grad. Sch. Pharm. Sci., Osaka Univ., ²Lab. Mol. Neuropharmacol., Grad. Sch. Pharm. Sci., Osaka Univ.)

Neuroendocrine System

P01-E-51 Estrogen enhances depolarization-induced glutamate release through activation of phosphatidylinositol 3-kinase in cultured hippocampal neurons

○Daisaku Yokomaku^{1,2}, Tadahiro Numakawa^{1,2,3}, Yumiko Numakawa¹, Naoki Adachi¹, Tomoya Matsumoto^{1,2}, Satoru Yamagishi^{1,3}, Chika Nishio¹, Takahisa Taguchi², Hiroshi Hatanaka¹

(¹Dept. Protein Biosynthesis, Inst. Protein Research, Osaka Univ., ²Neuronics RG, Special Div. Human Life Tech., AIST, ³Research Fellowships of the Japan Society for the Promotion of Science)

P01-E-52 Brainstem is a possible site of leptin's action in the brain

○Toru Kawagishi, Yasunobu Okuma, Toru Hosoi, Jun Tanaka, Yasuyuki Nomura
(Dept. Pharmacol., Grad. Sch. Pharm. Sci., Hokkaido Univ.)

Ion Channels and Excitable Membranes

P01-E-53 Augmentation of T-type Ca^{2+} -current by Neuropeptide Y in NG108-15 cells

○Masayoshi Okada, Ayumi Hasegawa, Hiroshi Higuchi
(Div. Pharmacol., Grad. Sch. Med. Sci., Niigata Univ.)

P01-E-54 Acid-sensing ion channels ASIC1a and ASIC3 are leading acid sensors expressed in polymodal nociceptors

○Shinya Ugawa, Takashi Ueda, Shoichi Shimada
(Dept. Anat. II, Nagoya City Univ. Med. Sch.)

Blood-Brain Barrier

P01-E-55 Transport mechanism of aluminum citrate across the blood-brain barrier

○Kazuki Nagasawa¹, Shingo Ito¹, Ikumi Tamai², Akira Tsuji³, Sadaki Fujimoto¹
(¹Dept. Environ. Biochem., Kyoto Pharm. Univ., ²Fac. Pharm. Sci., Tokyo Univ. Sci.,
³Fac. Pharm. Sci., Kanazawa Univ.)

Autonomic Nervous System

P01-E-56 Autocrine/paracrine regulation of voltage-dependent calcium channels in adrenal chromaffin cells

○Toshio Ohta, Takahiko Kai, Shigeo Ito
(Lab. Pharmacol., Grad. Sch. Vet. Med., Hokkaido Univ.)

Neuroimmunology

P01-E-57 Effect of central or peripheral IL-1 β on hydroxyl radical and nitric oxide productions in the medial prefrontal cortex in conscious rats

○Yuta Ishizuka¹, Yasushi Ishida¹, Hiroshi Kannan²
(¹Dept. Psychiatry, Miyazaki Med. College, ²Dept. Physiology, Miyazaki Med. College)

Neurotoxicity

P01-E-58 Disturbance of thyroid hormone-dependent synapse formation in cultured rat cortical neurons by environmental chemicals

○Ritsuko Hosoda^{1,3}, Midori Kato-Negishi¹, Kenji Nakayama², Kazuyo Muramoto¹, Masahiro Kawahara^{1,3}, Yoichiro Kuroda^{1,3}

(¹Dept. Mol. Cell. Neurobiol., TMIN, ²Dept. Clin. Pathol., Hokkaido Inst. Public Health,

³CREST, JST)

Psychiatric Diseases

P01-E-59 Neuronal toxicity of apoptotic injury induced by the long term treatment of typical and atypical antipsychotics

○Wataru Ukai, Hiroki Ozawa, Hiroshi Choei, Shingo Yamada, Rie Sakai, Masato Konno, Masaru Tatenno, Megumi Yamamoto, Toshikazu Saito

(Dept. Neuropsychiatry, Sapporo Med. Univ.)

P01-E-60 Short-term memory impairment in juvenile stroke-prone spontaneously hypertensive (MS4-7) rats, an animal model of attention-deficit/hyperactivity disorder, is improved by cholinergic intervention.

○Hiroko Togashi¹, Ken-ichi Ueno¹, Machiko Matsumoto¹, Hideya Saito², Mitsuhiro Yoshioka¹

(¹Dept. Pharmacol., Hokkaido Univ. Grad. Sch. Med., ²Dept. Basic Sci., Japan Red Cross Hokkaido College Nursing)

P01-E-61 Effect of valproate on agonist-induced intracellular calcium mobilization in human platelets

○Tatsuyuki Akimoto, Katsuzi Suzuki, Yuki Sasaki, Ichiro Kusumi, Tukasa Koyama

(Dept. Psychiatry., Grad. Sch. Med., Hokkaido Univ)

Epilepsy

P01-E-62 Dissociative role of GABA_B and GHB receptors in γ -butyrolactone induced absence epilepsy of developing rat brain

○Masayuki Tanaka¹, Noboru Takizawa¹, Kazuhiro Mawatari², Satoru Kato¹

(¹Dept. Mol. Neurobiol., Grad. Sch. Med, Kanazawa Univ., ²Dept. Lab. Sci., Fac. Med., Kanazawa Univ.)

Cerebral Blood Flow and Metabolism

- P01-E-63 Measurement of metabolic rates for glutamate and glutamine in occipito-parietal lobes of human brain by non-invasive 2T ^{13}C -magnetic resonance spectroscopy (^{13}C -MRS)**

○Tomoyuki Kanamatsu¹, Taisuke Otsuki², Kazuya Okamoto³, Hidehiro Watanabe³, Masaaki Umeda³, Yasuzo Tsukada¹

(¹Inst. Life Sci., Soka Univ., ²Dept. Neurosurgery, Musashi Hospital, Natl. Center of Neurology & Psychiatry, ³Toshiba Med. Systems Res. & Develop. Center)

Sleep and Biological Rhythms

- P01-E-64 Sleep-promoting system triggered by prostaglandin D₂ is involved in the homeostatic control of NREM sleep after sleep deprivation.**

○Yoshihiro Urade^{1,2}, Naomi Eguchi^{1,2}, Yuko Kuwahata^{1,2}, Wei-Min Qu¹, Zhi-Li Huang¹, Osamu Hayaishi¹

(¹Dept. Mol. Behav. Biol., Osaka Biosci. Inst., ²CREST, JST)

- P01-E-65 Prostaglandin E₂ activates histaminergic system via EP₄ receptor to induce wakefulness in rats**

○Zhi-Li Huang¹, Takatoshi Mochizuki¹, Tetsuya Okada^{1,2}, Wei-Min Qu¹, Atsushi Yamatodani³, Yoshihiro Urade^{1,4}, Osamu Hayaishi¹

(¹Dept. Molec. Behav. Biol., Osaka Biosci. Inst., ²Dept. Med. Sci. III, Sch. Health Sport, Osaka Univ., ³Dept. Med. Physics, Sch. Allied Health Sci., Osaka Univ., ⁴CREST, Jpn. Sci. Tech. Corp.)

- P01-E-66 Periodic nuclear/cytoplasmic shuttling of BMAL1 in the central and peripheral clocks**

○Teruya Tamaru¹, Yasushi Isojima², Ken Takamatsu¹

(¹Dept. Physiol., Toho Univ. Sch. of Med., ²Div. Protein Metabolism, Inst. Protein Res.)

Dementia and Alzheimer's Disease

- P01-F-1** Effect of MK-801, an NMDA receptor antagonist, on BACE and EP24.15 in rat brain
 ○Md. Mahiuddin Ahmed¹, Masaru Yamamoto¹, Toshiyuki Chikuma², Takeshi Kato¹
 (¹Grad. Sch. Int. Sci., Yokohama City Univ., ²Dept. Hygien. Chem., Showa Pharmaceut. Univ.)
- P01-F-2** Niemann-Pick C1 deficiency reduces the levels of cholesterol in detergent insoluble, (MS2-4) low-density membrane domains leading to activation of MAP kinase pathway and increased phosphorylation of tau
 ○Naoya Sawamura^{1,2}, Jian-Sheng Gong¹, Ta-Yuan Chang³, Katsuhiko Yanagisawa¹, Makoto Michikawa¹
 (¹Dept. Dementia Res., NILS, ²JSPS, ³Dept. Biochemistry, Dartmouth Med. Sch.)
- P01-F-3** p25 subunit, an activator of CDK5, induces intensive phosphorylation on Ser202/Thr205 residues of human tau40 *in vitro*.
 ○Mitsuko Hashiguchi¹, Taro Saito², Shin-ichi Hisanaga², Toshio Hashiguchi¹
 (¹Dept. Physiol. Sch. Med., Tokyo Med. Univ., ²Dept. Biol. Sci. Grad. Sch. Sci., Tokyo Met. Univ.)
- P01-F-4** Induction of differential responses in neurons and astrocytes by A β protein and non- (MS2-3) A β component (NAC) of Alzheimer's disease amyloid through generation of reactive oxygen species and activation of NF- κ B
 ○Seigo Tanaka, Masanori Takehashi, Kunihiro Ueda
 (Lab. Mol. Clin. Chem., Instit. Chem. Res., Kyoto Univ.)
- P01-F-5** CD36, a member of class B scavenger receptor family, serve as β -amyloid receptor and its expression on primary rat microglia stimulated with interleukine-4
 ○Kohichi Kawahara, Makoto Kajizono, Hitoshi Nakayama
 (Fac. Pharma. Sci., Kumamoto Univ.)
- P01-F-6** A novel function of monomeric amyloid β -protein serving as an antioxidant molecule against metal-induced oxidative damage
 ○Kun Zou, Jian-Sheng Gong, Katsuhiko Yanagisawa, Makoto Michikawa
 (Dept. Dementia Res., Natl. Inst. Longevity Sci.)
- P01-F-7** Isoform-dependent lipid release from astrocytes prepared from human apolipoprotein E3- and E4-knock-in mice
 ○Jian-Sheng Gong¹, Mariko Kobayashi², Hideki Hayashi¹, Kun Zou¹, Naoya Sawamura¹, Shinobu C Fujita², Katsuhiko Yanagisawa¹, Makoto Michikawa¹
 (¹Dept. Dementia Res., Natl. Inst. Longevity Sci., ²Mitsubishi Kagaku Inst. Life Sci.)

P01-F-8 Effect of the high expression level of Rab1A on A β generation

○ Hirohisa Shiraishi¹, Yuuki Kawamura², Ryo Suzuki¹, Xiaorei Sai¹, Katsuhiko Yanagisawa¹, Hiroto Komano¹

(¹Dept. Dementia Res., NILS, ²Dep. Pharmacol., Saitama Med. Sch.)

P01-F-9 Significance of glyceraldehyde-derived advanced glycation end-product (AGE-2) in Alzheimer's disease

○ Masayoshi Takeuchi¹, Nobuyuki Sasaki², Takayuki Watai¹, Seiji Kikuchi³, Sho-ichi Yamagishi⁴, Zenji Makita⁴, Yukihiro Kameda¹

(¹Dept. Biochemistry, Hokuriku Univ., ²Dept. Neuropsychiatry, Sapporo Med. Univ., ³Dept. Neurology, Hokkaido Univ. Sch. Med., ⁴Dept. Medicine, Kurume Univ. Sch. Med.)

P01-F-10 Transfected mutant presenilin-1 affects intracellular trafficking in PC12D cell

○ Fuyuki Kametani¹, Hideaki Kume¹, Hiroshi Mori²

(¹Dept. Mol. Neurobiol., Tokyo Inst. Psychiatry, ²Dept. Neurosci., Osaka City Univ. Med. Sch.)

P01-F-11 Analysis of the mechanism underlying Herp-mediated enhancement of the level of A β generation

○ Xiaorei Sai¹, Koichi Kokame², Hirohisa Shiraishi¹, Ryo Suzuki¹, Toshiyuki Miyata², Katsuhiko Yanagisawa¹, Hiroto Komano¹

(¹Dept. Dementia Res., Natl. Inst. Forlongevity Sci., ²Natl. Cardiovasc. Ctr. Res. Inst.)

P01-F-12 Immunohistochemical study of ubiquitin and deposition of β -amyloid protein in old dog brain

○ Tatsuki Itoh, Takao Satou, Yoshiyuki Hayashi, Shigeo Hashimoto

(Sec. Dep. Path., Kinki Univ. Sch. Med.)

P01-F-13 The preventive effect of propentofylline on the β -amyloid-induced apoptosis in rat hippocampal neurons

○ Yoshiki Koriyama¹, Matsumi Yamazaki¹, Kenzo Chiba¹, Satoru Kato², Tetsuro Mohri¹

(¹Dept. Biodynamics, Fac. Pharm. Sci., Hokuriku Univ., ²Dept. Mol. Neurobiol, Grad. Sch. Med., Kanazawa Univ.)

P01-F-14 Therapeutic effects of Zokumei-to on spatial learning impairment and synaptic loss in a mouse model of Alzheimer's disease

○ Takayuki Tamura, Chihiro Tohda, Katsuko Komatsu

(Res. Center for Ethnomed., Inst. Natural Med., Toyama Med. & Pharm. Univ.)

P01-F-15 Exogenous HSP90-induced microglial activation and amyloid- β clearance

○ Jun-ichi Kakimura¹, Yoshihisa Kitamura¹, Kazuyuki Takata¹, Shun Shimohama², Takashi Taniguchi¹

(¹Dept. Neurobiol., Kyoto Pharm. Univ., ²Dept. Neurol. Grad. Sch. Med., Kyoto Univ.)

Neurodegenerative Diseases (Parkinson's Disease and Others)

P01-F-16 Role of oxidative protein in neuronal cell death of Parkinson's disease.

- (MS4-2)** ○Wakako Maruyama¹, Masayo Shamoto-Nagai¹, Masashi Tanaka², Makoto Naoi³
(¹Lab. Biochem. Metabol., Dept. Basic Gerontol. NILS, ²Gifu International Biotechnol.,
³Dept. Brain Sci. Instit. Appl. Biochem.)

P01-F-17 Mechanism of neuroprotection by propargylamines

- (MS4-3)** ○Makoto Naoi¹, Wakako Maruyama², Yukihiro Akao³, Hong Yi³, Yoshihide Tsujimoto⁴
(¹Dept. Brain Sci. Instit. Appl. Biochem., ²Lab. Biochem. Metabol. Dept. Basic Gerontol.
NILS, ³Gifu International Biotechnol., ⁴Lab. Molecular Genetics and CREST of JST,
Osaka Univ. Grad. Sch. Med.)

**P01-F-18 ALS2 gene accounting for amyotrophic lateral sclerosis 2 encodes multi guanine
(MS4-6) exchanging factor (GEF) domains acting for small GTPases**

- Joh-E Ikeda, Asako Otomo, Ryota Kunita, Eri Yoshida, Shinji Hadano, Hitoshi Osuga
(SORST/JST, Dept. Mol. Neurosci., Inst. Med. Sci., Tokai Univ. Sch. Med.)

**P01-F-19 Functional analysis of the putative transcription factors for the human Huntington's
(MS4-5) disease gene**

- Kazunori Tanaka^{1,2}, Junko Shouguchi-Miyata², Natsuki Miyamoto¹, Joh-E Ikeda^{1,2}
(¹SORST, Japan Sci. Tech. Corporation (JST), ²Dept. Mol. Neurosci., Inst. Med. Sci.,
Tokai Univ. Sch. Med.)

**P01-F-20 Behavioral abnormality in tail suspension test in MPTP-treated mice model of
(MS4-1) Parkinson's disease**

- Atsushi Mori^{1,2}, Masami Nakai¹, Yasuhide Mitsumoto¹, Tetsuji Moriizumi²
(¹Second Inst. New Drug Discovery, Otsuka Pharmaceutical Co., Ltd., ²2nd Dep. of
Anat., Shinshu Univ. Sch. Med.)

**P01-F-21 Effects of isatin, an endogenous MAO inhibitor, on striatal dopamine level in rats of
Japanese encephalitis virus-induced Parkinson model**

- Masaru Minami¹, Naoya Hamaue¹, Mutsuko Terado¹, Toru Endo¹, Masahiko Hirafuji¹,
Akihiko Ogata², Kunio Tashiro²
(¹Dept. Pharmacol. Health Sci. Univ. Hokkaido, ²Dept. Neurol., Hokkaido Univ. Sch.
Med.)

P01-F-22 A role of MxA protein, anti-viral GTPase, in Parkinson's disease

- Daisuke Ishibashi, Kenichi Mishima, Naoya Aoo, Hisako Furukawa, Mitsuo
Takahashi, Michihiro Fujiwara
(Dept. Clin. Pharmacol., Facult. Pharm. Sci., Fukuoka Univ)

P01-F-23 Identification of genes that function in the ER stress using a randomized ribozyme library

○Reiko Onuki^{1,2}, Yoshio Bando^{3,4,5}, Eigo Suyama^{1,6}, Hiroaki Kawasaki^{1,6}, Taiichi Katayama^{3,5}, Masaya Tohyama^{3,5}, Kazunari Taira^{1,6}

(¹AIST, ²Grad. Sch. Life and Env. Sci., Tsukuba Univ., ³Dept. Anat. and Neurosci., Grad. Sch. Med., Osaka Univ., ⁴Dept. Anat., Asahikawa Med. college, ⁵CREST, JST, ⁶Dept. Chem. Biotech., Tokyo Univ.)

P01-F-24 Immunocytochemical analyses for subcellular localization of EAOH gene *aprataxin*

Hidetoshi Date, Shuichi Igarashi, Yasuteru Sano, Tetsuya Takahashi, Osamu Onodera,

○Shoji Tsuji

(Dept.Neurol., BRI Niigata University)

P01-F-25 The analysis of aprataxin, the product of causative gene of early-onset ataxia with ocular motor apraxia and hypoalbuminemia

○Yasuteru Sano¹, Shuichi Igarashi¹, Osamu Onodera¹, Hidetoshi Date¹, Mutsuo Oyake¹, Nobuyoshi Hukuhara³, Takao Makifuchi³, Mitsunori Morimatsu⁴, Hitoshi Takahashi², Shoji Tsuji¹

(¹Dept. Neuro., Brain, Niigata Univ., ²Dept. Patho., Brain, Niigata Univ., ³Dept. Neuro., Saigata Hp., ⁴Dept. Neuro, Yamaguchi Univ.)

P01-F-26 The role of PKR in neurodegenerative diseases induced by ER-stresses

○Yoshio Bando^{1,3}, Reiko Ohnuki^{4,6}, Taiichi Katayama^{2,3}, Shigetaka Yoshida¹, Kazanari Taira^{4,5}, Masaya Tohyama^{2,3}

(¹Dept. Anat., Asahikawa Med. college, ²Dept Anat. and Neurosci., Osaka Univ. Grad. Sch. Med., ³Core Res. Evolutional Sci. Tech., ⁴Gene Discovery Res. Center, AIST, ⁵Dept. Chem. Biotech., Tokyo Univ. Grad. Sch. of Engineering, ⁶Inst. Applied Biochem., Univ. Tsukuba)

P01-F-27 Molecular mechanism in neuroprotective properties of a dopamine agonist, cabergoline

Michiyo Yoshioka, Ken-ichi Tanaka, ○Naoko Fujita, Ikuko Miyazaki, Masato Asanuma, Norio Ogawa

(Dept. Brain Science, Okayama Univ. Grad. Sch. Med. & Dent.)

P01-F-28 Activation of CREB-dependent transcription system suppresses expanded (MS4-4) polyglutamine induced cytotoxicity

○Mitsuteru Shimohata, Takayoshi Shimohata, Shuichi Igarashi, Satoshi Naruse, Shoji Tsuji

(Dept.Neurol, Brain Res. Ins, Niigata Univ,)

Apoptosis

P01-F-29 ATF3 protects neurons from JNK induced apoptosis by upregulating Hsp27

○Saya Nakagomi¹, Yasuhiro Suzuki^{1,2}, Hiroshi Kiyama¹

(¹Dept. Anat. Grad. Sch. Med. Sci., Osaka City Univ., ²Dept Internal Med1. Med., Asahikawa Medical Coll.)

P01-F-30 Transcription factors induced in cultured cortical neurons by beta amyloid peptide

(MS2-1) ○Yoko Uchida, Fujiya Gomi

(Dept. Neuropathol. TMIG)

P01-F-31 Temporary damage by trimethyltin in dentate granule neurons of mouse

○Hiroaki Okuda¹, Norito Nishiyama¹, Yasuhiro Yamamoto¹, Yukio Yoneda², Kiyokazu Ogita¹

(¹Dept. Pharmacol. Setsunan Univ. Fac. Pharm. Sci., ²Dept. Mol. Pharmacol. Kanazawa Univ. Fac. Pharm. Sci.)

P01-F-32 Activity-dependent expression of *alivin 1* and its involvement in the survival of cerebellar granule neurons

○Tomio Ono^{1,2}, Naoko Suzuki¹, Yoshiaki Kikkawa², Hiromichi Yonekawa², Seiichi Kawashima¹

(¹Dept. Mol. Biol., Tokyo Metro. Inst. Med. Sci., ²Dept. Lab. Animal Sci., Tokyo Metro. Inst. Med. Sci.)

P01-F-33 Essential role of Rho/Rho-kinase signaling pathway in target-independent survival of developing motor neurons

○Kenta Kobayashi¹, Masanori Takahashi², Kazuaki Takahashi¹, Hiroyuki Yaginuma³, Noriko Osumi², Kozo Kaibuchi⁴, Kazuto Kobayashi¹

(¹Dept. Mol Genet. Inst. Biomed. Sci., Fukushima Medical Univ., ²Dept. Dev Neuro. Grad. Sch. Med., Tohoku Univ., ³Dept. Anat., Fukushima Medical Univ, ⁴Dept. Cell Pharmacol. Grad. Sch. Med., Nagoya Univ)

P01-F-34 Dlx1-1, a necdin/MAGE homologous protein, enhances E2F1-induced neuronal apoptosis

○Takaaki Kuwajima, Hideo Taniura, Kazuaki Yoshikawa

(Div. Regu.Macro.Mol.Func.,Inst.Protein Res.,Osaka Univ.)

P01-F-35 Nitric oxide(NO)-induced apoptosis is mediated by tyrosine kinase activation in PC12 cells

○Toshifumi Yamamoto¹, Kohei Yuyama¹, Hideko Yamamoto²

(¹Lab. Mol. Recognition. Grad. Sch. Integrated Sci., Yokohama City Univ., ²Dept. Mol. Psychiatry, Tokyo Inst. Psychiatry)

P01-F-36 The involvement of conversion of the p35 Cdk5 activator to p25 in neuronal cell death

○Taro Saito, Yuichi Fujita, Akiko Asada, Shin-ichi Hisanaga

(Dept. Biol. Sci., Grad. Sch. Sci., Tokyo Metropolitan Univ.)

P01-F-37 Mechanism of the inhibition of oligodendrocyte apoptosis by NF- κ B p50 subunit

○Makoto Hamanoue, Ken Takamatsu
(Dept. Physiol. Sch. Med., Toho Univ.)

P01-F-38 Effect of caspase-3 on the primary cultured mice cortical neurons with HSV-1 infection

○Jiawei Wang, Dexin Wang, Zijing Feng
(Dept. Neurology, Beijing Friendship Hospital)

Ischemia

P01-F-39 Activation of Forkhead transcription factor following transient forebrain ischemia in (MS4-8) gerbil hippocampus

○Kohji Fukunaga¹, Takayuki Kawano^{1,2}, Yukitaka Ushio², Eishichi Miyamoto¹
(¹Dept. Pharmacol., Kumamoto Univ. Sch. Med., ²Dept. Neurosurgery, Kumamoto Univ. Sch. Med)

P01-F-40 Leukotriene D₄ activates microglia through the CysLT₁ receptor

○Tetsuya Hirabayashi^{1,2}, Takao Shimizu¹
(¹Dept. Biochem. Mol. Biol., Grad. Sch. Med., Univ. Tokyo, ²Lab. Chem. Pharmacol., Grad. Sch. Pharm. Sci., Chiba Univ)

P01-F-41 Inhibitory effect of 17 β -estradiol administration on some factors implicating in progression of neuronal apoptosis in gerbil hippocampus induce by transient ischemia/recirculation insult

○Kimikazu Fujita¹, Toshiaki Kato¹, Masamitsu Yamauchi², Nobuko Yoshimoto³, Kenzo Shibayama¹, Yutaka Nagata¹, Eiichi Miyachi¹
(¹Dept. Physiol. Sch. Med., Fujita Health, Univ., ²Aichi Gakusen Coll., ³Nagoya Bunri Coll.)

Aging

P01-F-42 Subcellular localization of Sir2 NAD-dependent histone deacetylase homologues from brain

○Yoshiyuki Horio, Jun Sakamoto
(Dept. Pharmacol., Sapporo Med. Sch.)

P01-F-43 A novel gene containing ankyrin-repeat expressed in senescence accelerated mouse (SAMP8) hippocampus and cerebrum

○Naoshi Yamamura^{1,2}, Nobuya Koike¹, Yasuyuki Nomura³, Yasunobu Okuma³, Takashi Uehara³, Yoshiyuki Sakaki¹, Tomoko Tashiro², Chie Furihata^{1,2}
(¹Human Genome Cent., Insit. Med. Sci., Univ. Tokyo, ²Sch. Sci. Eng., Aoyama Gakuin Univ., ³Dept. Pharmacol., Grad. Sch. Pharm. Sci., Hokkaido Univ.)

- P01-F-44 Effect of Japanese herbal medicine (Toki-Shakuyaku-San) on DNA damage in aged rodent brain and C6 glia cell death**

○Midori Hiramatsu

(Tohoku Univ. Community Service and Science)

Drug Abuse and Psychopharmacology (Intoxication, Addiction)

- P01-F-45 Reduction of histamine-induced phosphoinositide hydrolysis in mouse frontal cortical slices after repeated methamphetamine treatment**

○Junichi Kitanaka, Nobue Kitanaka, Motohiko Takemura

(Dept. Pharmacol., Hyogo Col. Med.)

- P01-F-46 BDNF inhibits apoptosis induced by methamphetamine in primary cortical neurons through activation of Akt kinase**

○Hideo Matsuzaki¹, Kazuhiko Namikawa², Hiroshi Kiyama², Norio Mori³, Kohji Sato¹

(¹Dept. Anat. and Neurosci., Hamamatsu Univ. Sch. Med., ²Dept. Anat., Grad. Sch. Med., Osaka City Univ., ³Dept. Psychiatr. and Neurol., Hamamatsu Univ. Sch. Med.)

- P01-F-47 Paraquat induces long-lasting dopaminergic neurotoxicity through the excitotoxic pathway**

○Kazuo Matsubara¹, Keiko Shimizu², Ko-ichi Ohtaki¹, Osamu Saito², Hiroshi Shiono²

(¹Dept. Hospital Pharmacy & Pharmacol., ²Dept. Legal Med. Asahikawa Med. College)

Neuron and Glia

- P02-E-1 Compensatory neuroprotective events in kainic acid-induced neuronal cell death**
 ○Kazuyuki Takata, Yoshihisa Kitamura, Mitsuhiro Okazaki, Jun-ichi Kakimura, Takashi Taniguchi
 (Dept. Neurobiol., Kyoto Pharm. Univ.)
- P02-E-2 Association of protein kinase C alpha and of p38 mitogen-activated protein kinase with the induction of tumor necrosis factor alpha in lipopolysaccharide-stimulated microglia**
 ○Kazuyuki Nakajima^{1,2}, Yoko Tohyama¹, Shinichi Kohsaka², Tadashi Kurihara¹
 (¹Neurochem Div. Inst. Life Sci., Soka Univ., ²Dept. Neurochem. Natl Inst. Neurosci.)
- P02-E-3 Reciprocal localizations and functional aspects of CD9 and CD81 in the nervous system**
 ○Tomoko Ishibashi¹, Kazuhiro Ikenaka², Eisuke Mekada³, Hiroko Baba¹
 (¹Dept. Mol. Neurology, Tokyo Univ. Pharm. Life Sci., ²National Inst., Physiological Sci., ³Res. Inst. Microbial Disease, Osaka Univ.)
- P02-E-4 ATP selectively suppresses the synthesis of the inflammatory protein, MRF-1, through Ca²⁺-influx via P2X₇ receptors in cultured microglia**
 ○Shuuitsu Tanaka, Naoki Kaya, Tatsuro Koike
 (Div. Biol. Sci., Grad. Sch. Sci., Hokkaido Univ.)
- P02-E-5 Down-regulation of cholesterol metabolism by serotonin (5HT) in rat astrocytes**
 ○Sachiko Ueno^{1,2}, Jinichi Ito², Yuko Nagayasu², Schinji Yokoyama²
 (¹Dept. Psychiatry and Cognitive-Behavioral Sci., Nagoya City Univ. Grad. Sch. Med. Sci., ²Dept. Biochem., Cell Biol. and Metabolism, Nagoya City Univ. Grad. Sch. Med. Sci.)
- P02-E-6 Formation of floating cell aggregates from bipotential glial cells in the presence of primary microglia**
 ○Ryuuj Hisamura^{1,2}, Kenji F Tanaka^{1,3}, Makiko Kawakami¹, Haruo Kashima³, Makoto Sawada^{1,4}
 (¹JRDTAID, ICMS, Fujita Health Univ., ²Grad. Sch. Agri., Nagoya Univ., ³Dept. Neuropsychia. Grad. Sch. Med., Keio Univ., ⁴PRESTO, JST)
- P02-E-7 Origin of oligodendrocyte in mouse forebrain**
 ○Eiko Nakahira¹, Tetsushi Kagawa¹, Martyn M Guldin², Kazuhiro Ikenaka¹
 (¹Natl. Inst. Physiol. Sci., ²Salk Inst.)
- P02-E-8 Expression of P2Y₁₂ receptors in the brain**
 ○Yo Sasaki², Shizuyo Honda¹, Chihiro Akazawa¹, Kuninobu Johkohji¹, Yasuko Nakamura¹, Kazuhide Inoue³, Shinichi Kohsaka¹
 (¹Dept. Neurochem., Natl. Inst. Neurosci., ²Japan Sci. Tech. Corporation, ³Div. Pharmacol., Natl. Inst. Health Sci.)

P02-E-9 The basic helix-loop-helix factor Olig2 is essential for development of motoneuron and oligodendrocyte lineages

○Hirohide Takebayashi¹, Yoko Nabeshima², Shosei Yoshida², Osamu Chisaka³, Yo-ichi Nabeshima², Kazuhiro Ikenaka¹

(¹Natl. Inst. Physiol. Sci., ²Dept. Pathol., Grad. Sch. Med., Kyoto Univ., ³Grad. Sch. Biostudies., Kyoto Univ.)

P02-E-10 Influence of astrocyte-inducers on glial cell development in primary cultures of embryonic rat cerebral hemisphere

○Tatsumi Adachi^{1,2}, Hiromi Takanaga^{2,3}, Manabu Kunitomo^{2,3}, Hiroaki Asou⁴

(¹Dept. Basic Med. Sci., Natl. Inst. Minamata Dis., ²Natl. Inst. Environmental Studies, ³School Pharm. Sci., Kitasato Univ., ⁴Dept. Neurobiology, Tokyo Metropolitan Inst. Gerontology)

P02-E-11 Analysis of astrocyte lineage developmental

○Yasuhiro Ogawa¹, Matthew Rasband², Hirohide Takebayashi¹, Yasuno Iwasaki¹, James Trimmer², Kazuhiro Ikenaka¹

(¹Natl. Inst. Physiological Sci., ²SUNY Stony Brook)

P02-E-12 Control of cerebellar granule neuron precursor proliferation and differentiation

○Takayuki Okano-Uchida, Yoshiaki Komiya, Yasuki Ishizaki

(Dept. Mol. Cell. Neurobiol., Gunma Univ. Sch. Med.)

P02-E-13 Microglial apoptosis induced by a low concentration of polyamine

Katsura Takano¹, ○Yoichi Nakamura^{1,2}, Yukio Yoneda¹

(¹Dept. Mol. Pharmacol., Kanazawa Univ. Facul. Pharm. Sci., ²Dept. Physiol. Osaka Pref. Univ. Grad. Sch. Veterinary. Sci.)

P02-E-14 Neuroprotection against oxidative stress by microglia and astrocyte

○Satoru Oshiro¹, Chun Zhang¹, Shigetaka Kitajima¹, Chihiro Miyazawa³, Ken-ichi Kawamura³, Kazuyuki Nakajima²

(¹Dept. Biochem. Genet., Med. Res. Inst., Tokyo Med. & Dent. Univ., ²Dept. Neurochem., Life Sci. Inst., Soka Univ., ³Product Develp., TK Craft, Co. Ltd)

P02-E-15 Kainic acid induces leukemia inhibitory factor mRNA expression in the rat brain with different time courses between the dentate gyrus and hippocampal CA1/CA3 subfields

○Masabumi Minami, Keiko Maekawa, Hisashi Yamakuni, Takahiro Katayama, Junji Nakamura, Masamichi Satoh

(Dept. Mol. Pharmacol., Fac. Pharm. Sci., Kyoto Univ.)

P02-E-16 Functional expression of the monoamine transporter in cultured rat astrocytes

○Hiroshi Takeda, Masato Inazu, Teruhiko Matsumiya

(Dept. Pharmacol., and Intractable Diseases Research Center, Tokyo Med. Univ.)

P02-E-17 Generation of BRINP1 knock-out mice

○Toshiyuki Nakatani¹, Hiromi Kawano¹, Miwako Kobayashi¹, Ken-ichi Matsumoto², Hiroyoshi Ariga², Ichiro Matsuoka¹
(¹Dept. Neurosci. Grad. Sch. Pharm. Sci., Hokkaido Univ., ²Dept. Mol. Biol. Grad. Sch. Pharm. Sci., Hokkaido Univ.)

P02-E-18 Characterization of intercellular Ca²⁺ wave propagation by endogenous ATP in cultured rat hippocampus

○Schuichi Koizumi¹, Kayoko Fujishita^{1,2}, Kazuhide Inoue^{1,2}
(¹Div. Pharmacol., Natl. Inst. Hlth. Sci., ²Grad. Sch. Pharm. Sci., Kyushu Univ.)

P02-E-19 Perineuronal oligodendrocytes protect against neuronal apoptosis in a genetic demyelinating model by upregulation of lipocalin-type prostaglandin D synthase

○Naomi Eguchi^{1,2}, Masako Taniike³, Ikuko Mohri³, Kinuko Suzuki⁴, Yoshihiro Urade^{1,2}
(¹Dept. Mol. Behav. Biol., Osaka Biosc. Inst., ²CREST, JST, ³Dept. Developmental Medicine (Pediatrics), Osaka Univ. Grad. Sch. Med., ⁴Dept. Pathology and Labo. Med., and Neurosci. Center, Sch. Med., Univ. of North Carolina at Chapel Hill)

P02-E-20 Transcriptional regulation of the mouse 3-phosphoglycerate dehydrogenase (3-PGDH) gene in astrocytes

○Motohiro Shimizu^{1,2}, Shigeki Furuya¹, Yoko Shinoda¹, Kazuyuki Yoshida^{1,3}, Junya Mitoma⁴, Yasuo Suzuki², Yoshio Hirabashi¹
(¹Neuronal Circuit Mechanism Res. Group, BSI, RIKEN, ²Dept. Biochem. Sch. Pharm. Sci., Univ. Shizuoka, ³Unit. Grad. Sch. Agri. Sci., Tokyo Univ. Agriculture and Technology, ⁴Burnham Inst.)

P02-E-21 Establishment and characterization of immortalized Schwann cells from murine disease models

○Kazuhiko Watabe¹, Tsuyoshi Sakamoto¹, Yoko Kawazoe¹, Jin-Song Shen^{1,2}, Toya Ohashi², Yoshikatsu Eto², Makoto Michikawa³, Katsuhiko Yanagisawa³, Katsuichi Miyamoto⁴, Takashi Yamamura⁴, Norie Araki⁵
(¹Dept. Mol. Neuropathol., Tokyo Metropol. Inst. Neuroscience, ²Dept. Gene Therapy, Inst. DNA Medicine, Jikei Univ. Sch. Med., ³Dept. Dementia Res., Natl Inst. Longevity Sci., ⁴Dept. Demyelinating Disease and Aging, Natl Inst. Neuroscience, ⁵Dept. Tumor Genetics and Biol., Kumamoto Univ. Sch. Med.)

P02-E-22 Involvement of microglia activation and p38 phosphorylation in rat neuropathic pain

○Kazuhide Inoue^{1,3}, Makoto Tsuda¹, Akito Mizokoshi^{1,3}, Yukari Shigemoto-Mogami¹, Schuichi Koizumi¹, Shinichi Kohsaka²
(¹Div. Pharmacol., Natl. Inst. Health Sci., ²Dept. Neurochem., Natl. Inst. Neurosci., ³Grad. Sch. Pharmaceut. Sci., Kyusyu Univ.)

Signal Transduction and Modulation

P02-E-23 Binding of transcription factor AP-1 to the non-coding region in mitochondrial genome

○Yoshiaki Fujinami¹, Masahiro Kitano¹, Yukio Yoneda², Kiyokazu Ogita¹

(¹Dept. Pharmacol., Setsunan Univ. Fac. Pharm. Sci., ²Dept. Mol. Pharmacol., Kanazawa Univ. Fac. Pharm. Sci.)

P02-E-24 Effect of exposure to static magnetic field on AP1 DNA binding in cultured rat hippocampal neurons

○Takao Hirai, Yukio Yoneda

(Dept. Mol. Pharmacol., Fac. Pharm. Sci. Kanazawa Univ.)

P02-E-25 Augmentation of ethanol-induced cell damage and activation of NF κ B by over-expression of Annexin IV in C6 cells

○Hiroko Ohkawa^{1,2}, Hitoshi Sohma¹, Rie Sakai^{1,3}, Eri Hashimoto³, Wataru Ukai³, Toshikazu Saito³, Yoshio Kuroki¹

(¹Dept. Biochem. Sapporo Med. Univ. Sch. Med., ²Dept. Occup. Ther. Sapporo Med. Univ. Sch. Health Sci., ³Dept. Neuropsychiatry Sapporo Med. Univ. Sch. Med.)

P02-E-26 A novel human HRD1 involved in ER-associated degradation is induced by unfolded protein response and protects from ER stress-induced cell death via its ubiquitin ligase activity (MS6-4)

○Masayuki Kaneko, Masataro Ishiguro, Yoshifumi Niinuma, Yasuyuki Nomura

(Dept. Pharmacol., Grad. Sch. Pharm. Sci., Hokkaido Univ.)

P02-E-27 Induction of SEL1L, a human homolog of Hrd3p, by ER stress and identification of the region for transcriptional induction by ATF6

○Yoshifumi Niinuma, Masataro Ishiguro, Saori Yasui, Masayuki Kaneko, Yasuyuki Nomura

(Dept. Pharmacol., Grad. Sch. Pharm. Sci., Hokkaido Univ.)

P02-E-28 Expression of XRE binding proteins in rat cerebellum

○Noriko Sanada, Katsuhiko Baba, Nobuyuki Kuramoto, Yukio Yoneda

(Dept. Mol. Pharmacol., Kanazawa Univ. Pharm. Sci.)

P02-E-29 Estrogen elevates PKA activity via the putative membrane receptor in hippocampal neurons.

○Akiko S. Shingo, Shozo Kito

(Hyogo Univ. Sch. of Health Sci.)

P02-E-30 A novel FERM domain including guanine nucleotide exchange factor is involved in Rac signaling and regulates neurite remodeling

○Tateki Kubo, Toshihide Yamashita, Masaya Tohyama

(Dept. Anat. Neurosci., Grad. Sch. Med., Osaka Univ., CREST)

P02-E-31 Biological activity of neurotrophins is dependent on proper recruitment of Rac1 to lipid rafts

○Akiko Honda^{1,2,3}, Toshihide Yamashita^{1,2}, Tateki Kubo^{1,2}, Hiroyuki Tanaka^{1,2}, Taiichi Katayama^{1,2}, Masaya Tohyama^{1,2}

(¹Anat. Neurosci., Grad. Sch. Med., Osaka Univ., ²CREST,JST, ³Discovery Res. Lab., Tanabe Seiyaku Co.Ltd)

P02-E-32 Morphological changes in mouse neuroblastoma caused by disruption of glycosylation

○Kousuke Baba, Taiichi Katayama, Masaya Tohyama

(Dept. Anat. Neurosci., Grad. Sch. Med., Osaka Univ., CREST,JST)

P02-E-33 Identification of GRP78 as a type-1 sigma receptor (sigmaR1) -associated protein.

○Hideko Yamamoto¹, Fuyuki Kametani¹, Yoshiko Namiki¹, Toshifumi Yamamoto^{1,2}, Junnichi Karasawa^{1,3}, Ichiro Sora^{1,4}, Toshihide Nukada¹

(¹Tokyo Inst. Psychiatry, ²Yokohama City Univ., ³Nihon Shering Co., ⁴Tohoku Univ.)

P02-E-34 Regulation of exocytotic events and granular movements by protein kinase C in single chromaffin cells

○Nobuyuki Sasakawa, Sanae Hosaka, Mitsunori Hayashi, Konosuke Kumakura

(Life Sci. Inst. Sophia Univ.)

P02-E-35 Identification of a trans-acting protein interacting with cis-element for dendritic targeting of alpha-CAMKII transcript.

○Yasutake Mori, Shingo Miyata, Masaya Tohyama

(Dept. Anat. and Neurosci., Grad. Sch. Med., Osaka Univ., CREST)

P02-E-36 Influx of extracellular Ca²⁺ induced the expression of arc mRNA in PC12

○Hisashi Ichikawa, Eiichi Taira, Naomasa Miki

(Dept. Pharmacol., Osaka Univ. Med. Sch.)

P02-E-37 Activation and phosphorylation of Ca²⁺/calmodulin-dependent protein kinases in cultured rat hippocampal neurons

○Akiyoshi Uezu, Kohji Fukunaga, Jiro Kasahara, Eishichi Miyamoto

(Dept. Pharmacol., Kumamoto Univ. Sch. Med.)

P02-E-38 Activation of Cdk5 by the activation subunit p39

○Akio Sekigawa, Taro Saito, Yuko Hamazumi, Akiko Asada, Shin-ichi Hisanaga

(Dept. of Biol. Sci., Grad. Sch. of Sci., Tokyo Metro. Univ.)

P02-E-39 Characterization of a novel protein kinase that associate with Cdk5/p35

○Akiko ASADA, Naoyuki Honma, Sen Takeshita, Eiko Yamakawa, Mariko Enomoto, Taro Saito, Shin-ichi hisanaga

(Dept. Biol. Sci., Grad. Sch. Sci., Tokyo Metropolitan Univ.)

P02-E-40 Prostaglandin I₂ potentiates bradykinin-induced phosphorylation of synapsin I at site-3 in Neuro-2A cells.

○Yuri Matsuo, Keita Torihata, Hirotugu Kato, Yasuharu Sasaki
(Dept. Pharmacol., Sch. Pharmac. Sci., Kitasato Univ.)

P02-E-41 Neuronal cell type-specific expression of Ca²⁺/Calmodulin-dependent protein kinase II gene

○Kazuko Mima, Takashi Yamauchi
(Fac. Pharm. Sci., Tokushima Univ.)

P02-E-42 Antiapoptotic action of calcineurin via dephosphorylation of Bcl-2

○Hideyuki Yamamoto¹, Takeo Nomura², Hiromitsu Mimata², Miki Shitashige³, Futoshi Shibasaki³, Yoshio Nomura², Eishichi Miyamoto¹
(¹Dept. Pharmacol., Kumamoto Univ. Med. Sch., ²Dept. Urology, Oita Med. Univ., ³Mol. Cell Physiology, Tokyo Metro. Inst. Med. Sci.)

Axonal Transport, Cell Motility and Cytoskeleton

P02-E-43 Direct observation and characterization of gels formed from isolated neurofilaments *in vitro*

○Masashi Kurachi¹, Shinpei Haraikawa², Rei Ono², Jean-Francois Leterrier³, Yoshiaki Komiya¹, Tomoko Tashiro^{2,4}
(¹Dept. Mol. Cell. Neurobiol., Gunma Univ. Sch. Med., ²Dept. Biochem., Aoyama-Gakuin Univ. Coll. Sci. Eng., ³CNRS, Poitiers Univ., ⁴CREST, JST)

P02-E-44 A gene product binding to the 3'-untranslated region of *type1 inositol 1,4,5-trisphosphate receptor* mRNA and its involvement in mRNA localization in cerebellar Purkinje cells

○Takatoshi Iijima^{1,2,4}, Yuki Kimura³, Kanako Takatuki⁵, Takao Imai¹, Shigetaka Kawahara⁵, Aran Bernstein³, Yutaka Kirino⁵, Hideyuki Okano^{1,2}
(¹Dept. Physiology Keio Univ. Med. Sch., ²CREST, JST, ³Samuel Lunenfeld Res. Inst. Mount Sinai Hospital, CAN, ⁴Dept. Mol. Cellularbiology, Grad. Sch. Med., Osaka Univ., ⁵Lab. of Neurobiophysics, Grad. Sch. Pharmaceut. Sci., Tokyo Univ.)

P02-E-45 The roles of WAVES in filopodia formation at NG108 growth cones

(MS1-6) ○Shigeaki Miyamoto¹, Motohiro Nozumi¹, Hiroyuki Nakagawa¹, Hiroaki Miki², Tadaomi Takenawa²
(¹Dept. Biochem. Eng., Kyushu Inst. Tech., ²Dept. Biochem., Inst. Med. Sci., Univ. Tokyo)

P02-E-46 A role of semaphorin in newronal network formation in cerebral cortex

(MS1-5) ○Yoshio Goshima, Yukio Sasaki
(Dept. Pharmacol., Yokohama City Univ., Sch. Med.)

P02-E-47 Lysophospholipid signaling in the regulation of neuronal morphology and motility

(MS1-4) ○Nobuyuki Fukushima

(Dept. Biochem. Grad. Sch. Med., Hokkaido Univ.)

P02-E-48 Identification of fimbrin as a interacting molecule of microglia-specific protein, Iba1.

○Keiko Ohsawa, Yoshinori Imai, Yo Sasaki, Hiroko Kanazawa, Shinichi Kohsaka

(Dept. Neurochem., Natl. Inst. Neurosci.)

P02-E-49 Identification and characterization of drebrin binding protein, Drap1

○Hiroyuki Yamazaki¹, Hideto Takahashi¹, Eiji Hirose², Nozomu Mori², Tomoaki Shirao¹

(¹Dept. Neurobiol & Behav., Gunma Univ Sch Med, ²Dept. Molec Genetics., Natl. Inst. Longevity Sci. (NILS))

P02-E-50 Anterograde axonal transport of endopeptidase 24.15 in rat sciatic nerves

○Masaru Yamamoto¹, Toshiyuki Chikuma², Atsue Yamashita², Mitsune Yamaguchi², Hiroshi Hojo², Md Mahiuddin Ahmed¹, Takeshi Kato¹

(¹Grad. Sch. Int. Sci., Yokohama City Univ., ²Dept. Hygien. Chem., Showa Pharmaceut. Univ.)

Others

P02-E-51 Identification of a novel neural-specific protein family, BRINP

○Toda Fumiaki, Ueno Satoshi, Mori Takuya, Nakatani Toshiyuki, Matsuoka Ichiro

(Dept. Neurosci. Grad. Sch. Pharm. Sci., Hokkaido Univ.)

P02-E-52 Transgenic RNA interference in *Drosophila*

○Shunji Ohsako, Yoshiki Takamatsu

(Dept. Brain Struc., Tokyo Metropol. Inst. Neurosci.)

P02-E-53 Optimization of water purification technology for biological experiment

○Naoe Ishii¹, Ichiro Kano², Masanori Kanazawa¹

(¹Lab water Div., Nihon Millipore K.K., ²Lab water Div., Millipore S.A.)

P02-E-54 Identification and characterization of ER-stress sensor IRE1 associated proteins

○Kayoko Oono^{1,3}, Takunari Yoneda^{1,3}, Kazunori Imaizumi², Taiichi Katayama^{1,3}, Masaya Tohyama^{1,3}

(¹Dept. Anat. Neurosci., Osaka Univ. Grad. Sch. Med., ²Dept. Struct. Cell. Bio., NAIST, ³CREST)

P02-E-55 The 5' variants of p270, an integral member of SWI-SNF complexes, in the rat brain.

○Tsuyoshi Yamaguchi¹, Kazuaki Araki², Hiroyuki Nawa², Hiroshi Higuchi¹

(¹Dept. Mol. Genetics & Signal Transduction Res. Grad. Sch. Medical & Dental Sci. Niigata Univ., ²Dept Mol. Neurol., Brain Res. Inst., Niigata Univ.)

P02-E-56 Localization of prion-protein in cultured mouse neuroblastoma

○Reiko Kamata¹, Yuji Sakasegawa¹, Naomi S Hachiya^{1,2}, Kiyotoshi Kaneko¹
(¹Dept. Cortical Function Disorders, Natl. Inst. Neurosci., Natl. Center of Neurology and Psychiatry, ²CREST/JST)

P02-E-57 P38 α 2, a novel p38 α splice variant with kinase activity and unique C-terminal domain that regulates its cellular localization and cell death.

○Eiko Kusaka¹, Yasuyuki Ishikawa², Masato Okada¹
(¹Dept. Oncogene Res., Res. Inst. Microbial Diseases, Osaka Univ., ²Nara Inst. Sci. Tech.)

P02-E-58 Determination of fatty acid levels in erythrocyte membranes in patients with chronic fatigue syndrome

○Zhandong Liu, Dexin Wang, Jian Zhang
(Dept. Neurology, Binjing Friendship Hospital, Affiliated Hospital of Capital Univ. Med. Sci.)

Cell Adhesion Molecules

P02-E-59 Activation of p38 MAPK cascade by the activity-regulated neuronal cell adhesion molecule arcadlin

○Shin Yasuda¹, Hidekazu Tanaka², Hiroko Sugiura¹, Kyoko Suzuki¹, Takako Takemiya¹, Naomasa Miki², Kanato Yamagata¹
(¹Dept. Neuropharmacol., Tokyo Metropolitan Inst. Neurosci., ²Dept. Pharmacol., Osaka Univ. Sch. Med.)

P02-E-60 Interaction of locally modulated and de novo synthesized cadherins at the activated synaptic membranes

○Hidekazu Tanaka¹, Shin Yasuda², Takahiro Fujimoto¹, Akira Mizoguchi³, Hiroko Sugiura², Kanato Yamagata², Naomasa Miki¹
(¹Dept. Pharmacol., Osaka Univ. Sch. Med., ²Dept. Neuropharmacol., Tokyo Metropolitan Inst. Neuroscience, ³Dept. Anat., Mie Univ. Sch. Med.)

P02-E-61 GPI-anchored neural adhesion molecules contactin and NB-3 localized in post synaptic density

○Yasuo Takeda¹, Suni Lee^{1,2}, Vance Lemmon², Kazutada Watanabe³
(¹Dept. Cell Recog., Tokyo Metropol. Inst. Gerontol., ²Dept. Neurosci., Sch. Med., CWRU, ³Dept. BioEng., Nagaoka Univ. Tech.)

Infection

P02-E-62 Bacterial endotoxin induces IL-20 expression via p38 MAP kinase dependent mechanisms in the glial cells

○Toru Hosoi, Sachiyo Wada, Yasunobu Okuma, Yasuyuki Nomura
(Dept. Pharmacol., Grad. Sch. Pharm. Sci., Hokkaido Univ.)

Synapse

P02-E-63 A novel synaptic protein, ssLRP, with dendritic mRNA

○Tatsuo Suzuki¹, Qing Bao Tian¹, Akira Okano¹, Shoko Miyazawa¹, Nobuteru Usuda²
(¹Dept Neuroplasticity, Res Ctr on Aging & Adaptation, Shinshu Univ Sch Med, ²Dept Anatomy, Fujita Health Sci. Univ., Sch. Med.)

P02-E-64 Generation of the SNAP-25 mutant mice carrying a S187A mutation

○Masakazu Kataoka^{1,4}, Reiko Kuwahara², Yoko Nakahara², Rika Suzuki², Yoshiko Motegi², Yuko Kato-Fukui², Minesuke Yokoyama², Masami Takahashi^{3,4}
(¹Dept. Env. Sci. Tech. Fac. Eng., Shinsu Univ., ²Mitsubishi Kagaku Inst. Life Sci., ³Dep. Biochem. Sch. Med., Kitasato Univ., ⁴CREST, JST.)

P02-E-65 The binding of CaMKII to syntaxin isoforms

Kazushige Nomura^{1,2,3}, Yoshiaki Komiya³, ○Michihiro Igarashi¹
(¹Div. Mol. Cell. Biol., Grad. Sch. Med. Dent. Sci., Niigata Univ., ²Dept. Orthopedics, Gunma Univ. Sch. Med., ³Dept. Mol. Cell. Neurobiol., Gunma Univ. Sch. Med.)

Plasticity

P02-E-66 NMDA receptor-dependent recruitment of AMPA receptor and calnexin to plasma membrane in cultured rat hippocampal neurons

○Jun Tsujimura¹, Makoto Itakura², Mariko Sekiguchi³, Yoshihisa Kudo¹, Masami Takahashi^{2,4}
(¹Sch. Life Sci., Tokyo Univ. Pharm. and Life Sci., ²Dept. Biochem., Kitasato Univ. Sch. Med., ³Mitsubishi Kagaku Inst. Life Sci., ⁴CREST, JST)

P02-E-67 Kainate-induced neural activity alters the phosphorylation states of translation factors in rat hippocampus.

○Naoko Inamura, Hiroyuki Nawa, Nobuyuki Takei
(Dept. Mol. Neurobiol., Brain Res. Ins., Niigata Univ.)

Pattern Formation

P02-E-68 Reelin signaling and Cdk5 in the control of neuronal positioning

(MS1-1) ○Toshio Ohshima¹, Masaharu Ogawa², Katsuhiko Mikoshiba¹
(¹Lab. for Developmental Neurobiol., ²Lab. for Cell Culture Development, BSI, RIKEN)

Cellular Differentiation

- P02-F-1 Flow cytometric analysis of neural stem cells in the developing and adult mouse brain**
 ○Ayako Murayama^{1,2,3}, Yumi Matsuzaki-Iyoku^{1,3}, Ayano Kawaguchi⁴, Takaki Miyata⁵, Takuya Shimazaki^{1,3}, Hideyuki Okano^{1,3}
 (¹Dept. Physiol. Sch. Med., Keio Univ., ²Dept. Cell Biol. and Neurosci. Grad. Sch. Med., Osaka Univ., ³CREST-JST, ⁴Cell asymmetry. Center for Developmental Biol., RIKEN, ⁵Cell Culture Development. Brain Sci. Inst., RIKEN)
- P02-F-2 Interactions between necdin and E2F1 in terminal differentiation and survival of (MS3-4) sensory neurons**
 ○Risa Takazaki, Taichi Uetsuki, Hideo Taniura, Kazuaki Yosikawa
 (Div. Reg. Macromol. Func. Inst. Prot. Res. Osaka Univ.)
- P02-F-3 Upregulation of gene expression of PACAP and its receptors during differentiation of the ES cell-derived neural stem cells**
 ○Megumi Hirose¹, Hitoshi Hashimoto¹, Norihito Shintani¹, Naohisa Arakawa¹, Akemichi Baba^{1,2}
 (¹Lab. Mol. Neuropharmacol. Grad. Sch. Pharm. Sci., Osaka Univ., ²Lab. Mol. Pharmacol. Grad. Sch. Med., Osaka Univ.)
- P02-F-4 Genipin-induced neuritogenesis through long term phosphorylation of ERK via NO-cGMP-PKG signaling pathway in Neuro2a cells**
 ○Matsumi Yamazaki, Tadatoshi Ohkubo, Kenzo Chiba, Tetsuro Mohri, Shigeyuki Yoshifuji
 (Dept. Biodynam. Fac. Pharm. Sci., Hokuriku Univ.)
- P02-F-5 Cystatin C induces astrocyte differentiation by inhibition of cystein proteases**
 ○Akiko Hasegawa^{1,2}, Yasuno Iwasaki¹, Kazuhiro Ikenaka¹
 (¹Natl. Inst. Physiolog. Sci., ²The Grad. Univ. Advanced Studies)
- P02-F-6 Negative regulation of neural progenitor cell proliferation by necdin**
 ○Hideo Taniura, Noriko Fukunaga, Taichi Uetsuki, Kazuaki Yoshikawa
 (Div. Reg. Macromol. Func., Inst. Prot. Res., Osaka Univ.)
- P02-F-7 Involvement of small GTPase RhoA to STAT3 signaling pathway during astrocytic differentiation of rat C6 glial cells**
 ○Hiromi Takanaga¹, Tatsumi Adachi², Manabu Kunitomo¹
 (¹Sch. Pharm. Sci., Kitasato Univ., ²Dept. Basic Med. Sci., Natl. Inst. Minamata Dis.)
- P02-F-8 Roles of Gab1 in the maintenance and differentiation in neural stem cells**
 ○Yoshika Hayakawa^{1,3}, Keigo Nishida², Toshio Hirano², Takuya Shimazaki^{1,3}, Hideyuki Okano^{1,3}
 (¹Dept. Physiol. Sch. Med., Keio Univ., ²Dept. Mol. Onco. Grad. Sch. Med., Osaka Univ., ³CREST, JST)

P02-F-9 Roles of Musashi Family in mammalian nervous development

○Shinsuke Shibata^{1,3}, Shin-ichi Sakakibara², Takuro Kojima^{1,3}, Hideyuki Okano^{1,3}
(¹Dept. Physiol., Sch. Med., Keio Univ., ²Dept. Histol. Neurobiol., Sch. Med., Dokkyo Univ., ³CREST-JST)

P02-F-10 Inhibition of oligodendrocyte differentiation by Wnt signaling

○Takeshi Shimizu¹, Tamaki Wada¹, Tetsushi Kagawa¹, Shinji Takada^{2,3}, Yuko Muroyama^{2,3}, Kazuhiro Ikenaka¹
(¹Nat. Inst. Phys. Sci., Okazaki. Nat. Res. Inst., ²Cent. Integ. Biosci., Okazaki. Nat. Res. Inst., ³ERATO. Kondoh. Diff. Signal. Proj)

P02-F-11 The Wnt/calcium pathway activates NF-AT and promotes ventral cell fate in *Xenopus* embryos.

Takeo Saneyoshi¹, Shoen Kume¹, ○Katsuhiko Mikoshiba^{1,2,3}
(¹Dept. Mol. Neurobiol., The Inst. Med. Sci., Univ. Tokyo, ²Mikoshiba Calciosignal Net Project, ERATO and Calcium Oscillation Project, ICORP, JST, ³RIKEN Brain Sci. Inst.)

P02-F-12 Expression of a glutamine-rich forkhead transcription factor in the developing central nervous system.

○Hiroki Iwanishi, Yoshihiro Morikawa, Emiko Senba
(Dept. Anat. Neurobiol., Wakayama Med. Univ.)

P02-F-13 Kohjirin, A novel bone morphogenetic protein-4 antagonist derived from astrocytes.

○Kanna Kuriyama¹, Takatoshi Ueki¹, Masamitsu Tanaka², Naoyuki Miura³, Haruhiko Sugimura², Kohji Sato¹
(¹Dept. Anat. & Neurosci., Sch. Med., Hamamatsu Univ., ²Dept. Pathol. Sch. Med., Hamamatsu Univ., ³Dept. Biochem., Sch. Med., Hamamatsu Univ.)

Trophic Factor

P02-F-14 Mechanisms of riluzole-induced brain-derived neurotrophic factor synthesis in the rat (MS3-1) hippocampus

○Ritsuko Katoh-Semba, Kanefusa Kato
(Inst. Dev. Res., Aichi Human Service Center)

P02-F-15 bFGF evokes a rapid glutamate release through activation of the MAPK pathway in (MS3-2) cultured cortical neurons

○Tadahiro Numakawa^{1,2,3}, Daisaku Yokomaku^{1,2}, Kazuyuki Kiyosue², Naoki Adachi¹, Tomoya Matsumoto^{1,2}, Yumiko Numakawa^{1,2}, Takahisa Taguchi², Hiroshi Hatanaka¹, Masashi Yamada¹
(¹Div. Protein Biosynthesis, Inst. Prot. Res., Osaka Univ., ²Neuronics R.G. Div. Human Life Tech., Natl. Inst. Advanced Industrial Sci. Technol. (AIST), ³a JSPS Postdoctoral Fellow)

P02-F-16 Change of the expression of glial cell line-derived neurotrophic factor (GDNF) in the rat (MS3-3) sciatic nerve after its transection

○Yoshihisa Yamada¹, Atsumi Nitta², Katsuji Shimizu¹, Shoei Furukawa²
(¹Dept. Orthop. Surg., Gifu Univ. Sch. Med., ²Lab. of Mol. biol., Gifu Pharm. Univ.)

P02-F-17 cAMP-dependent protein kinase phosphorylates the p75 receptor and regulates its localization to lipid rafts

○Haruhisa Higuchi, Toshihide Yamashita, Masaya Tohyama
(Dept. Anat. and Neurosci. Grad. Sch. Med., Osaka Univ., CREST, JST)

P02-F-18 Responsiveness of embryonic striatal neurons to bone morphogenetic protein and neurotrophic factors

○Kikuchi Saya¹, Nakamura Takeshi², Mori Nozomu², Matsuoka Ichiro¹
(¹Dept. Neuroscience, Grad. Sch. Pharm. Sci., Hokkaido Univ., ²Dept. Mol. Gen., Nat. Inst. Longevity Sci.)

P02-F-19 Autocrine action of BMP regulates GDNF expression in Schwann cells

○Emi Kinameri, Ichiro Matsuoka
(Lab. Neurosci., Grad. Sch. Pharmaceut. Sci., Hokkaido Univ.)

P02-F-20 Levels of mRNA for brain derived neurotrophic factor are up-regulated in the urethra of diabetic rats

○Rintaro Machino¹, Ichiro Matsuoka², Hidehiro Kakizaki¹, Takashi Shibata¹, Tomohiko Koyanagi¹
(¹Dept. Urology, Grad. Sch. Med., Hokkaido Univ., ²Dept. Neurosci., Grad. Sch. Pharm. Sci., Hokkaido Univ.)

P02-F-21 Accumulation of nerve growth factor protein at both rostral and caudal stumps in the transected rat spinal cord

○Yoshiko Furukawa, Yutaka Murakami, Atsumi Nitta, Shoei Furukawa
(Lab. Mol. Biol., Gifu Pharm. Univ.)

P02-F-22 Effect of neurotrophins on the expression of bHLH transcription factors

○Hisanori Ito, Aki Nakazima, Shoei Furukawa
(Lab. of Mol. Biol., Gifu Pharmaceut. Univ.)

P02-F-23 Shp-2 tyrosine phosphatase regulates trophic effect of BDNF through activation of the PI3-K and MAPK pathways

○Satomi Takai, Masato Okada
(Dept. Oncogene Res., Ins. Microbial Diseases, Osaka Univ.)

P02-F-24 Autocrine action of acidicFGF to stimulate cholesterol metabolism and apoE secretion of rat astrocytes

○Jinichi Ito, Yuko Nagayasu, Shinji Yokoyama
(Dept. Biochem.1., Nagoya City Univ. Med. Sch.)

P02-F-25 A brain-specific EGF-like family protein, NELL2, is a novel survival factor for neurons

○Masato Horie¹, Koutoku Aihara¹, Katsuyuki Tanizawa², Shun'ichi Kuroda²
(¹Second Inst. New Drug Discovery, Otsuka Pharmaceutical Co., Ltd., ²Dept. Structural Mol. Biol., Inst. Sci. Industrial Res., Osaka Univ.)

P02-F-26 Changes of the gene expression in the injured site of the rat spinal cord

○Manabu Hashimoto, Takuya Hori, Shoei Furukawa
(Lab. of Mol. Bio., Gifu Pharm. Univ.)

Axonal Outgrowth and Network Formation

P02-F-27 Neurite outgrowth effects of withanolide A, withanoside IV and withanoside VI in A β (MS1-7) 25-35-induced atrophy of neurites

○Tomoharu Kuboyama, Chihiro Tohda, Katsuko Komatsu
(Res. Ctr. Ethnomed., Inst. Natural Med., Toyama Med. & Pharm. Univ.)

P02-F-28 Expression of tau isoforms containing exon2 and 3 during synaptogenesis *in vivo* and (MS1-8) *in vitro*

○Chikara Kitamura, Yuta Wakayama, Masaki Masuno, Miyuki Noro, Tomoko Tashiro
(Dept. Biochemistry, Coll. Science and Engineering, Aoyama-gakuin Univ.)

P02-F-29 Dynamic morphological changes of migrating neurons during the cortical (MS1-3) development

○Hidenori Tabata¹, Kazunori Nakajima^{1,2}
(¹Dept. Mol. Neurobiol., Inst. DNA Med., Jike Univ. Sch. Med., ²PRESTO, JST)

P02-F-30 Lateralization of thalamofugal pathway before light exposure in developing chicken visual pathway

○Mamiko Koshihara^{1,2}, Tateki Kikuchi², Masfumi Yohda¹, Shun Nakamura²
(¹Tokyo Univ. A&T, ²Natl. Inst. Neurosci.)

P02-F-31 Is Otx1 essential for the establishment of subocortical projections from the visual area?

Koichi Ando¹, Chikara Kubota¹, Hideshi Yagi¹, Takashi Nagano¹, Shinichi Aizawa², Toshio Terashima³, ○Makoto Sato¹
(¹Dept. Anat.2, Fukui Med. Univ., ²CARD, Kumamoto Univ., ³Dept. Brain Sci., Grad. Sch. Med., Kobe Univ.)

P02-F-32 CRMP-2 and axon formation

(MS1-2) ○Yuko Fukata, Kozo Kaibuchi
(Dept. Cell Pharmacol. Grad. Sch. Med., Nagoya Univ.)

- P02-F-33 Conformational changes of Pur α concomitantly increase in ssCRE binding activity**
 ○Che-Hui Kuo, Ling-Hui Zeng, Naomasa Miki
 (Dept.Pharmacol., Osaka Univ. Med. School)
- P02-F-34 Cytoplasmic p21^{Cip1/WAF1} regulates neurite remodeling by inhibiting Rho-kinase activity**
 ○Hiroyuki Tanaka, Toshihide Yamashita, Masaya Tohyama
 (Dept. Anatomy and Neuroscience., Grad. Sch. Med., Osaka Univ., CREST, JST)
- P02-F-35 Inactivity-induced synapse elimination preceding apoptosis**
 ○Akihiko Ogura^{1,2}, Norikazu Hashimoto¹, Keiko Tominaga-Yoshino^{1,2}
 (¹Osaka Univ. Grad. Sch. Sci., ²Osaka Univ. Grad. Sch. Frontier Biosci.)
- P02-F-36 Dopaminergic neurons derived from mouse midbrain maintain synchronous oscillation of intracellular calcium in primary culture**
 ○Fumie Yasumoto¹, Takayuki Negishi^{2,4}, Yoshiyuki Ishii², Shigeru Kyuwa², Yoichiro Kuroda^{3,4}, Yasuhiro Yoshikawa^{2,4}
 (¹Dept. Biomed. Sci., Faculty of Agri., The Univ. of Tokyo., ²Dept. Biomed. Sci., Grad. School of Agri. and Life Sci., The Univ. of Tokyo, ³Dept. of Mol. and Cell. Neurobiol., Tokyo Metropolitan Inst. for Neurosci., ⁴CREST,JST)
- P02-F-37 Disparity of cell death and mossy fiber sprouting in kainic acid-treated hippocampal slice cultures**
 ○Chunxi Han¹, Yuriko Furukawa^{1,2}, Keiichi Torimitsu^{1,2}
 (¹NTT Basic Res. Lab., ²C R E S T)
- P02-F-38 Measurement of neurite outgrowth in neuronal cell culture using cell morphological screening system**
 ○Fumiko Tanaka, Hiroki Shimizu, Toru Oki, Yoshikazu Hasegawa
 (Tsukuba Res. Lab., Eisai Co., Ltd.)

Regeneration, Transplantation

- P02-F-39 Transglutaminase expression and its implication in goldfish optic nerve regeneration**
 ○Kayo Sugitani^{1,2,3}, Toru Matsukawa^{2,3}, Keiko Homma^{1,2}, Satoru Kato^{2,3}
 (¹Dept. Lab. Sci., Fac. Med., Kanazawa Univ., ²Dept. Mol. Neurobiol., Grad. Sch. Med., Kanazawa Univ., ³CREST)
- P02-F-40 A member of collapsin response mediator protein(CRMP) family has a potent neurite elongation activity in nerve regeneration**
 ○Yasuhiro Suzuki^{1,2}, Saya Nakagomi¹, Naoyuki Inagaki³, Kozo Kaibuchi⁴, Hiroshi Kiyama¹
 (¹Dept. Anat. Grad. Sch. Med. Osaka-City Univ., ²Dept. Med. Asahikawa Med. Coll., ³Div. Sig. Trans. NAIST, ⁴Dept. Cell Pharm., Nagoya Univ. Grad. Sch. Med.)

P02-F-41 Repair of perineurium and nerve regeneration in end-to-side nerve graft

○Seiichiro Okajima¹, Ryo Oda¹, Hisanori Hirakawa¹, Hiroaki Kobashi¹, Toshikazu Kubo¹, Julia Terzis²

(¹Dept. Orthop. Surg, ²Virginia Med. Sch.)

P02-F-42 Damage induced neuronal endopeptidase (DINE/ECEL) expression is regulated by LIF and a deprivation of NGF

○Ryuichi Kato^{1,2}, Sumiko Kiryu-Seo², Hiroshi Kiyama²

(¹Dept. Urol., Sch. Med., Sapporo Med. Univ., ²Dept. Anat. Grad. Sch. Med., Osaka City Univ.)

P02-F-43 *in vitro* selection of brain-migrating cells from immature bone marrow cells

○Kenji Ono^{1,2}, Ken Yoshihara^{1,2}, Kenji Tanaka¹, Takemasa Takii², Kikuo Onozaki², Makoto Sawada¹

(¹JRDTAID, Inst. Comp. Med. Sci., Fujita Health Univ., ²Dept. Mol. Health. Sci. Grad. Sch. Pharm. Sci., Nagoya City Univ.)

P02-F-44 Challenge to raise monoclonal antibodies against galectin-1

○Koichiro Hori¹, Kayo Fujimaki¹, Kazunori Yoshimura¹, Toshihiko Kadoya², Hidenori Horie³, Masahiko Nomura¹

(¹Dept. Physiol., Saitama Med Sch., ²R&D Center, Prod. Dept., Pharmaceut. Div., Kirin Brewery Co. Ltd., ³Adav. Res. Center, Biol. Sci., Waseda Univ.)

P02-F-45 Differentiation of transplanted neural stem cells into oligodendrocyte in a mouse model of demyelinating disease

○Michio Matsumoto¹, Tetsushi Kagawa¹, Francois Lachapelle², Anne Baron-Van Evercooren², Kazuhiro Ikenaka¹

(¹Natl. Inst. Physiol. Sci., ²INSERM U546, France)

Myelin

P02-F-46 Two novel sulfated oligosaccharides derived from P0 of bovine peripheral nerve myelin

○Jianhong Yan, Kunio Kitamura, Masahiko Nomura

(Dept. Physiol., Saitama Med. Sch.)

P02-F-47 Structural studies on the glycoprotein-derived oligosaccharides in the peripheral nerves increased during maturation or by nerve crush injury

○Kunio Kitamura¹, Yuriko Yoshino², Jianhong Yan¹, Masahiko Nomura¹

(¹Dept. Physiol., Saitama Med. Sch., ²Dept. Gynecol., Saitama Med. Sch.)

P02-F-48 Mechanism of myelin sheath formation in *shiverer* mutant mouse

○Hiroaki Asou¹, Jin Nakahara^{1,2}, Chika Seiwa¹

(¹Dept. Neurobiol., TMIG, ²Dept. Anat., Keio Univ.)

特別講演

7月18日 (木) 9:00 - 10:00 A会場

座長: 三木 直正 (大阪大院・医)

- PL-1 神経機能と細胞内物質輸送: モーター分子群KIFsの遺伝子、構造、動態、機能および疾患
廣川 信隆
(東京大学大学院医学系研究科細胞生物学)

7月18日 (木) 10:00 - 11:00 A会場

座長: 高坂 新一 (国立精神・神経セ)

- PL-2 アポトーシスの分子メカニズム: Bcl-2蛋白の機能を中心に
辻本 賀英
(大阪大学大学院医学系研究科・遺伝子学)

7月18日 (木) 14:00 - 15:00 A会場

座長: 野村 靖幸 (北大院・薬)

- PL-3 Synaptic plasticity regulated by stargazin and PSD-95
David S. Bredt
(Department of Physiology, University of California at San Francisco)

7月18日 (木) 15:00 - 16:00 A会場

座長: 宮本 英七 (熊本大・医)

- PL-4 Tyrosine hydroxylase phosphorylation
Peter R. Dunkley
(School of Biomedical Sciences, Faculty of Health, The University of Newcastle)

招 待 講 演

7月17日 (水) 9:00-10:00 A会場

座長: 辻 省次 (新潟大・脳研)

IL-1 生物時計の分子機構と階層性

本間 研一

(北海道大学大学院医学研究科統合生理学講座)

7月17日 (水) 10:00-11:00 A会場

座長: 馬場 明道 (大阪大院・薬)

IL-2 変異マウスを用いた瞬目反射連合学習の分子神経機構の研究

桐野 豊

(東京大学大学院薬学系研究科・神経生物物理学教室)

7月19日 (金) 9:00-10:00 A会場

座長: 東田 陽博 (金沢大院・医)

IL-3 Ranサイクルによる核・細胞質間物質輸送と細胞複製制御。

西本 毅治

(九州大学大学院医学研究院、分子生命科学系専攻細胞工学講座)

7月19日 (金) 10:00-11:00 A会場

座長: 小宮 義璋 (群馬大・医)

IL-4 Bulk protein degradation - molecular dissection of autophagy-

大隅 良典

(基礎生物学研究所)

7月17日 (水)

14:00 - 17:00

A会場

国際神経化学会シンポジウム

Molecular Basis of Neuronal Signaling

座長：御子柴克彦 (東京大・医), Peter R. Dunkley (Univ. Newcastle)

ISN-1 AMPA receptor trafficking during plasticity

Robert Malinow

(Cold Spring Harbor Laboratory, USA)

ISN-2 シナプスの形成と機能の分子遺伝学

三品 昌美

(東京大学大学院医学系研究科分子神経生物学)

ISN-3 Cdk5: A new player at the neuromuscular synapse

Nancy Y. Ip

(Department of Biochemistry, Molecular Neuroscience Center, and Biotechnology Research Institute, Hong Kong University of Science and Technology, China)

ISN-4 Structure, regulation and function of PKB/Akt—a pleiotropic protein kinase involved in multiple signaling pathways

Brian A. Hemmings

(Friedrich Miescher-Institute, Switzerland)

ISN-5 Excitatory amino acid transporters: New insights into Structure, function and regulation

Susan G. Amara

(Vollum Institute and Howard Hughes Medical Institute, Oregon Health & Science University, USA)

7月17日 (水)

17:00 - 20:00

A会場

市民公開シンポジウム

心と行動を分子で読む

座長：武田雅俊（大阪大・医），西川 徹（東京医歯大）

- OS-1 遺伝子改変動物は精神疾患研究にどのように役立つか
 ○和田 圭司¹，山田 祐子¹，和田 恵津子^{1,2}，前野 浩巳¹，山田 一之^{1,3}
 （¹国立精・神セ・神経研，²学振，³理研脳セ）
- OS-2 心・行動と細胞内情報伝達系
 ○齋藤 利和
 （札幌医大・医・神経精神）
- OS-3 脳機能イメージングにより発達障害・学習過程を解析する
 ○渡辺 恭良
 （大阪市大・院医・システム神経科学）
- OS-4 遺伝と環境は人の性格形成にいかに関わるか—進化・発達生物学の視点から—
 ○神庭 重信
 （山梨医科大・精神神経医学）
- OS-5 精神分裂病研究の新しい芽生え
 ○西川 徹¹，梶井 靖^{1,2}，村岡 新一郎¹，海野 麻未¹，黒田 安計¹
 （¹東京医歯大・院・精神行動医科学，²三菱ファーマ）
- OS-6 うつ病治療薬の新しい展開
 ○小山 司
 （北大・院医・精神医学）

7月17日 (水)

9:00 - 11:00

D会場

奨励賞選考シンポジウム

座長：池中一裕（生理研）

シンポジウム

7月17日 (水)

14:30 - 17:00

B会場

S1 中枢神経系再生のストラテジー

座長：岡野栄之（慶応大・医）

- S1-1** **New strategies using adult neural stem cells in defined media and the absence of gene transfer**
 ○Tetsuro Shingo, Isao Date, Takeshi Ohmoto, Samuel Weiss
 (Dept. Neurol. Surg., Okayama Univ. Med. Sch., Genes Develop. Res. Group., Univ. Calgary Fac. Med.)
- S1-2** **Recent advance in the adenovirus mediated gene transfer system for neuronal regeneration**
 ○木山 博資, 濤川 一彦
 (大阪市大・院医・機能細胞形態)
- S1-3** **AAVベクターを用いたパーキンソン病の遺伝子治療**
 ○小澤 敬也¹, 村松 慎一², 王 立軍^{1,2}, 池口 邦彦², 藤本 健一², 岡田 尚巳¹, 水上 浩明¹, 寺尾 恵治³, 中野 今治²
 (¹自治医大・分子病態治療研究センター遺伝子治療, ²自治医大・神経内科, ³国立感染症研・筑波霊長類センター)
- S1-4** **骨髄間質細胞の神経・シュワン細胞への分化誘導および中枢神経再生への応用**
 ○出澤 真理
 (横浜市大・医・第一解剖)
- S1-5** **神経幹細胞移植による脊髄損傷モデル動物の治療**
 ○岡野 栄之
 (慶大・医・生理, CREST, JST)

7月17日 (水)

14:30 - 17:00

C会場

S2 PET・MRIを用いたヒト脳機能イメージング：現状と展望

座長：定藤規弘（生理研）

- S2-1 Cross-modal plasticity in the deaf and blind: studies with non-invasive human neuroimaging techniques
○定藤 規弘
(生理研)
- S2-2 生体におけるドーパミン受容体の定量とその変化
○須原 哲也
(放医研、科学技術振興事業団、戦略的基礎研究)
- S2-3 脳内コリン系神経の画像化
○難波 宏樹
(浜松医大・脳外)
- S2-4 PETによるヒスタミン神経系の機能研究：精神・神経疾患と創薬科学への応用
○谷内 一彦
(東北大・院医・病態薬理)
- S2-5 PET imaging of neural substrates for fatigue sensation
○渡辺 恭良
(大市大・院医・システム神経科学)

7月17日 (水)

14:30 - 17:00

D会場

S3 神経機能分子の構造的理解

座長：芳賀達也（学習院大・理）

- S3-1 電圧感受性Naチャンネルの3次元構造とそこから想像される開閉機構
○佐藤主税¹, 上野豊¹, 小椋俊彦¹, 佐藤雅彦², 藤吉好則³
(¹産業技術総合研究所, ²伊藤ハム, ³京大・生物物理)
- S3-2 G蛋白質共役受容体に結合したペプチドリガンドの立体構造
○白川 昌宏
(横浜市大・院総理・理)

- S3-3 Grb2を介するシグナル伝達の分子機構の解明**
 ○稲垣 冬彦
 (北大・院薬)
- S3-4 接着分子と細胞内シグナル伝達**
 ○箱嶋敏雄
 (奈良先端科学技術大学院)
- S3-5 モノマー型モーター分子KIF1Aの作動機構；分子生物物理学、クライオ電顕そしてX線結晶解析によるアプローチ**
 ○廣川信隆
 (東大・院医・分生)

7月18日 (木)

9:00 - 11:30

D会場

S4 脳の器官培養系の技術と応用を探る

座長：田中正彦（藤田保健衛生大・総医研）坂口卓也（塩野義製薬・創薬研）

- S4-1 Aspects and applications of organotypic brain slice cultures**
 ○坂口 卓也
 (塩野義・創薬研・中枢神経薬・神経生化学)
- S4-2 海馬苔状線維のネットワーク形成—explant coculture systemとslice overlay assay**
 ○池谷 裕二, 松木 則夫
 (東大・院薬・薬品作用学)
- S4-3 神経活動依存的な皮質ニューロン軸索の分枝形成：スライス培養法を用いた形態学・生理学的研究**
 ○山本 亘彦, 上阪 直史, 平井 聡, 大波 壮一郎
 (阪大・生命機能・脳神経)
- S4-4 切片培養系を用いた小脳プルキンエ細胞樹状突起の形態形成に関わる神経—グリア相互作用の解析**
 ○田中 正彦
 (藤田保健大・総医研・応用細胞学)
- S4-5 哺乳類胚長期培養系を用いた嗅球発生におけるPax6遺伝子の機能解析**
 ○野村 真, 大隅 典子
 (東北大・院医・器官構築)

7月18日 (木)

16:00 - 18:30

A会場

S5 協調的分子配置によるシナプスの機能発現

座長：阿部輝雄（新潟大・脳研），白尾智明（群馬大・医）

S5-1 シナプス小胞の微小管への結合機構

○阿部 輝雄

（新潟大・脳研・細胞神経生物）

S5-2 シナプス前終末におけるCa²⁺チャネルの多様性

○八尾 寛

（東北大・院生命科学・脳機能解析，CREST，JST）

S5-3 ロイシンジッパーモチーフを有するPSD足場蛋白質（PSD-Zip45とPSD-Zip70）のシナプスターゲティング

○祖父江 憲治，今野 大治郎，白井 審一，堀 啓，丸岡 久人

（阪大・院医・未来医療開発専攻・神経幹細胞医科学）

S5-4 Differential dynamic behavior of postsynaptic density proteins and its regulation by neuronal activity

○岡部 繁男

（東京医歯大・院医・細胞相関機構）

S5-5 スパイン内シグナル伝達におけるドレブリンの役割

○白尾 智明，田中 聡一，山崎 博幸，関野 祐子

（群大・医・行動分析）

7月18日 (木)

14:30 - 17:00

B会場

S6 脳神経系細胞系譜決定とその可塑的変化の分子メカニズム

座長：池田一裕（生理研）

S6-1 神経幹細胞の運命決定の分子機構

○中福 雅人，長尾 元史

（東大・院医・神経生物）

S6-2 Neurogenin 1 as a positive bHLH regulator of neuronal differentiation

So-Yeon Kim¹, Yong-Soo Yoon¹, Yong-Jin Jeon¹, Mi-Young Jung¹, Seong-Soo Kim¹, Jiwon Kim¹, Seung-Up Kim², Young-Don Lee¹, ○Haeyoung Suh-Kim^{1,2}

(¹Dept. Anat., Ajou Univ., Sch. Med., ²BDRC, Ajo Univ. Sch. Med.)

- S6-3** 胎生期中枢神経系の細胞系譜制御に関わる細胞外来性シグナルと細胞内在性プログラム
○田賀 哲也, 滝沢 琢己, 中島 欽一
(熊本大・発生医学研究センター・転写制御)
- S6-4** オリゴデンドロサイト発生の分子機構
○鹿川 哲史, 池中 一裕
(生理研・神経情報)
- S6-5** 脳入出力神経細胞の分化と軸索伸展の遺伝解析
○岡本 仁
(理研・脳センター・発生遺伝子, 科技団・戦略)

7月18日 (木)

14:30 - 17:00

C会場

S7 チロシンリン酸化によるシナプス機能の制御

座長: 武井延之 (新潟大・脳研), 高橋正身 (北里大・医)

- S7-1** Srcファミリーチロシンキナーゼの機能と制御
○岡田 雅人
(阪大・微生物病研・発癌制御)
- S7-2** チロシンフォスファターゼによる神経伝達物質放出の制御
○板倉 誠¹, 山森 早織¹, 大西 浩史², 高橋 正身^{1,3}
(¹北里大・医, ²群馬大・生体調節, ³クレスト)
- S7-3** チロシンキナーゼによるNMDAレセプターとPSD-95の相互作用の調節
○乾 誠
(山口大・医・病態薬理)
- S7-4** レセプター型チロシンキナーゼによるシナプス機能の正負の調節
○武井 延之, 難波 寿明, 斎藤 真子, 永野 忠聖, 那波 宏之
(新潟大・脳研・分子神経生物)
- S7-5** ErbBレセプター型チロシンキナーゼによるシナプス機能の制御機構
○尾崎 美和子, 端川 勉
(理研・脳センター・神経構築)

7月18日 (木)

14:30 - 17:00

D会場

S8 グリア細胞の機能と病態：神経疾患の治療に向けて

座長：下濱 俊（京大・医），北村佳久（京都薬大），馬場広子（東京薬大・薬）

S8-1 グリア細胞の機能と病態：オーバービュー

○下濱 俊

（京大・院医・神経内科）

S8-2 アミロイドβ蛋白に対するアストログリア特有の応答

○阿部 和穂，三澤 美和

（星薬大・薬理）

S8-3 ミクログリアにおけるアミロイドβクリアランスの調節機構

○北村 佳久，柿村 順一，谷口 隆之

（京都薬大・病態生理）

S8-4 グリア・ニューロン相互作用を利用した神経変性疾患の治療法の開発

○和田 圭司¹，原田 高幸¹，原田 知加子¹，王玉来¹，野田 百美²，小坂 仁^{1,3}

（¹国立精・神セ・神経研・4部，²九大・院薬・病態生理，³科技団・さがけ21）

S8-5 神経再生におけるニューロン・グリアの相互作用

○濤川 一彦，福嶋 宗久，村上 公一，木山 博資

（大阪市大・院医・機能形態学（解剖学第一））

S8-6 髄鞘形成障害と軸索タンパク質のオーガナイゼーション

○馬場 広子

（東薬大・薬・機能形態）

7月19日 (金)

9:00 - 11:30

B会場

S9 mRNA制御と神経疾患

座長：今泉和則（奈良先端大），塩見春彦（徳島大・ゲノム研究センター）

S9-1 ショウジョウバエ脆弱X遺伝子により仲介される概日リズムの発現

井上 俊介¹，霜田 政美²，西ノ首 いづみ³，塩見 美喜子¹，石田 直理雄^{3,4,5}，○塩見 春彦¹

（¹徳島大・ゲノム機能研究センター，²農業生物資源研，³産総研・生物時計，⁴東工大・生命理工，⁵東北大・学際）

- S9-2** 雄同士の求愛行動を惹起するショウジョウバエのfruitless変異とその分子基盤
○Daisuke Yamamoto
(早稲田大・人間科学部)
- S9-3** リボソームタンパク質L38変異は、マウス神経管形成異常を誘導する
○城石俊彦
(遺伝研・系統生物研究センター・哺乳動物遺伝)
- S9-4** The RNA-binding protein causes aberrant splicing of presenilin-2 pre-mRNA in sporadic Alzheimer's disease
○片山 泰一^{1,3}, 眞部 孝幸^{1,3}, 松崎 伸介^{1,3}, 今泉 和則^{2,3}, 遠山 正彌^{1,3}
(¹阪大・院医・プロセシング機能形態, ²奈良先端科学技術大学院大・細胞構造, ³科学技術振興事業団)
- S9-5** スプライシング障害と神経難病ータウおよびSMNの異常スプライシング
○今泉 和則
(奈良先端大・バイオ・細胞構造)

7月19日 (金)

9:00 - 11:30

C会場

S10 プロテオグリカンによる神経機能調節機構

座長：前田信明 (都神経研), 大平敦彦 (愛知コロニー研)

- S10-1** バーシカンV2・脳リンクプロテイン会合体の中樞神経ランビエ絞輪での発現とその機能
○大橋 俊孝
(岡山大・院医歯・分子医化学)
- S10-2** PTP ζ の情報伝達とプルキンエ細胞の形態形成
○前田 信明¹, 田中 正彦²
(¹都神経研・分子発生生物, ²藤田保健衛生大・総医研)
- S10-3** フォスファカンの脂質ラフトにおけるTAG-1/Lynを介するシグナル伝達
○笠原 浩二, 佐内 豊
(東京都臨床研・生命情報)
- S10-4** Structure and expression pattern of neuroglycan C, a brain-specific transmembrane chondroitin sulfate proteoglycan, in the developing brain
○青野 幸子, 時田 義人, 山内 忍, 周尾 卓也, 大平 敦彦
(愛知コロニー研・周生)

S10-5 視神経軸索誘導に関する特異的ヘパラン硫酸構造

○入江 敦

(都臨床研・生命情報)

7月19日 (金)

9:00-11:30

D会場

S11 神経・内分泌系と免疫系間におけるクロストーク

座長：川島紘一郎 (共立薬大), 大熊康修 (北海道大・薬)

S11-1 迷走神経およびレプチンを介する脳内サイトカインの発現制御

○大熊 康修, 細井 徹, 野村 靖幸

(北大・院薬・薬理)

S11-2 IL-1 β による一次知覚神経機能の制御

○森岡 徳光¹, 井上 敦子², 仲田 義啓²

(¹広島大・院医歯薬学総合・病態探究医科学 (歯科薬理), ²広島大・院医歯薬学総合・薬効解析科学)

S11-3 糖質コルチコイドによるリンパ球分布調節のメカニズム

○永富 良一

(東北大・院医・運動)

S11-4 リンパ球におけるコリン作動系発現と機能調節への関与

○藤井 健志, 川島 紘一郎

(共立薬大・薬理)

S11-5 Th1/Th2バランスからみた炎症性腸疾患マウスモデルに対するニコチンの影響

○村田 有志¹, 伊東 重豪²

(¹青森県立中央病院・消化器内科, ²弘大・第一内科)

ミニシンポジウム

7月17日(水)

9:00 - 11:30

B会場

MS1 細胞形態および運動の制御と神経系形成

座長：福島 伸之 (北大院・医)
五嶋 良郎 (横浜市大・医)
田代 朋子 (青山学院大・理工)

MS1-1 神経細胞の移動を制御するリーリングナルとCdk5キナーゼ

(P02-E-68) ○大島 登志男¹, 小川 正晴², 御子柴 克彦¹
(¹理研・脳総研・発生神経, ²同・細胞培養)

MS1-2 CRMP-2 and axon formation

(P02-F-32) ○深田 優子, 貝淵 弘三
(名大・院医・薬理)

MS1-3 発生期大脳皮質における移動神経細胞のダイナミックな形態変化

(P02-F-29) ○田畑 秀典¹, 仲嶋 一範^{1,2}
(¹慈恵医大・DNA医学研究所・分子神経生物学, ²PRESTO, JST)

MS1-4 神経系の細胞形態および運動性とリゾリン脂質シグナル

(P02-E-47) ○福島 伸之
(北大・院医・分子医化学)

MS1-5 セマフォリンと皮質神経回路形成

(P02-E-46) ○五嶋 良郎, 佐々木 幸生
(横浜市大・医・薬理)

MS1-6 The roles of WAVES in filopodia formation at NG108 growth cones

(P02-E-45) ○Shigeaki Miyamoto¹, Motohiro Nozumi¹, Hiroyuki Nakagawa¹, Hiroaki Miki², Tadaomi Takenawa²
(¹九州工大・情報工・生化システム, ²東京大・医科研・腫瘍分子生物)

MS1-7 Aβ 25-35誘導性の突起形成不全に対するwithanolide A, withanoside IV, withanoside VIの神経突

(P02-F-27) 起伸展効果
○久保山 友晴, 東田 千尋, 小松 かつ子
(富山医薬大・和漢薬研・薬効解析センター)

MS1-8 Expression of tau isoforms containing exon2 and 3 during synaptogenesis *in vivo* and *in vitro*

(P02-F-28) ○北村 主税, 若山 勇太, 舩野 正樹, 野呂 美幸, 田代 朋子
(青山学院大学理工学部化学科)

7月17日(水)

9:00 - 10:15

C会場

MS2 神経細胞死

座長：和中 明生 (奈良県立医大)
木山 博資 (大阪市大院・医)

MS2-1 Transcription factors induced in cultured cortical neurons by beta amyloid peptide

(P01-F-30) ○内田 洋子, 五味 不二也
(都老人研・神経病理)

MS2-2 神経細胞の生存に関連する新規遺伝子 *alivin 1*

(P01-F-32) ○小野 富男^{1,2}, 鈴木 直子¹, 吉川 欣亮², 米川 博通², 川島 誠一¹
(¹都臨床研・分子制御, ²都臨床研・実験動物)

- MS2-3 A β 蛋白とNACによる活性酸素の産生とNF- κ Bの活性化：神経細胞とアストロサイトの反応性の相違**
(P01-F-4) ○田中 静吾, 竹橋 正則, 上田 國寛
(京大・化研・生体反応設計3)
- MS2-4 Niemann-Pick C1 deficiency reduces the levels of cholesterol in detergent insoluble, low-density membrane domains leading to activation of MAP kinase pathway and increased phosphorylation of tau**
(P01-F-2) ○澤村 直哉^{1,2}, キョウ 建生¹, Ta-Yuan Chang³, 柳澤 勝彦¹, 道川 誠¹
(¹長寿研・痴呆疾患研究部, ²日本学術振興会・科学技術特別研究員, ³Dept. Biochemistry, Dartmouth Med. Sch.)

7月17日(水)

10:15 - 11:30

C会場

MS3 神経栄養因子

座長：高松 研 (東邦大・医)
古川 昭栄 (岐阜薬大・分子生物)

- MS3-1 リルゾールによるラット海馬における脳由来神経栄養因子の合成機構**
(P02-F-14) ○仙波 りつ子, 加藤 兼房
(愛知県コロニー・研究所)
- MS3-2 bFGFによる培養中枢ニューロンからのMAPK活性化依存的な興奮性神経伝達物質の放出機構。**
(P02-F-15) ○沼川 忠広^{1,2,3}, 横幕 大作^{1,2}, 清末 和之², 安達 直樹¹, 松本 知也^{1,2}, 沼川 裕美子^{1,2}, 田口 隆久², 畠 中 寛¹, 山田 雅司¹
(¹大阪大学蛋白質研究所生合成, ²産業技術総合研究所関西センター, ³日本学術振興会特別研究員)
- MS3-3 Change of the expression of glial cell line-derived neurotrophic factor (GDNF) in the rat sciatic nerve after its transection**
(P02-F-16) ○山田 喜久¹, 新田 淳美², 清水 克時¹, 古川 昭栄²
(¹岐大・医・整外, ²岐薬大・分生)
- MS3-4 E2F1に誘導されるニューロンの細胞死に対するNecdinの抑制作用**
(P02-F-2) ○高崎 理紗, 植月 太一, 谷浦 秀雄, 吉川 和明
(阪大・蛋白研・機能制御)

7月18日(木)

9:00 - 11:30

B会場

MS4 神経変性疾患

座長：柳澤 勝彦 (国立長寿研)
遠山 正彌 (大阪大院・医)

- MS4-1 尾懸垂試験を用いたMPTP誘発パーキンソン病モデルマウスの行動評価**
(P01-F-20) ○森 厚詞^{1,2}, 中井 正三¹, 光本 泰秀¹, 森泉 哲次²
(¹大塚製薬(株)探索第二研究所, ²信州大・医・第二解剖)
- MS4-2 Role of oxidative protein in neuronal cell death of Parkinson's disease.**
(P01-F-16) ○丸山和佳子¹, 社本 (永井) 雅代¹, 田中雅嗣², 直井信³
(¹長寿研 老化機構生化学・代謝, ²岐阜国際バイオ 遺伝子治療, ³応用生化学 脳科学)
- MS4-3 Mechanism of neuroprotection by propargylamines**
(P01-F-17) ○直井信¹, 丸山和佳子², 赤尾幸博³, 伊紅³, 辻本賀英⁴
(¹応用生化学 脳科学, ²長寿研 基礎老化 生化学・代謝, ³岐阜国際バイオ 遺伝子診断, ⁴大阪大学 医学部 未来医療開発専攻ポストゲノム疾患解析学細胞死制御分野)

- MS4-4** Activation of CREB-dependent transcription system suppresses expanded polyglutamine induced cytotoxicity.
(P01-F-28) ○Mitsuteru Shimohata, Takayoshi Shimohata, Shuichi Igarashi, Satoshi Naruse, Shoji Tsuji
(新大・脳研・神内)
- MS4-5** Functional analysis of the putative transcription factors for the human Huntington's disease gene
(P01-F-19) ○田中 一則^{1,2}, 将口 (宮田) 淳子², 宮本 なつき¹, 池田 穰衛^{1,2}
(¹基礎的研究発展推進事業、科学技術振興事業団, ²東海大学総合医学研究所 分子神経科学部門)
- MS4-6** ALS2 gene accounting for amyotrophic lateral sclerosis 2 encodes multi guanine exchanging factor (GEF) domains acting for small GTPases
(P01-F-18) ○池田 穰衛, 大友 麻子, 国田 竜太, 吉田 英理, 秦野 伸二, 大須賀 等
(SORST/ 科技団、東海大学総合医学研究所 分子神経科学部門)
- MS4-7** ニコチンは注意欠陥多動性障害モデルにおける短期記憶障害を改善する
(P01-E-60) ○富樫 広子¹, 上野 健一¹, 松本 真知子¹, 齋藤 秀哉², 吉岡 充弘¹
(¹北海道大学大学院医学研究科機能薬理, ²日本赤十字北海道看護大・基礎科学)
- MS4-8** 秒ネズミ海馬における一過性前脳虚血に伴う Forkhead transcription factor の活性化反応
(P01-F-39) ○福永 浩司¹, 河野 隆幸^{1,2}, 生塩 之敬², 宮本 英七¹
(¹熊本大・医学部・第一薬理, ²熊本大・医学部・脳神経外科)

7月18日(木)

9:00 - 10:15

C会場

MS5 神経伝達物質

座長：熊倉 鴻之助 (上智大・生命科学研)
野村 正彦 (埼玉医大)

- MS5-1** ラット大脳皮質においてD-セリンによって発現調節される遺伝子の解析
(P01-E-33) ○山本 直樹¹, 土田 英人¹, 海野 麻未¹, 嶋津 奈^{1,2}, 桜井 新一郎^{1,2}, 谷口 豪^{1,2}, 西川 徹^{1,2}
(¹国立精・神セ・神経研・疾病3部, ²医歯大院・医歯総合・精神行動)
- MS5-2** Biochemical properties and partial purification of pChAT, a novel splice variant of choline acetyltransferase.
(P01-E-34) ○Jean-Pierre Bellier, 相見 良成, 朴 真紗美, 久野 正, Petra Minnasch, 松尾 明典, 安原 治, 遠山 育夫, 木村 宏
(滋賀医大・分子神経センター)
- MS5-3** 連続経頭蓋磁気刺激によるラットの抗不安作用の検討: 高架式十字迷路法を用いた行動ならびに神経化学的变化
(P01-E-42) ○菅野 学¹, 松本 真知子², 富樫 広子², 吉岡 充弘², 眞野 行生¹
(¹北大・院医・リハビリテーション医学, ²北大・院医・機能薬理学)
- MS5-4** Stimulation of phospholipase A₂ activity by anandamide in PC12 cells
(P01-E-41) ○染谷 晃好, 堀江 俊治, 村山 俊彦
(千葉大・院薬・薬効薬理)

7月18日(木)

10:15 - 11:30

C会場

MS6 受容体シグナリング

座長：村山 俊彦 (千葉大・薬)
山脇 成人 (広島大・医)

- MS6-1** Neurospheres isolated from hippocampus of adult mice
(P01-E-2) ○北山 友也, 米山 雅紀, 米田 幸雄
(金沢大・薬・薬物)

MS6-2 ヒトアストロサイトにおける gabapentin の輸送機構と膜電位依存性 Ca^{2+} channel との相互作用

(P01-E-1) ○藤田 卓也¹, 山中 理¹, 四宮 賢治¹, 和田 美由紀¹, 福井 美由紀¹, 山本 昌¹, 岡 美智子²
(¹京都薬大・薬剤, ²日本新薬)

MS6-3 M5 muscarinic receptors mediate the vasodilator actions of acetylcholine in cerebral arterioles and arteries.

(P01-E-4) ○Yamada Masahisa¹, Noguchi Takanori², Lamping G Kathryn³, Duttaroy Alokesh⁴, Zhang Weilie⁴, Faraci M Frank³, Ogawa Masaharu¹, Wess Jüen⁴

(¹Lab. Cell Culture Develop., RIKEN Brain Sci. Inst., ²Lab. Behavioral Genetics, RIKEN Brain Sci. Inst., ³Dept. Internal Med. Pharmacol., Cardiovascular Center, Univ. Iowa, ⁴Lab. Bioorganic Chem., Natl. Inst. Diabetes and Digestive and Kidney Diseases)

MS6-4 ERAD (小胞体関連分解) 関連分子Hrd1pのヒトホモログの単離・同定とその機能・誘導機構の解析

(P02-E-26) ○金子 雅幸, 石黒 正太郎, 新沼 芳文, 野村 靖幸
(北大・院薬・薬理)

ポスター

受容体、輸送体

- P01-E-1 ヒトアストロサイトにおける gabapentin の輸送機構と膜電位依存性 Ca^{2+} channel との相互作用 (MS6-2)**
 ○藤田 卓也¹, 山中 理¹, 四宮 賢治¹, 和田 美由紀¹, 福井 美由紀¹, 山本 昌¹, 岡 美智子²
 (1京都薬大・薬剤, 2日本新薬)
- P01-E-2 Neurospheres isolated from hippocampus of adult mice (MS6-1)**
 ○北山 友也, 米山 雅紀, 米田 幸雄
 (金沢大・薬・薬物)
- P01-E-3 Functional expression of GABA_B receptors in cultured calvarial osteoblasts**
 ○藤森 さゆ美, 檜井 栄一, 米田 幸雄
 (金沢大・薬・薬物)
- P01-E-4 M5 muscarinic receptors mediate the vasodilator actions of acetylcholine in cerebral arterioles and arteries. (MS6-3)**
 ○Yamada Masahisa¹, Noguchi Takanori², Lamping G Kathryn³, Duttaroy Alokesh⁴, Zhang Weilie⁴, Faraci M Frank³, Ogawa Masaharu¹, Wess Jugen⁴
 (1Lab. Cell Culture Development, RIKEN Brain Sci. Inst., 2Lab. Behavioral Genetics, RIKEN Brain Sci. Inst., 3Dept. Internal Med. Pharmacol., Cardiovascular Ctr., Univ. Iowa, 4Lab. Bioorganic Chem., Natl. Inst. Diabetes and Digestive and Kidney Diseases)
- P01-E-5 Expression pattern of oncostatin M receptor β in the murine central nervous system**
 ○田村 志宣¹, 森川 吉博¹, 田中 稔², 宮嶋 篤², 仙波 恵美子¹
 (1和医大・二解, 2東大・分生研・細胞合成)
- P01-E-6 PC12細胞におけるATPによる細胞内 Ca^{2+} 増加反応について -ミトコンドリアの関与- 丸山 浩二, 太田 利男, ○伊藤 茂男**
 (北大・院獣医・薬理)
- P01-E-7 PSD-95及びMALS-2のNR1/NR2B サブタイプ受容体チャンネル活性に及ぼす効果の比較**
 ○岩本 敬^{1,2}, 山田 康枝¹, 小田 隆将¹, 渡辺 義文², 祖父江 憲治³, 乾 誠¹
 (1山口大・医・薬理, 2山口大・医・神経精神, 3阪大・院医・神経生化)
- P01-E-8 Accumulation of [³H]L-serine in rat brain synaptosomal fractions**
 ○宝田 剛志, Vladimir J. Balcar, 馬場 勝弘, 高本 晃子, 米田 幸雄
 (金沢大・薬・薬物)
- P01-E-9 Functional heteromeric NMDA receptors expressed in cultured rat calvarial osteoblasts**
 ○米田 幸雄, 檜井 栄一, 藤森 さゆ美
 (金沢大・薬・薬物)

P01-E-10 Functional metabotropic glutamate receptor group III subtype expressed in primary cultures of rat calvarial osteoblasts

○井上 真希, 檜井 栄一, 藤森 さゆ美, 竹森 章浩, 米田 幸雄
(金沢大・薬・薬物)

P01-E-11 嗅球系球体における endophilin-3 によるドーパミン D2 受容体エンドサイトーシスの調節

○杉浦 弘子¹, 岩田 健², 松岡 勝人³, 車田 正男³, 山内 卓⁴, 市川 真澄⁵, 芳賀 達也², 山形 要人¹
(¹都神経研・神経薬理, ²学習院大・理・生命分子科学研, ³新潟大・院医歯学総合・神経生物解剖, ⁴徳大・薬・生化, ⁵東京都神経研・発生形態)

P01-E-12 Expression of dominant negative isoform of rat norepinephrine transporter

○北山 滋雄¹, 熊谷 圭², 森田 克也², 土肥 敏博²
(岡山大・院医歯学総合・歯科薬理, ²広島大・院医歯学総合・歯科薬理)

P01-E-13 Effects of atypical antipsychotics on 5-HT_{1A} receptor-mediated [³⁵S]GTP γ S binding in native brain membranes

○小田垣 雄二, 山内 俊雄
(埼玉医大・精神)

P01-E-14 デルフィリン：小脳プルキンエ細胞シナプスでグルタミン酸受容体 δ 2 と共存し結合する新規PDZタンパク質

○川本 進¹, 宮城 洋平², 山下 哲司¹, 深谷 昌弘³, 園田 智子¹, 奥野 利明⁴, 山田 和之³, 渡辺 雅彦³, 奥田 研爾¹, 三品 昌美⁴
(¹横浜市大・医・細菌, ²神奈川がんセンター, ³北大・院医・生体構造解析, ⁴東大・院医・分子神経生物/CREST)

P01-E-15 Molecular cloning of human p40BBP and its distribution in human brain

○濱 登希子, 本多 友希子
(三菱化学生命研)

P01-E-16 中性アミノ酸トランスポーターASCT2の培養アストロサイトと神経細胞における発現

○古屋 茂樹¹, 篠田 陽子¹, 遠藤 昌吾², Thomas Launey², 吉田 一之^{1,3}, 渡辺 雅彦⁴, 三苦 純也⁵, 平林 義雄¹
(¹理研・脳総研セ・神経回路メカニズム, ²理研・脳総研セ・記憶学習機構, ³東京農工大・連合農学研究科, ⁴北大・院医・生体構造解析, ⁵Glycobiology Program, The Burnham Institute)

P01-E-17 パーキンソン病モデル線条体グリア細胞におけるドーパミントランスポーターの発現誘導

○浅沼 幹人, 宮崎 育子, 東 洋一郎, 小川 紀雄
(岡山大・院医歯・神経情報学)

P01-E-18 Differential regulation of aquaporin 5 and 9 expression in astrocytes by protein kinase A

○祖父江 和哉¹, 山本 直樹², 稲垣雅昭¹, 伊藤弘晃¹, 藤田義人¹, 勝屋 弘忠¹, 浅井 清文²
(¹名市大・院医・危機管理医学, ²名市大・院医・分子神経生物学)

P01-E-19 アストロサイトにおけるドパミントランスポーターを介したドパミン取込み機構

○宮崎 育子, 浅沼 幹人, 東 洋一郎, 小川 紀雄
(岡山大・院医歯・神経情報学)

P01-E-20 イノシトール3リン酸受容体の開口機構の解析

○内田 敬子^{1,2,3}, 宮内 浩¹, 古市 貞一⁴, 道川 貴章^{1,5}, 御子柴 克彦^{1,2,5}
(¹東大・医科研・基礎医・脳神経発生・分化, ²理研・BSI・発生神経生物, ³慶應大・医・小児科, ⁴理研・BSI・分子神経形成, ⁵科技団・カルシウム振動プロジェクト)

P01-E-21 カルシウムによるIP₃受容体の構造変化

○濱田 耕造¹, 御子柴 克彦^{1,2,3}
(¹理研・脳セ・発生神経, ²東大・医科研・脳神経, ³科技団)

P01-E-22 Agonist-specific translocation of μ -opioid receptor and protein kinase C in living cells

○吉田 明¹, 井上 誠¹, 斉藤 尚亮², 植田 弘師¹
(¹長崎大・薬・分子薬理, ²神戸大・バイオシグナル研)

P01-E-23 ヒト苦味受容に関わるT2R遺伝子の組織発現と遺伝子多型の同定

○植田 高史¹, 鶴川 真也¹, 石田 雄介², 柴田 泰宏¹, 村上 信五², 島田 昌一¹
(¹名古屋市立大・院医・分子形態, ²名古屋市立大・医・耳鼻咽喉)

P01-E-24 Developmental expression pattern of TROY in mouse central nervous system

○久岡 朋子¹, 森川 吉博¹, 北村 俊雄², 仙波 恵美子¹
(¹和医大・二解, ²東大・医科研・先端医療・細胞療法)

P01-E-25 Identification and characterization of molecules that interact with melanin-concentrating hormone (MCH) receptor 1

○斎藤 祐見子, 川村 勇樹, 手塚 満恵, 李 月, 丸山 敬
(埼玉医大・薬理)

P01-E-26 網膜内グループIIIグルタメート受容体のADPリボシールシクラーゼへのカップリング

○東田 陽博¹, 陳 小良¹, 張 家生¹, 野田 百美², 星 直人¹, 橋井 美奈子¹, 横山 茂¹
(¹金大・院医・脳細胞遺伝子, ²九大・院薬・病態生理)

P01-E-27 Methamphetamine反復投与による移所移動活性増加と海馬 α 2A -アドレナリン受容体との関連について

○西尾 昌洋, 諫田 泰成, 水野 勝重, 渡辺 康裕
(防衛医大・薬理)

P01-E-28 Regulation of P2X receptor by ubiquitin C-terminal hydrolase L1

○野田 百美¹, 金掘 佳子¹, 寫田 亜希¹, 青木 俊介^{2,4}, 王 玉来², 小坂 仁^{2,3}, 和田 圭司²
(¹九大・院薬・病態生理, ²国立精・神・セ・神経研・4部, ³科技団・さきがけ21, ⁴学振)

P01-E-29 Protein kinase C regulates aquaporin expression in astrocytes

○山本 直樹¹, 祖父江 和哉², 河合 洋子³, 三浦 裕¹, 勝屋 弘忠², 浅井 清文¹
(¹名市大・院医・分子神経生物学, ²名市大・院医・危機管理医学, ³名市大・看護)

P01-E-30 C6 グリオーマ細胞における cAMP による Na⁺ 依存性 vitamin C トランスポーターの発現制御機構

○山中 理¹, 森 宏平¹, 山本 昌¹, 岡 美智子², 藤田 卓也¹
(¹京都薬大・薬剤, ²日本新薬)

P01-E-31 PC-12 細胞にける norepinephrine transporter を介した MPP⁺ の輸送機構

○四宮 賢治, 山中 理, 山本 昌, 藤田 卓也
(京都薬大・薬剤)

P01-E-32 GFPを利用したIP3受容体の細胞内動態の解析

○立石 陽子¹, 服部 光治¹, 中山 智博^{1,2}, 中村 健⁴, 坂内 博子^{1,3}, 岩井 美和子¹, 内田 敬子^{1,5}, 道川 貴章^{1,4}, 井上 貴文^{1,4}, 御子柴 克彦^{1,3,4}
(¹東大・医科研・脳神経発生分化, ²東京女子医大・小児科, ³理研・脳研・発生神経, ⁴科技団・カルシウム振動プロジェクト, ⁵慶應大・医学部・小児科)

神経伝達物質、修飾物質

P01-E-33 ラット大脳皮質においてD-セリンによって発現調節される遺伝子の解析

(MS5-1) ○山本 直樹¹, 土田 英人¹, 海野 麻未¹, 嶋津 奈^{1,2}, 桜井 新一郎^{1,2}, 谷口 豪^{1,2}, 西川 徹^{1,2}
(¹国立精・神セ・神経研・疾病3部, ²医歯大院・医歯総合・精神行動)

P01-E-34 Biochemical properties and partial purification of pChAT, a novel splice variant of choline acetyltransferase.

(MS5-2) ○Jean-Pierre Bellier, 相見 良成, 朴 真紗美, 久野 正, Petra Minnasch, 松尾 明典, 安原 治, 遠山 育夫, 木村 宏
(滋賀医大・分子神経センター)

P01-E-35 メタンフェタミンによるドーパミン放出に対するトリヘキシフェニールの抑制効果：依存治療に向けて

○霜里 和朗¹, 渡辺 悟¹, 北山 滋雄²
(¹川崎医大・薬理, ²岡山大院・医歯総合・歯科薬理)

P01-E-36 Serotonergic signaling inhibits hyperalgesia induced by spinal cord damage

○堀内 秀樹, 尾形 直則, 森野 忠夫, 山本 晴康
(愛媛大・整形外科)

P01-E-37 Nicotine inhibits ERE binding via MAPK cascade in the hippocampus.

○鬼頭 昭三, 新郷 S. 明子
(兵庫大・健康科学部)

- P01-E-38 皮質線条体神経経路を介したGABAならびにDA神経の機能的相互作用**
 ○松本 真知子¹, 富樫 広子¹, 菅野 学², 大橋 聡¹, 上野 健一¹, 吉岡 充弘¹
 (1北大・院医・機能薬理, 2北大・院医・リハビリテーション医学)
- P01-E-39 Effect of different species of aluminum on glutamate-induced overexpression of PLC- δ 1 protein level in primary cultured rat cortical neurons**
 ○藤本 貞毅¹, 西田 健太郎¹, 下濱 俊², 長澤 一樹¹
 (1京都薬大・衛生化学, 2京大・院医・神経内科)
- P01-E-40 5HT-1 and 5HT-3 serotonin receptor subtypes regulate pain sensation in the spinal cord level: Normal and hyperalgesic animal model**
 ○尾形 直則, 堀内 秀樹, 森野 忠夫, 山本 晴康
 (愛媛大・医・整形)
- P01-E-41 Stimulation of phospholipase A₂ activity by anandamide in PC12 cells (MS5-4)**
 ○染谷 晃好, 堀江 俊治, 村山 俊彦
 (千葉大・院薬・薬効薬理)
- P01-E-42 連続経頭蓋磁気刺激によるラットの抗不安作用の検討: 高架式十字迷路法を用いた行動ならびに神経化学的变化 (MS5-3)**
 ○菅野 学¹, 松本 真知子², 富樫 広子², 吉岡 充弘², 眞野 行生¹
 (1北大・院医・リハビリテーション医学, 2北大・院医・機能薬理学)
- P01-E-43 The mutation of two amino acid residues of tyrosine hydroxylase dramatically enhances the ability of neuroendocrine AtT-20 cells to synthesize L-DOPA**
 ○中島 昭¹, 金子 葉子¹, 森 啓至¹, 藤原 健太郎², 継 敏光¹, 鈴木 崇弘³, 永津 俊治³, 太田 明¹
 (1藤田保健衛生大・医・第1生理, 2藤田保健衛生大・医・内科, 3藤田保健衛生大・総医研・分子遺伝)
- P01-E-44 The expression of GTP cyclohydrolase I mRNA in murine locus coeruleus is enhanced by peripheral administration of lipopolysaccharide**
 ○金子 葉子¹, 森 啓至¹, 中島 昭¹, 永津 郁子², 太田 明¹
 (1藤田保健衛生大・医・生理1, 2藤田保健衛生大・医・解剖2)
- P01-E-45 神経伝達物質放出へのカルモジュリン阻害剤の影響**
 ○安藤 公祐¹, 工藤 佳久¹, 高橋 正身^{2,3}
 (1東京薬大・生命科学研究科, 2北里大・医, 3クレスト)
- P01-E-46 Effect of peripheral lipopolysaccharide administration on dopamine content in murine anterior olfactory nucleus**
 ○森 啓至¹, 金子 葉子¹, 中島 昭¹, 藤原 健太郎², 永津 郁子³, 太田 明¹
 (1藤田保健衛生大・医・生理, 2藤田保健衛生大学病院・内科, 3藤田保健衛生大・医)

P01-E-47 坐骨神経結紮ラット後根神経節におけるサブスタンスPとそのプロセッシング酵素レベルの変化

○中島 良子¹, 土田 健¹, 知久馬 敏幸², 加藤 武¹
(¹横浜市大・院総合理学, ²昭和薬大・衛生化)

P01-E-48 ビスフェノールAの胎仔期・乳仔期曝露におけるモノアミン神経系への影響

○廣井 豊子, 岡田 和嗣, 米田 卓史, 舩江 良彦
(大阪市大・院医・生体機能解析)

疾患モデル

P01-E-49 PACAP遺伝子欠損マウスにおける脳セロトニン神経系の機能異常

○新谷 紀人¹, 田中 一裕¹, 川口 ちひろ¹, 橋本 均¹, 松田 敏夫², 馬場 明道^{1,3}
(¹阪大・院薬・神経薬理, ²阪大・院薬・複合薬物, ³阪大・院医・分子医薬)

P01-E-50 マウス皮質ドーパミン遊離に関わるセロトニン_{1A}受容体機能の隔離飼育による選択的低下

○松田 敏夫¹, 吾郷 由希夫¹, 阪上 匡紀², 小山 豊¹, 馬場 明道²
(¹阪大・院薬・複合薬物, ²阪大・院薬・神経薬理)

神経内分泌

P01-E-51 Estrogen enhances depolarization-induced glutamate release through activation of phosphatidylinositol 3-kinase in cultured hippocampal neurons

○横幕 大作^{1,2}, 沼川 忠広^{1,2,3}, 沼川 裕美子¹, 安達 直樹¹, 松本 知也^{1,2}, 山岸 寛^{1,3}, 西尾 チカ¹, 田口 隆久², 畠中 寛¹
(¹阪大・蛋白研・生合成, ²産業技術総合研究所・人間系・ニューロニクス, ³日本学術振興会)

P01-E-52 脳幹部がレプチンの直接の脳内作用部位である可能性について

○川岸 亨, 大熊 康修, 細井 徹, 田中 淳, 野村 靖幸
(北大・院薬・薬理)

イオンチャンネル、興奮膜

P01-E-53 T型カルシウム電流のNeuropeptide Y による増強

○岡田 誠剛, 長谷川 歩未, 樋口 宗史
(新潟大・院医・薬理)

P01-E-54 Acid-sensing ion channels ASIC1a and ASIC3 are leading acid sensors expressed in polymodal nociceptors

○鵜川 真也, 植田 高史, 島田 昌一
(名市大・医・2解剖)

血液脳関門

P01-E-55 Transport mechanism of aluminum citrate across the blood-brain barrier

○長澤 一樹¹, 伊藤 慎悟¹, 玉井 郁巳², 辻 彰³, 藤本 貞毅¹
(¹京都薬大・衛生化学, ²東京理科大・薬, ³金沢大・薬)

自立神経系

P01-E-56 副腎髄質細胞の電位依存性Caチャンネル活性に対する自己/傍分泌性制御

○太田 利男, 甲斐 隆彦, 伊藤 茂男
(北大・院獣医・薬理)

神経免疫

P01-E-57 中枢性および末梢性インターロイキン-1 β 投与による前頭前野のヒドロキシルラジカルおよび一酸化窒素代謝産物の動態の変化

○石塚 雄太¹, 石田 康¹, 河南 洋²
(¹宮崎医科大・精神医学, ²宮崎医科大・第一生理学)

中毒症

P01-E-58 Disturbance of thyroid hormone-dependent synapse formation in cultured rat cortical neurons by environmental chemicals

○細田 律子^{1,3}, 根岸・加藤 みどり¹, 中山 憲司², 村本 和世¹, 川原 正博^{1,3}, 黒田 洋一郎^{1,3}
(¹都神経研・分子神経生物, ²道衛研・臨床病理, ³科技団)

精神疾患

P01-E-59 定型・非定型抗精神病薬慢性処置による神経細胞障害性について

○鶴飼 渉, 小澤 寛樹, 長栄 洋, 山田 真吾, 酒井 理恵, 紺野 雅人, 館農 勝, 山本 恵, 斉藤 利和
(札幌医大・医・神経精神医学)

P01-E-60 ニコチンは注意欠陥多動性障害モデルにおける短期記憶障害を改善する

(MS4-7) ○富樫 広子¹, 上野 健一¹, 松本 真知子¹, 齋藤 秀哉², 吉岡 充弘¹
(¹北大・院医・機能薬理, ²日本赤十字北海道看護大・基礎科学)

P01-E-61 Effect of valproate on agonist-induced intracellular calcium mobilization in human platelets

○秋本 達之, 鈴木 克治, 佐々木 由紀, 久住 一郎, 小山 司
(北大・院医・精神)

てんかん

P01-E-62 ラットの発達脳での γ -ブチロラクトン誘発欠神てんかんにおけるGABA_B受容体とGHB受容体の分離的役割

○田中 聖之¹, 滝沢 昇¹, 馬渡 一浩², 加藤 聖¹

(¹金沢大・院医・脳情報分子, ²金沢大・医・保健)

脳血流、代謝

P01-E-63 Measurement of metabolic rates for glutamate and glutamine in occipito-parietal lobes of human brain by non-invasive 2T ¹³C-magnetic resonance spectroscopy (¹³C-MRS)

○金松 知幸¹, 大槻 泰介², 岡本 和也³, 渡邊 英宏³, 梅田 匡朗³, 塚田 裕三¹

(¹創価大学・生命科学研究所, ²国立精・神センター武蔵病院、脳神経外科, ³東芝医用機器・システム開発センター)

睡眠、生体リズム

P01-E-64 プロスタグランジンD2による睡眠誘発機構は断眠後のノンレム睡眠のホメオスタシスに関与する

○裏出 良博^{1,2}, 江口 直美^{1,2}, 桑幡 裕子^{1,2}, Wei-Min Qu¹, Zhi-Li Huang¹, 早石 修¹

(¹大阪バイオサイエンス研・分子行動生物, ²科技団・CREST・脳を知る)

P01-E-65 Prostaglandin E₂ activates histaminergic system via EP₄ receptor to induce wakefulness in rats

○黄 志力¹, 望月 貴年¹, 岡田 哲也^{1,2}, 曲 衛敏¹, 大和谷 厚³, 裏出 良博^{1,4}, 早石 修¹

(¹大阪バイオサイエンス・分子行動生物, ²阪大・健康体育・健康医学第三, ³阪大・医・保健・医用物理, ⁴科技団・CREST)

P01-E-66 中枢／末梢時計におけるBMAL1の周期的核細胞質間移行

○田丸輝也¹, 磯島康史², 高松 研¹

(¹東邦大・医・第2生理, ²阪大・蛋白研・代謝)

痴呆、アルツハイマー病

- P01-F-1** Effect of MK-801, an NMDA receptor antagonist, on BACE and EP24.15 in rat brain
 ○Md. Mahiuddin Ahmed¹, 山本 賢¹, 知久馬 敏幸², 加藤 武¹
 (1横浜市大・院総合理, 2昭和薬大・衛生化)
- P01-F-2** Niemann-Pick C1 deficiency reduces the levels of cholesterol in detergent insoluble, (MS2-4) low-density membrane domains leading to activation of MAP kinase pathway and increased phosphorylation of tau
 ○澤村 直哉^{1,2}, キョウ 建生¹, Ta-Yuan Chang³, 柳澤 勝彦¹, 道川 誠¹
 (1長寿研・痴呆疾患, 2日本学術振興会・科学技術特別研究員, 3Dept. Biochemistry. Dartmouth Med. Sch.)
- P01-F-3** p25 subunit, an activator of CDK5, induces intensive phosphorylation on Ser202/Thr205 residues of human tau40 *in vitro*.
 ○橋口 美津子¹, 斎藤 太郎², 久永 真市², 橋口 利雄¹
 (1東京医大・医・第一生理, 2都立大・院理・生物)
- P01-F-4** Aβ蛋白とNACによる活性酸素の産生とNF-κBの活性化：神経細胞とアストロサイトの (MS2-3) 反応性の相違
 ○田中 静吾, 竹橋 正則, 上田 國寛
 (京大・化研・生体反応設計3)
- P01-F-5** CD36, a member of class B scavenger receptor family, serve as β-amyloid receptor and its expression on primary rat microglia stimulated with interleukine-4
 ○川原 浩一, 鍛冶園 誠, 中山 仁
 (熊大・薬・生体機能化学)
- P01-F-6** A novel function of monomeric amyloid β-protein serving as an antioxidant molecule against metal-Induced oxidative damage
 ○ゾウ クン, キョウ 建生, 柳澤 勝彦, 道川 誠
 (長寿研・痴呆疾患)
- P01-F-7** Isoform-dependent lipid release from astrocytes prepared from human apolipoprotein E3- and E4-knock-in mice
 ○キョウ 建生¹, 小林 まり子², 林 秀樹¹, ゾウ クン¹, 澤村 直哉¹, 藤田 忍², 柳澤 勝彦¹, 道川 誠¹
 (1長寿研・痴呆疾患, 2三菱生命科学研究)
- P01-F-8** small GTPase Rab1Aの過剰発現によるAβ産生の促進
 ○白石 博久¹, 川村 勇樹², 鈴木 亮¹, 蔡 曉蕊¹, 柳澤 勝彦¹, 駒野 宏人¹
 (1長寿研・痴呆疾患, 2埼玉医大・薬理)
- P01-F-9** アルツハイマー病におけるグリセルアルデヒド由来終末糖化産物 (AGE-2) の意義
 ○竹内 正義¹, 佐々木 信幸², 渡井 孝幸¹, 菊地 誠志³, 山岸 昌一⁴, 牧田 善二⁴, 亀田 幸彦¹
 (1北陸大・薬・生化, 2札幌医大・医・神経精神, 3北大・医・神経内科, 4久留米大・医・内分泌代謝)

P01-F-10 PC12D細胞に導入した変異プレセリンは細胞内輸送に影響する

○亀谷 富由樹¹, 久米 秀明¹, 森 啓²

(¹都精神研・分子神経生物, ²大阪市大・医・老年医学)

P01-F-11 Herp によるA β 産生増加機構の解析

○蔡曉蕊¹, 小亀浩市², 白石博久¹, 鈴木亮¹, 宮田敏行², 柳澤勝彦¹, 駒野宏人¹

(¹長寿研・痴呆疾患, ²国立循環器病セ・研)

P01-F-12 Immunohistochemical study of ubiquitin and deposition of β -Amyloid protein in old dog brain

○伊藤 龍生, 佐藤 隆夫, 林 義之, 橋本 重夫

(近大・医・病理二)

P01-F-13 The preventive effect of propentofylline on the β -amyloid-induced apoptosis in rat hippocampal neurons

○郡山 恵樹¹, 山崎 眞津美¹, 千葉 賢三¹, 加藤 聖², 毛利 哲郎¹

(¹北陸大・薬・生物活性, ²金沢大院・医・脳情報分子学)

P01-F-14 アルツハイマーモデルマウスの空間学習障害とシナプス減少に対する統命湯の治療的作用

○田村 隆幸, 東田 千尋, 小松 かつ子

(富山医薬大・和漢薬研・薬効解析センター)

P01-F-15 HSP90によるミクログリアの活性化およびアミロイド β (1-42)の取込促進

○柿村 順一¹, 北村 佳久¹, 高田 和幸¹, 下濱 俊², 谷口 隆之¹

(¹京都薬大・病態生理, ²京大・医・神経内科)

変性疾患

P01-F-16 Role of oxidative protein in neuronal cell death of Parkinson's disease.

(MS4-2) ○丸山和佳子¹, 社本 (永井) 雅代¹, 田中雅嗣², 直井信³

(¹長寿研・老化機構・生化学・代謝, ²岐阜国際バイオ 遺伝子治療, ³応用生化研・脳科学)

P01-F-17 Mechanism of neuroprotection by propargylamines

(MS4-3) ○直井信¹, 丸山和佳子², 赤尾幸博³, 伊紅³, 辻本賀英⁴

(¹応用生化研・脳科学, ²長寿研・基礎老化・生化学・代謝, ³岐阜国際バイオ 遺伝子診断, ⁴阪大・医・細胞死制御)

P01-F-18 ALS2 gene accounting for amyotrophic lateral sclerosis 2 encodes multi guanine exchanging factor (GEF) domains acting for small GTPases

(MS4-6) ○池田 穰衛, 大友 麻子, 国田 竜太, 吉田 英理, 秦野 伸二, 大須賀 等

(SORST/ 科技団、東海大・総合医学研・分子神経科学)

P01-F-19 Functional analysis of the putative transcription factors for the human Huntington's (MS4-5) disease gene

○田中 一則^{1,2}, 将口 (宮田) 淳子², 宮本 なつき¹, 池田 穰衛^{1,2}

(¹基礎的研究発展推進事業、科技団, ²東海大・総合医学研・分子神経科学)

P01-F-20 尾懸垂試験を用いたMPTP誘発パーキンソン病モデルマウスの行動評価

(MS4-1) ○森 厚詞^{1,2}, 中井 正三¹, 光本 泰秀¹, 森泉 哲次²

(¹大塚製薬 (株) 探索第二研究所, ²信州大・医・第二解剖)

P01-F-21 パーキンソン病モデルラットの脳内ドパミン濃度変化に及ぼすイサチンの影響

○南 勝¹, 浜上 尚也¹, 寺戸 睦子¹, 遠藤 泰¹, 平藤 雅彦¹, 緒方 昭彦², 田代 邦雄²

(¹北海道医療大・薬・薬理, ²北大・医・神経内科)

P01-F-22 A role of MxA protein, anti-viral GTPase, in Parkinson's disease

○石橋 大輔, 三島 健一, 青尾 直也, 古川 ひさ子, 高橋 三津雄, 藤原 道弘

(福岡大・薬・臨薬)

P01-F-23 小胞体ストレスにおける細胞死促進因子の探索

○大貫 玲子^{1,2}, 板東 良雄^{3,4,5}, 須山 英悟^{1,6}, 川崎 広明^{1,6}, 片山 泰一^{3,5}, 遠山 正彌^{3,5}, 多比良 和誠^{1,6}

(¹産総研・ジーン, ²筑波大・院生命環境科学, ³阪大・院ポストゲノム, ⁴旭川医科大・解剖学第一, ⁵CREST, JST, ⁶東京大・院工)

P01-F-24 EAOH遺伝子 *apratxin* の細胞局在解析

伊達 英俊, 五十嵐 修一, 佐野 泰照, 高橋 哲哉, 小野寺 理, ○辻 省次

(新潟大・脳研・神経内科)

P01-F-25 The analysis of aprataxin, the product of causative gene of early-onset ataxia with ocular motor apraxia and hypoalbuminemia

○佐野 泰照¹, 五十嵐 修一¹, 小野寺 理¹, 伊達 英俊¹, 小宅 睦郎¹, 福原 信義³, 卷淵 隆夫³, 森松 光紀⁴, 高橋 均², 辻 省次¹

(¹新潟大・脳研・神内, ²新潟大・脳研・病理, ³犀潟病院・神内, ⁴山口大・神内)

P01-F-26 ERストレスによって誘導される因子PKRの神経変性疾患への関与

○板東 良雄^{1,3}, 大貫 玲子^{4,6}, 片山 泰一^{2,3}, 吉田 茂孝¹, 多比良 和誠^{4,5}, 遠山 正彌^{2,3}

(¹旭川医大・第一解剖, ²阪大・院医・ポストゲノム疾患解析学, ³クレスト, ⁴ジーン ディスカバリー研究センター・産総研, ⁵東大・院工・生命科学, ⁶筑波大・院農)

P01-F-27 ドパミンアゴニストcabergolineが示す神経保護効果の作用機序

吉岡 真世, 田中 健一, ○藤田 尚子, 宮崎 育子, 浅沼 幹人, 小川 紀雄

(岡山大院・医歯・神経情報)

P01-F-28 Activation of CREB-dependent transcription system suppresses expanded (MS4-4) polyglutamine induced cytotoxicity.

○Mitsuteru Shimohata, Takayoshi Shimohata, Shuichi Igarashi, Satoshi Naruse, Shoji Tsuji

(新潟大・脳研・神内)

プログラム細胞死

P01-F-29 ストレス応答性転写因子ATF3によるHSP27発現誘導と神経細胞死防御

○中込 咲綾¹, 鈴木 康博^{1,2}, 木山 博資¹

(¹大阪市大・院医・機能細胞形態, ²旭川医大・医・1内)

P01-F-30 Transcription factors induced in cultured cortical neurons by beta amyloid peptide

(MS2-1) ○内田 洋子, 五味 不二也

(都老人研・神経病理)

P01-F-31 トリメチルスズによるマウス歯状回顆粒細胞の一過性障害

○奥田洋明¹, 西山徳人¹, 山本泰弘¹, 米田幸雄², 荻田喜代一¹

(¹摂南大・薬・薬理, ²金沢大・薬・薬物)

P01-F-32 神経細胞の生存に関連する新規遺伝子 *alivin 1*

(MS2-2) ○小野 富男^{1,2}, 鈴木 直子¹, 吉川 欣亮², 米川 博通², 川島 誠一¹

(¹都臨床研・分子制御, ²都臨床研・実験動物)

P01-F-33 Essential role of Rho/Rho-kinase signaling pathway in target-independent survival of developing motor neurons

○小林 憲太¹, 高橋 将文², 高橋 一彰¹, 八木沼 洋行³, 大隅 典子², 貝淵 弘三⁴, 小林 和人¹

(¹福島医大・生体機能, ²東北大院・医・器官構築, ³福島医大・解剖1, ⁴名大・院医・細胞情報薬理)

P01-F-34 Dlxin-1, a necdin/MAGE homologous protein, enhances E2F1-induced neuronal apoptosis

○桑島 孝明, 谷浦 秀夫, 吉川 和明

(阪大・蛋白研・機能制御)

P01-F-35 PC12細胞の一酸化窒素(NO)によるアポトーシスはチロシンキナーゼの活性化を介する

○山本 敏文¹, 湯山 耕平¹, 山本 秀子²

(¹横浜市大・院総理・分子認識, ²都精神研・分子精神医学)

P01-F-36 Cdk5活性化サブユニットp35のp25への限定分解と神経細胞死に関する検討

○斎藤 太郎, 藤田 悠一, 浅田 明子, 久永 真市

(都立大・院理・生物科学)

P01-F-37 NF- κ Bによるオリゴデンドロサイト細胞死阻害機構の解析

○浜之上 誠, 高松 研

(東邦大・医・第2生理)

P01-F-38 Effect of caspase-3 on the primary cultured mice cortical neurons with HSV-1 infection

○Jiawei Wang, Dexin Wang, Zijing Feng

(Dept. Neurology, Beijing Friendship Hospital)

虚 血

P01-F-39 砂ネズミ海馬における一過性前脳虚血に伴う Forkhead transcription factor の活性化反応 (MS4-8) ○福永 浩司¹, 河野 隆幸^{1,2}, 生塩 之敬², 宮本 英七¹

(¹熊本大・医・第一薬理, ²熊本大・医・脳神経外科)

P01-F-40 ロイコトリエンD₄によるCysLT₁受容体を介したミクログリアの活性化

○平林 哲也^{1,2}, 清水 孝雄¹

(¹東大・院医・細胞情報, ²千葉大・院薬・薬効薬理学)

P01-F-41 脳虚血再灌流負荷後に投与した17β-estradiolによる海馬組織内の神経細胞死抑制メカニズム

○藤田 公和¹, 加藤 寿章¹, 山内 理充², 芳本 信子³, 柴山 健三¹, 永田 豊¹, 宮地 栄一¹

(¹保衛大・医・生理, ²愛知学泉短大, ³名古屋文理短大)

老 化

P01-F-42 脳に発現するNAD依存性ヒストン脱アセチル化酵素SIR2ホモログの細胞内局在

○堀尾嘉幸, 坂本 淳

(札幌医大・薬理)

P01-F-43 老化促進モデルマウス(SAMP8)の海馬と大脳に発現するAnkyrin-repeatを含む新規遺伝子の解析

○山村 尚志^{1,2}, 小池 宣也¹, 野村 靖幸³, 大熊 康修³, 上原 孝³, 榊 佳之¹, 田代 朋子², 降旗 千恵^{1,2}

(¹東大・医科研・ヒトゲノム解析セ, ²青山学院大・理工・化学, ³北大・院薬・薬理)

P01-F-44 高齢ラットおよびSAMP8の脳内DNA損傷とC6グリア細胞死に対する当帰芍薬散の効果

○平松 緑

(東北公益文科大)

薬物依存、精神薬理

P01-F-45 メタンフェタミン慢性投与によるマウスの行動感作形成と脳情報伝達系の変化

○北中 純一, 北中 順恵, 竹村 基彦

(兵庫医大・薬理)

P01-F-46 BDNF inhibits apoptosis induced by methamphetamine in primary cortical neurons through activation of Akt kinase

○松崎 秀夫¹, 瀧川 一彦², 木山 博資², 森 則夫³, 佐藤 康二¹

(¹浜松医大・第1解剖学, ²大阪市大・院医・機能細胞形態学, ³浜松医大・精神神経医学)

P01-F-47 パラコートはドパミン神経に対し興奮毒性を示し長時間に及ぶドパミン放出を惹起する

○松原 和夫¹, 清水 恵子², 大滝 康一¹, 斉藤 修², 塩野 寛²

(¹旭川医大・医・病院薬剤部, ²旭川医大・法医学)

ニューロン、グリア

P02-E-1 カイニン酸海馬障害モデルにおける代償性保護作用の解析

○高田 和幸, 北村 佳久, 岡崎 充宏, 柿村 順一, 谷口 隆之
(京都薬大・病態生理)

P02-E-2 Association of protein kinase C alpha and of p38 mitogen-activated protein kinase with the induction of tumor necrosis factor alpha in lipopolysaccharide-stimulated microglia

○中嶋 一行^{1,2}, 遠山 陽子¹, 高坂 新一², 栗原 正¹
(¹創価大・生命研・神経化学, ²国立精神・神経センター・神経研・代謝)

P02-E-3 神経系における4回膜貫通蛋白質CD9およびCD81の相補的分布と機能分担

○石橋智子¹, 池中一裕², 目加田英輔³, 馬場広子¹
(¹東京薬大・薬, ²生理研, ³阪大・微研)

P02-E-4 培養ミクログリアにおけるATPによる炎症関連遺伝子*mrf-1*の選択的発現抑制: P2X₇リセプターからのCa²⁺流入の関与

○田中 秀逸, 賀谷 直樹, 小池 達郎
(北大・院理・行動知能)

P02-E-5 セロトニンによるラットアストロサイトのコレステロール代謝制御

○上野 幸子^{1,2}, 伊藤 仁一², 長安 祐子², 横山 信治²
(¹名市大・院医・精神認知行動医学, ²名市大・院医・代謝細胞生化学)

P02-E-6 グリオblast様株化細胞OS3はミクログリア存在下で浮遊spheroidを形成する

○久村 隆二^{1,2}, 田中 謙二^{1,3}, 川上 真紀子¹, 鹿島 晴雄³, 澤田 誠^{1,4}
(¹藤田保健衛生大・総医研・難病治療, ²名大・院農・生物情報制御, ³慶応大・院医・精神, ⁴さががけ21)

P02-E-7 オリゴデンドロサイトの発生の起源

○中平 英子¹, 鹿川 哲史¹, Martyn M Guldin², 池中 一祐¹
(¹生理研・神経情報, ²The salk institute)

P02-E-8 Expression of P2Y₁₂ receptors in the brain

○佐々木 洋², 本田 静世¹, 赤澤 智宏¹, 城光寺 邦信¹, 中村 泰子¹, 井上 和秀³, 高坂 新一¹
(¹国立精神・神経センター 神経研・代謝, ²科技団・科技特, ³国立医薬品食品衛生研・薬理部)

P02-E-9 The basic helix-loop-helix factor Olig2 is essential for development of motoneuron and oligodendrocyte lineages

○竹林 浩秀¹, 鍋島 曜子², 吉田 松生², 千坂 修³, 鍋島 陽一², 池中 一裕¹
(¹生理研・神経情報, ²京大・院医・腫瘍生物, ³京大・院理)

P02-E-10 Influence of astrocyte-inducers on glial cell development in primary cultures of embryonic rat cerebral hemisphere

○足立 達美^{1,2}, 高永 博実^{2,3}, 国本 学^{2,3}, 阿相 皓晃⁴

(¹国立水俣病研セ・基礎研, ²国立環境研, ³北里大・薬, ⁴都老人研・神経生物)

P02-E-11 アストロサイト系譜の発生の解析

○小川 泰弘¹, Matthew Rasband², 竹林 浩秀¹, 岩崎 靖乃¹, James Trimer², 池田 一裕¹

(¹生理研・神経情報, ²SUNY Stony Brook)

P02-E-12 小脳顆粒ニューロン前駆細胞の増殖・分化調節

○岡野 (内田) 孝幸, 小宮 義璋, 石崎 泰樹

(群馬大・医・分子病態)

P02-E-13 低濃度ポリアミンによるミクログリアのアポトーシス誘導

高野 桂¹, ○中村 洋一^{1,2}, 米田 幸雄¹

(¹金沢大・薬・薬物, ²大阪府大・院農・統合生理)

P02-E-14 ミクログリアおよびアストロサイトによる酸化ストレスに対する神経細胞死抑制作用

○大城 聰¹, 張 純¹, 北嶋 繁孝¹, 宮澤 ちひろ³, 川村 憲一³, 中嶋 一行²

(¹東医歯大・難研・遺伝生化, ²創価大・生命研・神経化学, ³テークークラフト・研究開発)

P02-E-15 Kainic acid induces leukemia inhibitory factor mRNA expression in the rat brain with different time courses between the dentate gyrus and hippocampal CA1/CA3 subfields

○南 雅文, 前川 京子, 山國 尚志, 片山 貴博, 中村 純治, 佐藤 公道

(京都大・薬・生体機能解析)

P02-E-16 培養ラットアストロサイトにおけるモノアミントランスポーターの機能的発現

○武田 弘志, 稲津 正人, 松宮 輝彦

(東京医大・薬理・難治研センター)

P02-E-17 Generation of BRINP1 knock-out mice

○中谷 俊幸¹, 川野 裕美¹, 小林 三和子¹, 松本 健一², 有賀 寛芳², 松岡 一郎¹

(¹北大・院薬・神経科学, ²北大・院薬・分子生物)

P02-E-18 海馬アストロサイトのCa²⁺ wave伝播の性質

○小泉 修一¹, 藤下 加代子^{1,2}, 井上 和秀^{1,2}

(¹国立衛研・薬理, ²九大・院・分子制御)

P02-E-19 Perineuronal oligodendrocytes protect against neuronal apoptosis in a genetic demyelinating model by upregulation of lipocalin-type prostaglandin D synthase

○江口 直美^{1,2}, 谷池 雅子³, 毛利 育子³, 鈴木 衣子⁴, 裏出 良博^{1,2}

(¹(財)大阪バイオサイエンス研・分子行動生物, ²科技団・CREST・脳を知る, ³阪大・院医・小児, ⁴Dept. Pathology and Labo. Med., and Neurosci. Center, Sch. Med., Univ. North Carolina at Chapel Hill)

P02-E-20 L-Serine合成酵素3PGDH遺伝子のアストロサイトにおける遺伝子発現制御

○清水 基宏^{1,2}, 古屋 茂樹¹, 篠田 陽子¹, 吉田 一之^{1,3}, 三苫 純也⁴, 鈴木 康夫², 平林 義雄¹
(¹理研・脳総研・神経回路メカニズムG, ²静岡県大・薬・生化学, ³東京農工大・連合農学, ⁴The Burnham Institute)

P02-E-21 疾患モデルマウスからの培養シュワン細胞株の樹立

○渡部 和彦¹, 坂本 剛¹, 川添 陽子¹, 沈 勁松^{1,2}, 大橋 十也², 衛藤 義勝², 道川 誠³, 柳澤 勝彦³, 宮本 勝一⁴, 山村 隆⁴, 荒木 令江⁵
(¹都神経研・分子神経病理, ²慈恵医大DNA医研・遺伝子治療, ³長寿研・痴呆疾患, ⁴国立精神・神経センター神経研・免疫, ⁵熊本大・医・腫瘍医学)

P02-E-22 Involvement of microglia activation and p38 phosphorylation in rat neuropathic pain

○井上 和秀^{1,3}, 津田 誠¹, 溝腰 朗人^{1,3}, 重本-最上 由香里¹, 小泉 修一¹, 高坂 新一²
(¹国立医薬品食品衛生研・薬理, ²国立精・神・センター神経研・代謝, ³九大・院薬・化学療法分子制御学)

情報伝達、変換、修飾

P02-E-23 転写因子AP-1のミトコンドリア遺伝子非翻訳領域への結合

○藤波義明¹, 北野雅大¹, 米田幸雄², 荻田喜代一¹
(¹摂南大・薬・薬理, ²金沢大・薬・薬物)

P02-E-24 Effect of exposure to static magnetic field on AP1 DNA binding in cultured rat hippocampal neurons

○平居 貴生, 米田 幸雄
(金沢大・薬・薬物)

P02-E-25 アネキシンIVの強制発現によるC6細胞のエタノール細胞障害の増大とNF-κBの活性化

○大川 浩子^{1,2}, 相馬 仁¹, 酒井 理恵^{1,3}, 橋本 恵理³, 鶴飼 渉³, 齋藤 利和³, 黒木 由夫¹
(¹札幌医大・医・生化学第一, ²札幌医大・保健医療・作業療法, ³札幌医大・医・神経精神)

P02-E-26 ERAD (小胞体関連分解) 関連分子Hrd1pのヒトホモログの単離・同定とその機能・誘導 (MS6-4) 機構の解析

○金子 雅幸, 石黒 正太郎, 新沼 芳文, 野村 靖幸
(北大・院薬・薬理)

P02-E-27 HRD3p ヒトホモログである SEL1L の小胞体ストレスによる誘導とATF6 による転写誘導を制御する領域の同定

○新沼 芳文, 石黒 正太郎, 安井 沙織, 金子 雅幸, 野村 靖幸
(北大・院薬・薬理)

P02-E-28 Expression of XRE binding proteins in rat cerebellum

○眞田 法子, 馬場 勝弘, 倉本 展行, 米田 幸雄
(金沢大・薬・薬物)

P02-E-29 Estrogen elevates PKA activity via the putative membrane receptor in hippocampal neurons.

○新郷 S. 明子, 鬼頭 昭三
(兵庫大・健康科学)

P02-E-30 A novel FERM domain including guanine nucleotide exchange factor is involved in Rac signaling and regulates neurite remodeling

○久保 盾貴, 山下 俊英, 遠山 正彌
(阪大・院医・二解, 科学技術振興事業団)

P02-E-31 Biological activity of neurotrophins is dependent on proper recruitment of Rac1 to lipid rafts

○本田 章子^{1,2,3}, 山下 俊英^{1,2}, 久保 盾貴^{1,2}, 田中 啓之^{1,2}, 片山 泰一^{1,2}, 遠山 正彌^{1,2}
(¹阪大・院医・ポストゲノム疾患解析学, ²CREST,JST, ³田辺製薬(株) 創薬研究所)

P02-E-32 Morphological changes in mouse neuroblastoma caused by disruption of glycosylation

○馬場孝輔, 片山泰一, 遠山正彌
(阪大・院医・ポストゲノム疾患解析学, CREST, JST)

P02-E-33 Identification of GRP78 as a type-1 sigma receptor (sigmaR1) -associated protein.

○山本 秀子¹, 亀谷 富由樹¹, 並木 芳子¹, 山本 敏文^{1,2}, 唐沢 淳一^{1,3}, 曾良 一郎^{1,4}, 額田 敏秀¹
(¹都精神研, ²横浜市大・院, ³日本シェーリング, ⁴東北大・医)

P02-E-34 Regulation of exocytotic events and granular movements by protein kinase C in single chromaffin cells

○笹川 展幸, 保坂 早苗, 林 光紀, 熊倉 鴻之助
(上智大・生命研・神経化学)

P02-E-35 α CAMKII mRNA の 3' 非翻訳領域に結合し樹状突起への局在を決定する蛋白因子の同定

○森 泰丈, 宮田 信吾, 遠山 正彌
(阪大・院医・ポストゲノム疾患解析, 科学技術振興事業団)

P02-E-36 細胞外Ca流入によるarc mRNAの発現誘導

○市川 久詞, 平 英一, 三木 直正
(阪大・院医・情報薬理)

P02-E-37 培養ラット海馬神経細胞におけるCaM kinase のリン酸化、活性化反応

○上江洲 章吉, 福永 浩司, 笠原 二郎, 宮本 英七
(熊本大・医・薬理学一)

P02-E-38 Cdk5活性化サブユニットp39のCdk5の活性化

○関川 明生, 斎藤 太郎, 浜住 由子, 浅田 明子, 久永 真市
(都立大・院理・生物)

P02-E-39 p35/Cdk5と結合する新規蛋白質の解析

○浅田 明子, 本間 直幸, 竹下 亘, 山川 英子, 榎本 真理子, 斉藤 太郎, 久永 真市
(都立大・院・生物科学)

P02-E-40 Neuro-2A細胞におけるプロスタグランジン₁₂によるブラジキニン誘発シナプシン1のサイト3リン酸化の増強について

○松尾 由理, 鳥畑 啓太, 加藤 浩嗣, 佐々木 泰治
(北里大・薬・薬理)

P02-E-41 α CaM kinase II 遺伝子の神経特異的発現

○美馬 佳寿子, 山内 卓
(徳島大・薬)

P02-E-42 Antiapoptotic action of calcineurin via dephosphorylation of Bcl-2

○山本 秀幸¹, 野村 威雄², 三股 浩光², 下重 美紀³, 芝崎 太³, 野村 芳雄², 宮本 英七¹
(¹熊本大・医・一薬理, ²大分医大・医・泌尿器, ³都臨床研・細胞生理)

軸索輸送、細胞運動、細胞骨格

P02-E-43 Direct observation and characterization of gels formed from isolated neurofilaments *in vitro*

○倉知 正¹, 萩川 慎平², 小野 れい², Jean-Francois Leterrier³, 小宮 義璋¹, 田代 朋子^{2,4}
(¹群馬大・医・分子病態, ²青山学院大・理工・化, ³CNRS, Poitiers Univ., ⁴CREST, JST)

P02-E-44 IP_3 receptor type 1 mRNA の3'末端非翻訳領域に結合する遺伝子産物と小脳Purkinje細胞における樹状突起へのmRNA局在化機構

○飯島 崇利^{1,2,4}, 木村 友喜³, 高月 香菜子⁵, 今井 貴雄¹, 川原 茂敬⁵, Aran Bernstein³, 桐野 豊⁵, 岡野 栄之^{1,2}
(¹慶応大・医・生理学, ²科技団・CREST, ³Samuel Lunenfeld Res. Inst. Mount Sinai Hospital, CAN, ⁴阪大・院医・機能形態学, ⁵東大・院薬・神経生物物理学)

P02-E-45 The roles of WAVES in filopodia formation at NG108 growth cones

(MS1-6) ○Shigeaki Miyamoto¹, Motohiro Nozumi¹, Hiroyuki Nakagawa¹, Hiroaki Miki², Tadaomi Takenawa²
(¹九州工大・情報工・生化システム, ²東大・医科研・腫瘍分子生物)

P02-E-46 セマフォリンと皮質神経回路形成

(MS1-5) ○五嶋 良郎, 佐々木 幸生
(横浜市大・医・薬理)

P02-E-47 神経系の細胞形態および運動性とリゾリン脂質シグナル

(MS1-4) ○福嶋 伸之
(北大・院医・分子医化学)

P02-E-48 ミクログリア特異的Iba1結合タンパク質としてのフィブリンの同定と相互作用の解析

○大澤 圭子, 今井 嘉紀, 佐々木 洋, 金澤 裕子, 高坂 新一
(精神神経セ・神経研・代謝)

P02-E-49 ドレブリン結合タンパク質Drap1の同定及び機能解析

○山崎 博幸¹, 高橋 秀人¹, 広瀬 英司², 森 望², 白尾 智明¹
(¹群馬大・医・行動分析, ²長寿研・分子遺伝)

P02-E-50 Anterograde axonal transport of endopeptidase 24.15 in rat sciatic nerves

○山本 賢¹, 知久馬 敏幸², 山下 厚重², 山口 光峰², 北條 博史², Md Mahiuddin Ahmed¹,
加藤 武¹
(¹横浜市大・院総合理, ²昭和薬大・衛生化)

その他

P02-E-51 Identification of a novel neural-specific protein family, BRINP

○戸田 史明, 上野 哲, 森 卓哉, 中谷 俊幸, 松岡 一郎
(北大・院薬・神経科学)

P02-E-52 ショウジョウバエでのトランスジーンによるRNAiを用いた遺伝子機能解析

○大迫 俊二, 高松 芳樹
(都神経研・脳構造)

P02-E-53 バイオ実験に用いられる水の最適精製方法の検討

○石井 直恵¹, 加納 一郎², 金沢 旬宣¹
(¹日本ミリポア・ラボラトリーウォーター事, ²Lab water Div., Millipore S.A.)

P02-E-54 小胞体ストレストランスデュースIRE1に相互作用する因子の同定と機能解析

○大野 加代子^{1,3}, 米田 託成^{1,3}, 今泉 和則², 片山 泰一^{1,3}, 遠山 正彌^{1,3}
(¹阪大・院医・ポストゲノム疾患解析, ²奈良先端大・バイオ・細胞構造, ³科技団)

P02-E-55 The 5' variants of p270, an integral member of SWI-SNF complexes, in the rat brain.

○山口 剛¹, 荒木 一明², 那波 宏之², 樋口 宗史¹
(¹新潟大・院・分子細胞医学・薬理, ²新潟大・脳研・分子神経生物)

P02-E-56 マウス神経芽細胞腫由来N2a細胞におけるプリオンタンパク質の局在

○鎌田 礼子¹, 逆瀬川 裕二¹, 八谷 如美^{1,2}, 金子 清俊¹
(¹精神・神経センター 神経研・疾病研究第7部, ²科技団・戦略・脳を守る)

P02-E-57 p38 α の新規スプライス産物p38 α 2 の細胞内局在と細胞死の誘導は、そのキナーゼ活性とC末領域が制御する。

○日下 永子¹, 石川 保幸², 岡田 雅人¹
(¹阪大・微研・発癌制御, ²奈良先端科学技術大学院大)

P02-E-58 Determination of fatty acid levels in erythrocyte membranes in patients with chronic fatigue syndrome.

○Zhandong Liu, Dexin Wang, Jian Zhang

(Dept. Neurology, Binjing Friendship Hospital, Affiliated Hospital of Capital Univ. Med. Sci.)

細胞接着因子

P02-E-59 Activation of p38 MAPK cascade by the activity-regulated neuronal cell adhesion molecule arcadlin

○安田 新¹, 田中 秀和², 杉浦 弘子¹, 鈴木 香子¹, 竹宮 孝子¹, 三木 直正², 山形 要人¹

(¹都神経研・神経薬理, ²阪大・院医・情報薬理)

P02-E-60 Interaction of locally modulated and de novo synthesized cadherins at the activated synaptic membranes

○田中 秀和¹, 安田 新², 藤本 崇宏¹, 溝口 明³, 杉浦 弘子², 山形 要人², 三木 直正¹

(¹阪大・医・薬理, ²都神経研・神経薬理, ³三重大・医・一解剖)

P02-E-61 GPI-アンカー型神経接着分子コンタクチン及びNB-3の後シナプス肥厚部における局在

○武田 泰生¹, 李 順姫^{1,2}, Vance Lemmon², 渡邊 和忠³

(¹都老人研・細胞認識, ²Dept. Neurosci., Sch. Med., CWRU, ³長岡技科大・生物)

感染症

P02-E-62 バクテリアエンドトキシンによるグリア細胞での p38 MAP キナーゼを介する IL-20 の誘導

○細井 徹, 和田 吉世, 大熊 康修, 野村 靖幸

(北大・院薬・薬理)

シナプス

P02-E-63 シナプス膜に局在する新規のDem protein, ssLRP

○鈴木 龍雄¹, 田 慶宝¹, 岡野 照¹, 宮沢 昌子¹, 臼田 信光²

(¹信州大・医・神経可塑性, ²藤田保健衛生大・医・解剖)

P02-E-64 SNAP-25(S187A)変異マウスの作製

○片岡 正和^{1,4}, 桑原 玲子², 中原 陽子², 鈴木 理可², 茂手木 淑子², 福井 由宇子², 横山 峯介², 高橋 正身^{3,4}

(¹信州大・工・環境機能, ²三菱化学生命研, ³北里大・医・生化学, ⁴科技団・CREST)

P02-E-65 シンタキシンアイソフォームとCaMKIIの結合

野村 和重^{1,2,3}, 小宮 義璋³, ○五十嵐 道弘¹

(¹新潟大・院医歯・分子細胞機能, ²群馬大・整形外科, ³群馬大・医・分子病態)

可塑性

P02-E-66 NMDA receptor-dependent recruitment of AMPA receptor and calnexin to plasma membrane in cultured rat hippocampal neurons

○辻村 淳¹, 板倉 誠², 関口 真理子³, 工藤 佳久¹, 高橋 正身^{2,4}

(¹東京薬大・生命科学, ²北里大・医, ³三菱化学生命研, ⁴CREST, JST)

P02-E-67 カイニン酸刺激による興奮性神経活動における海馬ニューロンでの翻訳調節因子的変化

○稲村 直子, 那波 宏之, 武井 延之

(新潟大・脳研究所・分子神経生物)

パターン形成

P02-E-68 神経細胞の移動を制御するリーリンシグナルとCdk5キナーゼ

(MS1-1) ○大島 登志男¹, 小川 正晴², 御子柴 克彦¹

(¹理研・脳総研・発生神経, ²同・細胞培養)

細胞分化

- P02-F-1 胎児／成体マウス脳における神経幹細胞のフローサイトメーターを用いた解析**
 ○村山 綾子^{1,2,3}, 松崎—伊谷 有未^{1,3}, 川口 綾乃⁴, 宮田 卓樹⁵, 島崎 琢也^{1,3}, 岡野 栄之^{1,3}
 (1慶大・医・生理, 2阪大・院・医・機能形態, 3CREST-JST, 4理研・発生再生センター・神戸, 5理研・脳センター・和光)
- P02-F-2 E2F1に誘導されるニューロンの細胞死に対するNecdinの抑制作用**
(MS3-4) ○高崎 理紗, 植月 太一, 谷浦 秀雄, 吉川 和明
 (阪大・蛋白研・機能制御)
- P02-F-3 ES細胞由来神経幹細胞の分化に伴うPACAPおよびその受容体の発現上昇**
 ○廣瀬 恵¹, 橋本 均¹, 新谷 紀人¹, 荒川 尚久¹, 馬場 明道^{1,2}
 (1阪大・院薬・神経薬理, 2阪大・院医・分子医薬)
- P02-F-4 genipin による Neuro2a 細胞の NO-cGMP-PKG 経路から ERK のリン酸化を介した突起伸展**
 ○山崎 眞津美, 大窪 忠利, 千葉 賢三, 毛利 哲郎, 吉藤 茂行
 (北陸大・薬・生物活性)
- P02-F-5 Cystatin C induces astrocyte differentiation by inhibition of cystein proteases**
 ○長谷川 明子^{1,2}, 岩崎 靖乃¹, 池中 一裕¹
 (1生理研・神経情報, 2総合研究大学院大)
- P02-F-6 Negative regulation of neural progenitor cell proliferation by necdin**
 ○谷浦秀夫, 福永典子, 植月太一, 吉川和明
 (阪大・蛋白研・機能制御)
- P02-F-7 ラットC6グリオーマ細胞のアストロサイト様分化におけるSTAT3シグナル伝達経路への低分子量Gタンパク質RhoAの関与**
 ○高永 博実¹, 足立 達美², 国本 学¹
 (1北里大・薬, 2水俣病研セ・基礎研究部)
- P02-F-8 神経幹細胞の維持と分化におけるGab1の役割**
 ○早川 佳芳^{1,3}, 西田 圭吾², 平野 俊夫², 島崎 琢也^{1,3}, 岡野 栄之^{1,3}
 (1慶大・医・生理, 2阪大・院医・腫瘍病理, 3科技団・戦略)
- P02-F-9 哺乳類の神経発生におけるRNA結合蛋白質Musashiの機能解析**
 ○芝田 晋介^{1,3}, 榊原 伸一², 小島 拓郎^{1,3}, 岡野 栄之^{1,3}
 (1慶大・医・生理, 2獨協医大・解剖, 3科技団・戦略)
- P02-F-10 Wntシグナリングによるオリゴデンドロサイト分化抑制**
 ○清水 健史¹, 和田 圭樹¹, 鹿川 哲史¹, 高田 慎治^{2,3}, 室山 優子^{2,3}, 池中 一裕¹
 (1生理研・神経情報, 2岡崎統合バイオ, 3ERATO近藤分化)

P02-F-11 The Wnt/calcium pathway activates NF-AT and promotes ventral cell fate in *Xenopus* embryos.

実吉 岳郎¹, 糸 昭苑¹, ○御子柴 克彦^{1,2,3}

(¹東大医科研・脳神経発生・分化, ²科技団 カルシウム振動プロジェクト, ³理研 脳科学総合研究セ)

P02-F-12 Expression of a glutamine-rich forkhead transcription factor in the developing central nervous system.

○岩西 宏樹, 森川 吉博, 仙波 恵美子

(和医大・第二解剖)

P02-F-13 Kohjirin, A novel bone morphogenetic protein-4 antagonist derived from astrocytes.

○栗山 寛奈¹, 植木 孝俊¹, 田中 正光², 三浦 直行³, 梶村 春彦², 佐藤 康二¹

(¹浜松医大・医・解剖¹, ²浜松医大・医・病理¹, ³浜松医大・医・生化²)

栄養因子

P02-F-14 リルゾールによるラット海馬における脳由来神経栄養因子の合成機構

(MS3-1) ○仙波 りつ子, 加藤 兼房

(愛知コロニー研)

P02-F-15 bFGFによる培養中枢ニューロンからのMAPK活性化依存的な興奮性神経伝達物質の放出機構。

○沼川 忠広^{1,2,3}, 横幕 大作^{1,2}, 清末 和之², 安達 直樹¹, 松本 知也^{1,2}, 沼川 裕美子^{1,2}, 田口 隆久², 畠中 寛¹, 山田 雅司¹

(¹阪大・蛋白研・生合成, ²産総研関西センター, ³日本学術振興会特別研究員)

P02-F-16 Change of the expression of glial cell line-derived neurotrophic factor (GDNF) in the rat sciatic nerve after its transection

(MS3-3) ○山田 喜久¹, 新田 淳美², 清水 克時¹, 古川 昭栄²

(¹岐阜大・医・整外, ²岐阜薬大・分生)

P02-F-17 cAMP-dependent protein kinase phosphorylates the p75 receptor and regulates its localization to lipid rafts

○樋口 晴久, 山下 俊英, 遠山 正彌

(阪大・院医・未来医療開発・ポストゲノム疾患解析, 科学技術振興事業団)

P02-F-18 Responsiveness of embryonic striatal neurons to bone morphogenetic protein and neurotrophic factors

○菊池 紗綾¹, 中村 岳志², 森 望², 松岡 一郎¹

(¹北大・院薬・神経科学, ²長寿研・分子遺伝学)

P02-F-19 Autocrine action of BMP regulates GDNF expression in Schwann cells

○木滑 恵未, 松岡 一郎
(北大・院薬・神経科学)

P02-F-20 Levels of mRNA for brain derived neurotrophic factor are up-regulated in the urethra of diabetic rats

○町野 倫太郎¹, 松岡 一郎², 柿崎 秀宏¹, 柴田 隆¹, 小柳 知彦¹
(¹北大・院医・泌尿器外科, ²北大・院薬・神経科学)

P02-F-21 脊髄の切断損傷部位における神経成長因子 (NGF) の蓄積

○古川 美子, 村上 豊, 新田 淳美, 古川 昭栄
(岐阜薬大・分子生物)

P02-F-22 Effect of neurotrophins on the expression of bHLH transcription factors

○伊藤 久則, 中嶋 亜紀, 古川 昭栄
(岐阜薬大・分子生物)

P02-F-23 脳由来神経栄養因子 (BDNF) 細胞内シグナルにおける Shp-2 の役割

○高井 里美, 岡田 雅人
(阪大・微研・発癌制御)

P02-F-24 Autocrine action of acidicFGF to stimulate cholesterol metabolism and apoE secretion of rat astrocytes

○伊藤 仁一, 長安 祐子, 横山 信治
(名市大・医・生化I)

P02-F-25 A brain-specific EGF-like family protein, NELL2, is a novel survival factor for neurons

○堀江 正人¹, 相原 公德¹, 谷澤 克行², 黒田 俊一²
(¹大塚製薬(株)探索第2研究所, ²阪大・産研)

P02-F-26 Changes of the gene expression in the injured site of the rat spinal cord

○橋本 学, 堀 琢弥, 古川 昭栄
(岐阜薬大・分子生物)

突起進展、回路網形成

P02-F-27 A β 25-35誘導性の突起形成不全に対するwithanolide A、withanoside IV、withanoside (MS1-7) VIの神経突起伸展効果

○久保山 友晴, 東田 千尋, 小松 かつ子
(富山医薬大・和漢薬研・薬効解析センター)

P02-F-28 Expression of tau isoforms containing exon2 and 3 during synaptogenesis *in vivo* and (MS1-8) *in vitro*

○北村 主税, 若山 勇太, 舩野 正樹, 野呂 美幸, 田代 朋子
(青山学院大・理工・化学)

P02-F-29 発生期大脳皮質における移動神経細胞のダイナミックな形態変化

(MS1-3) ○田畑 秀典¹, 仲嶋 一範^{1,2}

(¹慈恵医大・DNA医学研・分子神経生物, ²PRESTO, JST)

P02-F-30 Lateralization of thalamofugal pathway before light exposure in developing chicken visual pathway

○小柴 満美子^{1,2}, 菊池 建機², 養王田 正文¹, 中村 俊²

(¹東京農工大・生命工, ²精神セ神経研)

P02-F-31 Otx1は視覚野からの皮質下投射路形成に必須か?

安藤 康一¹, 久保田 力¹, 八木 秀司¹, 永野 隆¹, 相沢 慎一², 寺島 俊雄³, ○佐藤 真¹

(¹福井医大・医・解剖2, ²熊本大・発生医学研究センター, ³神戸大・院医・脳科学)

P02-F-32 CRMP-2 and axon formation

(MS1-2) ○深田 優子, 貝淵 弘三

(名大・院医・薬理)

P02-F-33 Conformational changes of Pur α concomitantly increase in ssCRE binding activity

○郭 哲輝, 曾 玲暉, 三木 直正

(阪大・院医・情報薬理)

P02-F-34 Cytoplasmic p21^{Cip1/WAF1} regulates neurite remodeling by inhibiting Rho-kinase activity

○田中 啓之, 山下 俊英, 遠山 正彌

(阪大・院医・ポストゲノム疾患解析学, 科技団)

P02-F-35 活動抑制による細胞死に先行して起こるシナプス廃止

○小倉 明彦^{1,2}, 橋本 典和¹, 富永(吉野) 恵子^{1,2}

(¹阪大・院理, ²阪大・院・生命機能)

P02-F-36 Dopaminergic neurons derived from mouse midbrain maintain synchronous oscillation of intracellular calcium in primary culture

○安本 史恵¹, 根岸 隆之^{2,4}, 石井 寿幸², 久和 茂², 黒田 洋一郎^{3,4}, 吉川 泰弘^{2,4}

(¹東大・農・実験動物, ²東大・院農・実験動物, ³都神経研・分子神経生物, ⁴CREST, JST)

P02-F-37 Disparity of cell death and mossy fiber sprouting in kainic acid-treated hippocampal slice cultures

○韓 春錫¹, 古川 由里子^{1,2}, 鳥光 慶一^{1,2}

(¹N T T物性科学基礎研, ²科技団・戦略)

P02-F-38 細胞形態測定装置を用いた神経細胞の突起伸展評価

○田中 史子, 清水 博喜, 隠岐 徹, 長谷川 嘉一

(エーザイ(株)筑波研)

再生、移植

P02-F-39 金魚視神経再生過程におけるトランスグルタミナーゼの変動とその役割

○杉谷 加代^{1,2,3}, 松川 通^{2,3}, 本間 啓子^{1,2}, 加藤 聖^{2,3}

(¹金大・医・保健, ²金大・院医・脳情報分子, ³CREST)

P02-F-40 A member of collapsin response mediator protein(CRMP) family has a potent neurite elongation activity in nerve regeneration

○鈴木 康博^{1,2}, 中込 咲綾¹, 稲垣 直之³, 貝淵 弘三⁴, 木山 博資¹

(¹大市大・院医・機能細胞形態, ²旭医・一内, ³奈良先端・院・バイオ・細胞情報, ⁴名大・院医・細胞情報薬理)

P02-F-41 神経周膜の修復・端側縫合移植モデルにおける神経再生

○岡島 誠一郎¹, 小田 良¹, 平川 永徳¹, 小橋 裕明¹, 久保 俊一¹, Julia Terzis²

(¹京府医大・整形, ²バージニア医学校)

P02-F-42 Damage induced neuronal endopeptidase (DINE/ECEL) expression is regulated by LIF and a deprivation of NGF

○加藤 隆一^{1,2}, 瀬尾 (桐生) 寿美子², 木山 博資²

(¹札医大・医・泌, ²大阪市大・院医・機能細胞形態)

P02-F-43 脳に移行する細胞を骨髓細胞から選択する方法の検討

○小野 健治^{1,2}, 吉原 賢^{1,2}, 田中 謙二¹, 瀧井 猛将², 小野 寄 菊夫², 澤田 誠¹

(¹藤田保健大・総医研・難病治療, ²名市大・院薬・生体防御)

P02-F-44 ガレクチン-1に対するモノクローナル抗体の作製の試み

○堀 浩一郎¹, 藤牧 香代¹, 吉村 和法¹, 門屋 利彦², 堀江 秀典³, 野村 正彦¹

(¹埼玉医大・医・生理, ²医薬開発研究所, ³早稲田大・先端バイオ研究所)

P02-F-45 脱髄モデルマウスへの神経幹細胞の移植

○松本 路生¹, 鹿川 哲史¹, Francois Lachapelle², Anne Baron-Van Evercooren², 池中 一裕¹

(¹生理研・神経情報, ²INSERM U546, France)

ミエリン

P02-F-46 Two novel sulfated oligosaccharides derived from P0 of bovine peripheral nerve myelin

○閻 剣虹, 北村 邦男, 野村 正彦

(埼玉医大・生理)

P02-F-47 Structural studies on the glycoprotein-derived oligosaccharides in the peripheral nerves increased during maturation or by nerve crush injury

○北村 邦男¹, 吉野 百合子², 閻 剣虹¹, 野村 正彦¹

(¹埼玉医大・生理, ²埼玉医大・産婦人科)

P02-F-48 シバラーミュータントマウス由来オリゴデンドロサイト前駆細胞を用いたミエリン形成機構の解析

○阿相 皓晃¹, 中原 仁^{1,2}, 清和 千佳¹

(¹老人研・神経生物, ²慶大・解剖)

発表抄録

(Abstracts)

Plenary Lectures (PL-1~PL-4)	269
Invited Lectures (IL-1~IL-4)	273
2002 ISN Symposium (ISN-1~ISN-5)	277
Open Symposium (OS-1~OS-6)	282
Symposium (S1~S11)	285
Poster	
(P01-E-1~P01-E-66)	313
(P01-F-1~P01-F-47)	346
(P02-E-1~P02-E-68)	370
(P02-F-1~P02-F-48)	404

PL-1

Neuronal Function and Intracellular Transport: Gene, Structure, Dynamics, Function and Diseases of Kinesin superfamily motor proteins, KIFs.

Nobutaka Hirokawa

University of Tokyo, Graduate School of Medicine, Tokyo, Japan

In neurons many proteins are transported and sorted to axon and dendrites as membranous organelles or protein complexes along microtubules. This mechanism plays fundamental role on neuronal morphogenesis, function and survival and also relates deeply with neuronal diseases. To understand this mechanism we identified all members, 45, of microtubule based molecular motors, KIFs in mammals. Using multiple molecular cell biological and molecular genetical approaches we have characterized these members. In this lecture I will focus on KIF1A, 1Bb and 17. KIF1A is the fastest anterograde monomeric motor for transport of precursors of synaptic vesicles and essential for neuronal function and survival. KIF1Bb conveys also synaptic vesicle precursors and is a responsible gene of Charcot-Marie-Tooth type 2A hereditary neuropathy. KIF17, expressed specifically in neurons, transports vesicles containing NMDA receptors in dendrites. KIF17 recognizes and binds to NMDA receptor via interaction composed of KIF17-mLin10-mLin2-mLin7-NR2B. In this lecture I will also introduce the mechanism of transport of mRNAs and AMPA receptors. The mechanisms how the functional molecules are sorted specifically to axon and dendrites will be presented, too. Thus, our studies revealed that transport of important functional molecules is accomplished very precisely by these new molecular motors.

PL-2

Molecular basis of apoptosis: Bcl-2 family proteins as a life-or-death switch

Yoshihide Tsujimoto

Osaka University Medical School, Dept. of Post-Genomics & Diseases, CREST of JST

Programmed cell death or apoptosis, a cellular destruction mechanism plays an integral part in a variety of biological events including morphogenesis, tissue homeostasis, and removal of unwanted or harmful cells. Because dysregulation of programmed cell death leads to a variety of human diseases including various neurodegenerative diseases, the knowledge of the molecular mechanism of cell death is important not only for basic research but also for developing therapeutic strategy for these diseases.

Extensive studies performed in the last 10 years have unveiled a considerable amount of the molecular basis of cell death including apoptosis and some forms of necrosis. Accumulating evidence indicates that a change in mitochondrial membrane permeability is one of the most critical events in cell death in mammals because it leads to mitochondrial dysfunction (necrosis) as well as the translocation of apoptogenic factors, including the translocation of cytochrome c into the cytoplasm, which in turn activates apoptosis-driving proteases called caspases. The Bcl-2 family of proteins, consisting of anti-cell death and pro-cell death members, regulate cell death by forming heterodimers between members with opposite activities as well as by functioning independently from each other. This family of proteins determines survival-or-death of cells mainly by controlling mitochondrial membrane permeability.

We have recently shown that VDAC (mitochondrial porin) is required for apoptotic and necrotic mitochondrial changes, and is one of the targets for Bcl-2 family members: pro-apoptotic Bax and Bak induce a conformational change of VDAC to allow the passage of apoptogenic factors such as cytochrome c through the channel, while anti-apoptotic Bcl-2 and Bcl-x_L through direct binding close the VDAC to preserve the mitochondria, which implies a role of Bcl-2 family proteins as channel regulators. This provides us with a crucial basis from which we could dissect in details the functions of Bcl-2 family of proteins. We have also previously reported that Bcl-2 defect is involved in pathogenesis of one neurodegenerative disease, which implies that Bcl-2 is an important player in neuroprotection.

By presenting additional recent studies, I will discuss how death signals are transmitted in a cell, how life-or-death of a cell is determined, and finally how we could contribute to developing a therapeutic strategy for the diseased with dysregulated cell death.

PL-3

Synaptic plasticity regulated by stargazin and PSD-95

David S. Bredt

Department of Physiology, University of California at San Francisco

Excitatory synaptic transmission is mediated primarily by glutamate acting on two classes of ligand gated ion channels: AMPA receptors and NMDA receptors. The AMPA receptors are involved in moment-to-moment signaling, whereas NMDA receptors play an important role in initiating synaptic plasticity. Recent cell biological analyses have emphasized remarkable differences in regulation of the synaptic expression of these two classes of receptors. NMDA receptors are stable components of the postsynaptic density (PSD), whereas AMPA receptors cycle on and off the synaptic membrane in a manner that is tightly controlled by neuronal activity. Regulated insertion and removal of AMPA receptors at the synapse provides a mechanism for altering synaptic efficacy and for storing information in brain. This has led to intensive study of the molecular architecture that recruits and anchors glutamate receptors at the synapse.

In this symposium, I will describe experiments showing that synaptic AMPA receptor levels are regulated by a synaptic complex of the tetraspanin, stargazin and the postsynaptic density protein, PSD-95. Stargazin, which is mutated in epileptic stargazer mice, is the first transmembrane protein found to interact with glutamate receptors. In addition to suffering epilepsy, stargazer mice exhibit cerebellar ataxia. Physiological studies showed that synaptic AMPA receptor currents are selectively absent from synapses on cerebellar granule cells, though NMDA-mediated responses are normal at these same synapses. Despite this lack of functional AMPA receptors, the levels of the AMPA receptor subunits GluR2 and GluR4 appear normal in stargazer cerebellum. The lack of synaptic AMPA responses reflects a block in AMPA receptor trafficking to the synapse as electron microscopic analysis with immunogold labeling shows a profound loss of synaptic AMPA receptors. In addition to disrupting synaptic AMPA receptors, lack of stargazin also abolishes AMPA receptor responses at extrasynaptic sites.

The structure of stargazin helps explain these dual roles in regulation of AMPA receptors. Stargazin is a tetraspanin, a protein with four transmembrane domains. The cytosolic C-terminal tail of stargazin contains a PDZ binding site, which binds to PSD-95. This PDZ binding site is critical for synaptic function of AMPA receptors, as transfecting mutant granule cells with a stargazin construct lacking the extreme C-terminus (stargazin Δ C) does not rescue synaptic responses. Interestingly, stargazin Δ C, which also binds to AMPA receptors, does rescue extrasynaptic AMPA receptor responses. These data suggest that stargazin mediates two distinct steps in trafficking AMPA receptors to the synapse. First, the transmembrane domains of stargazin interact with AMPA receptors and regulate the delivery of AMPA receptors to the cell surface. Second, interaction of the C-terminal PDZ binding site of stargazin with PSD-95 targets the AMPA receptors to synapses. Expression of a mutant stargazin lacking the PDZ-binding domain in hippocampal pyramidal cells disrupts synaptic AMPA receptors, suggesting that stargazin-like AMPA receptor targeting mechanisms are widespread in the CNS.

By binding to stargazin, PSD-95 participates in synapse development and plasticity. Overexpression of PSD-95 in hippocampal neurons drives maturation of excitatory synapses as evidenced by enhanced synaptic clustering and function of AMPA receptors. Interestingly, PSD-95 overexpression also mediates a retrograde signal to enhance maturation and function of presynaptic nerve terminals that synapse upon neurons overexpressing PSD-95.

How might PSD-95 dynamically regulate synaptic glutamate receptors and synaptic plasticity? PSD-95 contains two critical cysteine residues at positions 3 and 5, which are sites of protein palmitoylation. This post-translational modification involves addition of palmitate, a 16-carbon fatty acid, via a labile thioester linkage. Palmitoylation of PSD-95 is essential for clustering at the PSD. Akin to protein phosphorylation, palmitoylation is a reversible process that is dynamically regulated by specific cellular stimuli. We therefore asked whether this lipidation of PSD-95 is activity-dependent at the synapse and whether this might regulate glutamate receptor function.

Using cultured hippocampal neurons, we found palmitate cycling on PSD-95 at the synapse and find that palmitate turnover on PSD-95 is regulated by glutamate receptor activity. Acutely blocking palmitoylation disperses synaptic clusters of PSD-95, and causes a selective loss of synaptic AMPA receptors. We also find that rapid glutamate-mediated AMPA receptor internalization requires depalmitoylation of PSD-95. In a non-neuronal model system, clustering of PSD-95, stargazin and AMPA receptors is also regulated by on-going palmitoylation of PSD-95 at the plasma membrane. These studies suggest that palmitate cycling on PSD-95 can regulate synaptic strength and regulates aspects of activity-dependent plasticity.

PL-4

Tyrosine Hydroxylase Phosphorylation

Peter R Dunkley

School of Biomedical Sciences, Faculty of Health, The University of Newcastle, NSW 2308, Australia.

When catecholamines are secreted there is a need to replace the lost catecholamines. The most important short-term mechanism is the activation of Tyrosine Hydroxylase (TH), the rate limiting enzyme in catecholamine biosynthesis. TH activity is normally inhibited by catecholamine binding. Stimuli that lead to an increase in intracellular calcium and catecholamine secretion also increase the phosphorylation of TH. This phosphorylation releases bound catecholamines and "activates" TH. The three sites on TH phosphorylated in response to a range of stimuli are Ser19, Ser31 and Ser40. Ser19 is generally phosphorylated first, followed by Ser40 and finally Ser31. It is established in vitro that phosphorylation of Ser40 is the most important site for activation of TH. Phosphorylation of TH on Ser31 has only a minor effect on TH activity and phosphorylation of Ser19 has no effect.

What protein kinases and protein phosphatases act on TH to explain the sequence of phosphorylation events? In bovine adrenal chromaffin cells it is established that Ser19 is phosphorylated by CaMPK-II and by MAPKAPK2 (Bobrovskaya et al., J Neurochemistry 78, 490-498, 2001). CaMPK-II is rapidly activated by an increase in intracellular calcium induced by the stimuli that lead to catecholamine secretion and TH activation. However, Ser40 can also be phosphorylated by CaMPK-II and the delayed phosphorylation of Ser40 is therefore likely to be due to other reason(s). Ser31 is phosphorylated by ERK. Activation of this kinase takes some time to occur due to a sequence of protein kinases needing to be activated and this explains the delayed phosphorylation of Ser31. Ser40 is phosphorylated by a large number of protein kinases, but it is generally agreed that PKA predominates. Protein phosphatases 2A and 2C both dephosphorylate Ser19, Ser31 and Ser40. In recent studies we have established that dephosphorylation of Ser40 by PP2C is prominent under certain circumstances.

Why does Ser19 phosphorylation precede Ser40 phosphorylation on TH? It is possible that hierarchical phosphorylation of TH can explain this sequence of events. Hierarchical phosphorylation is defined as the rate of phosphorylation of a protein at one site being dependent on prior phosphorylation at another site on the same protein. We provided the first evidence for hierarchical phosphorylation of TH whereby the phosphorylation of TH on Ser19 increases the rate of phosphorylation of TH at Ser40 (Bevilaqua et al. J Biological Chemistry, 276, 40411-40416, 2001). We have used electrospray ionization mass spectrometry to determine the rate constants for the phosphorylation of Ser19 and Ser40 using a consecutive reaction model. The rate constant for the phosphorylation of Ser40 is approximately 3 fold higher if Ser19 is already phosphorylated. Our results suggest that the phosphorylation of Ser19 alters the conformation of TH to allow increased accessibility of Ser40 to kinases. Hierarchical phosphorylation of TH could therefore explain the delayed phosphorylation of Ser40 relative to Ser19. We have recently extended these studies to bovine adrenal chromaffin cells and the data suggest that hierarchical phosphorylation of TH also occurs in intact cells.

IL-1

Molecular Mechanism and Hierarchical Structure of Circadian Clock

Ken-ichi Honma

Hokkaido University Graduate School of Medicine, Department of Physiology

The circadian clock in mammals regulates the time structure of physiology and behaviors by generating the circadian rhythms in the suprachiasmatic nucleus (SCN) and synchronizing them with environmental periodicities, especially a light-dark cycle.

In the last few years, our knowledge on the molecular mechanism of circadian clock was rapidly accumulated and there seems to be a general agreement that at least 6 clock genes, *Per1*, *Per2*, *Clock*, *BMAL1*, *Cry1*, *Cry2*, are involved in the core of rhythm generation system. The protein products of *Clock* and *BMAL1*, bHLH-PAS type transcription factors, form heterodimers which activate the transcription of *Per* gene. *Per* protein products in turn inhibit the transactivation by *CLOCK/BMAL1* together with the protein products of *Cry1* and *Cry2*, closing a auto-regulatory feedback loop. Recently, the third factor is proposed to be involved in the auto-feedback loop, which represses the transactivation of *Per* genes by *CLOCK/BMAL1*. It is a bHLH-PAS type transcription factor and expressed in the SCN. Its expression is enhanced by light stimulus, suggesting an intracellular transmitter of light in the circadian system.

There is evidence that the SCN contains multiple oscillating neurons. Circadian rhythms of individual neurons in the dispersed cell culture are different in their periodicity but become identical in the slice culture in which the normal assemblies of SCN are preserved. The finding suggests an important role of cell-to-cell communication in mutual synchronization of the oscillating neurons. The synchronization of oscillating neurons is critical for the SCN to function as the circadian pacemaker. Clock mutation abolishes the circadian rhythms in behaviors under constant darkness. However, individual SCN neurons in *Clock* mutant mice exhibit robust circadian rhythms for a substantial length, indicating that the dynamics of multi-oscillator structure strongly effects the final outputs of circadian clock.

There is also evidence that more than one pacemakers are operating in the SCN. The SCN neurons secrete two major peptide hormones, vasopressin (AVP) and vasoactive intestinal peptide (VIP). In the slice culture, AVP and VIP in the culture medium show robust circadian rhythms with the peak phases at almost the same time of day. However, the peak phases of peptide rhythms become separate and even antiphasic, when antimetabolites is administered in the culture medium. These findings suggest two different pacemakers in the SCN which regulate AVP and VIP separately. Dissociation of circadian rhythms in different functions are also known in the level of whole body. Chronic treatment of methamphetamine (MAP) in rats induces an internal desynchronization between the behavioral rhythm and circadian rhythms in plasma melatonin. In these animals, the circadian rhythms in *Per1* and *Per2* expression in the SCN are always synchronized with the melatonin rhythms, while those in the striatum and cerebral cortexes are in parallel with the behavioral rhythm. These findings indicate that there are peripheral oscillators which directly drive the behavioral rhythms. These peripheral oscillators are normally under the control of the central SCN pacemaker but dissociated from it under particular conditions.

In conclusion, the circadian clock in mammals consists of multiple oscillators. Although a molecular auto-feedback loop is a core of subcellular rhythm generation system in the SCN, a hierarchical structure of the circadian clock provide a stable and adaptable rhythmicities to the body functions.

IL-2

Molecular and Neural Mechanisms of Eyeblink Conditioning Studied with Mutant Mice

Yutaka Kirino

Laboratory of Neurobiophysics, School of Pharmaceutical Sciences, The University of Tokyo

Classical eyeblink conditioning is a form of associative motor learning and critically depends on the cerebellum. It typically uses a tone as the conditioned stimulus (CS), and a periorbital shock or corneal air puff as the unconditioned stimulus (US). By repeated presentations of the CS paired with the US, the CS comes to elicit an eyeblink, which is called the conditioned response (CR). The memory trace is believed to be formed and stored in the anterior interpositus nucleus and overlying cerebellar cortex. As a key mechanism in the cerebellar cortex, the long-term depression (LTD) at parallel fiber-Purkinje cell synapses has been proposed. The glutamate receptor subunit $\delta 2$ (GluR $\delta 2$) is selectively expressed at the dendritic spines of PCs and essential to the induction of cerebellar LTD. Therefore, GluR $\delta 2$ -null mice, in which cerebellar LTD is specifically impaired, provide a useful means to test the cerebellar LTD hypothesis.

Mutant mice lacking GluR $\delta 2$ showed severe learning impairment in *delay* conditioning in which the US was delayed and co-terminated with the CS, supporting the hypothesis [Kishimoto, *et al.*, *Eur J Neurosci* 13, 1249 (2001)]. However, we also found that these mutant mice learned normally in trace conditioning in which a stimulus-free trace interval intervened between the CS and US, and even when we used a "trace 0 paradigm" (in which the US started just after termination of the CS) [Kishimoto, *et al.*, *Eur J Neurosci* 14, 1515 (2001)]. This surprising finding was confirmed in experiments with another line of mutant mice lacking phospholipase C $\beta 4$ [Kishimoto, *et al.*, *Neuroreport* 12, 2919 (2001)] or wild type mice subjected to intracerebellar injection of an NO synthase inhibitor L-NAME [Suzuki, *et al.*, submitted], both lacking cerebellar LTD. Therefore, there may be variations in the cerebellar neural substrates for eyeblink conditioning depending on the CS-US temporal overlap.

We recently have found that the muscarinic acetylcholine receptor antagonist scopolamine impairs learning in trace 0 paradigm experiments with GluR $\delta 2^{-/-}$ mice [K. Takatsuki, *et al.*, *Neuroreport* 13, 159 (2002)] and that mGluR1 outside the cerebellum is essential for trace conditioning but not for delay conditioning [Kishimoto *et al.*, submitted]. These findings suggest a contribution of the hippocampus to the LTD-independent learning mechanism. To examine this possibility further, we looked at the effects of hippocampal lesions on learning in GluR $\delta 2^{-/-}$ mice [Takatsuki *et al.*, submitted]. The GluR $\delta 2^{-/-}$ mice whose dorsal hippocampi were aspirated exhibited severe learning impairment in the trace 0 paradigm experiments, while the control GluR $\delta 2^{-/-}$ mice that received a lesion in the cortex overlying the hippocampus were able to learn promptly. Wild-type mice did not show such hippocampal dependency in the trace 0 paradigm. We therefore concluded that the hippocampus is essential for learning with a trace 0 paradigm when cerebellar LTD is disrupted. In contrast, GluR $\delta 2^{-/-}$ mice that had received post-training hippocampal lesions retained the memory as well as the control GluR $\delta 2^{-/-}$ mice did. The hippocampal lesion also did not affect memory retention in the wild-type mice. These results suggest that the hippocampus is *not* essential for *retention* of motor memory in LTD-deficient mice. Thus, the present studies clearly argue that the hippocampus is essential for memory formation in cerebellar motor learning when cerebellar LTD is disrupted. These studies suggest that the neural network which underlies learning and memory is both flexible and robust.

IL-3

Regulation of nucleocytoplasmic transport and cell proliferation by Ran GTPase.

Takeharu Nishimoto

Department of Molecular biology, graduate School of Medical science, Kyushu University

Ran is a Ras-like nuclear small GTPase. Its GTPase activating protein, RanGAP1, is localized in the cytoplasm and the RanGDP/GTP exchange factor, RCC1, is on the chromosome. Thus, Ran shuttles between the nucleus and the cytoplasm to complete its GTPase cycle, making a gradient of RanGTP concentration from the nucleus to the cytoplasm. Along this gradient of RanGTP concentration, mRNA and proteins were transported from the nucleus to the cytoplasm by the aid of Ran and exportins. In addition to nucleocytoplasmic transport, Ran is involved in microtubule organization. Since nuclear membrane is absent in M-phase of mammalian cells, microtubule assembly stimulated by Ran is thought to be independent from nucleocytoplasmic transport. It is a matter of debate as to how the nucleocytoplasmic transport is related to the cell cycle progression. We think that RCC1 may play an important role for coupling the nucleocytoplasmic transport with the cell cycle progression, since the coupling of S-phase completion and M-phase initiation is abolished upon loss of RCC1 in hamster tsBN2 cells arrested with hydroxyurea, an inhibitor of ribonucleotide reductase.

RCC1 comprises of sevenfold repeated antiparallel β -sheets arranged in a circular fashion, a fold called a β -propeller. Its affinities to RanGDP and RanGTP are similar. Thus, the extra factors such as either RCC1- or Ran-binding proteins may shift the equilibrium further toward formation of the RanGTP complex. Since a RCC1 mutant without DNA-binding activity still locates on the chromatin and rescues a temperature-sensitive lethality of hamster tsBN2 cells, we assume that there must be some proteins which anchor RCC1 on the chromatin. Such RCC1-binding proteins may play a role for coupling the cell cycle progression with the nucleocytoplasmic transport, along with RCC1. Ran-binding proteins are grouped into RanGTP and RanGDP binding proteins. RanBP1, RanBP2 and RanBP3 bind to RanGTP, and Ntf2p/p10 binds to RanGDP. Mog1p which has been identified as a suppressor of ts mutants of *S. cerevisiae* Ran homologue Gsp1p, binds efficiently to RanGTP, while the crystal structure of Mog1p is similar to Ntf2p/p10 that specifically binds to RanGDP. Other Ran-binding proteins thus far identified are Dis3p, RanBPM and proteins belonging to the Importin β family.

In addition to Importin β family proteins, RanBP1, RanBP2 and RanBP3 are required for nucleocytoplasmic transport. RanBP1 that stimulates the RanGAP activity, is in the cytoplasm and RanBP3 in the nucleus. RanBP2 possesses four RanBP1 domains, in addition to a zinc finger domain and a cyclophilin-homologous domain. RanBP2 is localized on the cytoplasmic filament of the nuclear pore complex (NPC). Disruption of the MOG1 gene make *S. cerevisiae* temperature-sensitive for cell proliferation, causing defects in nuclear protein import, but not export. It is a matter of debate as to how Mog1p is involved in the nucleocytoplasmic transport. Ntf2p/p10 is reported to be required for nuclear import of RanGDP. In contrast to these facts related to nucleocytoplasmic transport, Dis3p is one of components comprising the exosome which is required for 3' processing of 7S-to-5.8S rRNA. Indeed, *S. cerevisiae* gsp1 mutants are defective in rRNA processing. Originally, we thought that Ran regulates microtubule assembly through RanBPM since a truncated form of RanBPM was localized in the centrosome. But it is unlikely since the full-length RanBPM which has a long stretch of glutamine and proline in the N-terminal region, is not localized in the centrosome. In order to further clarify the *in vivo* function of the Ran GTPase cycle, we are currently preparing a mouse defective in the RCC1 gene.

IL-4

Bulk Protein Degradation - Molecular Dissection of Autophagy -

Yoshinori Ohsumi

National Institute for Basic Biology

Cellular activities are maintained in balance of continuous synthesis and degradation of many proteins. Bulk degradation of cytosol and organelles is crucial for cellular homeostasis under nutrient limitation, cell differentiation and development. This process occurs in a lytic compartment, lysosome/vacuole. Major route for lysosome/vacuole, autophagy, proceeds by dynamic membrane reorganization. To elucidate the molecular mechanism of the autophagic process we have used the yeast, *S. cerevisiae*, as a model system. Under various nutrient starvation conditions, yeast cells induce active sequestration of the cytoplasmic components to the vacuole. This whole process is topologically the same as macroautophagy known in mammalian cells. Taking advantage of yeast system, we isolated total 15 autophagy-defective mutants (*apg*). All mutants showed a similar phenotypes, reduced protein degradation, loss of viability under starvation, and defect in sporulation. Most *APG* genes have mammalian homologues, suggesting that autophagy is well conserved from yeast to human.

We are currently working on the structures and functions of these yeast *Apg* proteins and applying these findings to higher eukaryotes. Among them we reported a unique protein conjugation system. The C-terminal glycine residue of *Apg12p*, a 186 amino acid protein, conjugates to a lysine residue of *Apg5p*, a 294-amino acid protein via an isopeptide bond. The conjugation reaction is mediated *Apg7p*, E1 like activating enzyme and *Apg10p*, conjugating enzyme. This conjugate is constitutively synthesized and is essential for autophagosome formation. Furthermore recently we discovered another ubiquitin like protein, *Apg8*, is activated by *Apg7* and transferred to *Apg3* through a thioester intermediate. Interestingly *Apg8* finally conjugates with one of major phospholipid, phosphatidylethanolamine via an amido bond. *Apg8* is synthesized as a precursor and processed to expose a C-terminal glycine by a novel cysteine proteinase, *Apg4*. *Apg4* has another role to deconjugate *Apg8-PE*, which is also important of autophagy. These findings have expanded the concept of UBL system to conjugation reaction with not only protein but other molecules such as lipids. Recent analyses show that these two systems are working very closely and interacting each other. Other *Apg* proteins form a protein kinase complex and autophagy-specific PI3 kinase complex. These four systems play concerted function during autophagosome formation.

Still we have many problems to be solved such as mechanism and signal transduction of induction of autophagy, origin of autophagosome membrane, mechanism of membrane formation, fusion and degradation of autophagosome and reutilization of degradation products. Furthermore it is remained to be uncovered what is physiological role of autophagy in mammals. *Apg* proteins give us many clues to answer these problems. Present stage of our studies and perspective will be discussed.

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ISN-1

AMPA receptor trafficking during plasticity

Robert Malinow

Cold Spring Harbor Laboratory, USA

The molecular mechanisms underlying synaptic plasticity are becoming more clear. Activity-induced synaptic enhancement in hippocampus is accomplished at different developmental ages by delivery of AMPA receptors containing the different but homologous subunits GluR4 or GluR1. Here we show that activity-driven phosphorylation of GluR4 by PKA is necessary and sufficient for delivery of receptors to synapses during early development. Such phosphorylation relieves a retention interaction that, in the absence of synaptic activity, maintains GluR4-containing receptors away from the synapse. In contrast, phosphorylation of GluR1 by PKA is necessary but not sufficient for delivery of receptors to synapses in older animals. For GluR1, CaMKII is also necessary. Thus, a mechanism that mediates plasticity early in development becomes a gate for plasticity later in development. Increasing requirements over development may be one way that plasticity becomes more specific and also recalcitrant with age.

ISN-2

Molecular Genetics of the Synapse Formation and Function

Masayoshi Mishina

Department of Molecular Neurobiology and Pharmacology, Graduate School of Medicine, University of Tokyo

Cumulative evidence suggests that the dynamic structure-function relationship of the brain largely depends on the experience-dependent development and functioning of synapses. Studies on the glutamate receptor (GluR) channel shed light on the molecular basis of neural input-dependent synapse dynamics. The *N*-methyl-D-aspartate (NMDA) subtype of GluR plays key roles in synaptic plasticity as a molecular coincidence detector. We revealed that the multiple GluR/NR2 subunits carrying the glutamate binding site are major determinants of the NMDA receptor molecular diversity. We examined the physiological roles of respective GluR subunits by gene targeting. Mutant mice lacking GluR1 showed increased thresholds for both hippocampal long-term potentiation and contextual learning. Heterozygous mutant mice with reduced GluR2 exhibited exaggerated startle responses to acoustic stimuli, indicating forebrain-specific GluR2 regulates the reflex because the primary circuit of the startle response is located in the lower brainstem. GluR2 mutant mice died shortly after birth and failed to form the whisker-related neural pattern (barrelles) in the brainstem trigeminal complex. These results suggest that the molecular diversity of GluR underlies the diverse physiological roles of NMDA receptor channels. GluR δ is a novel member of the GluR channel family we found by molecular cloning. GluR δ 2 is selectively localized in cerebellar Purkinje cells. Analyses of GluR δ 2 mutant mice revealed that the GluR δ 2 subunit plays essential roles in motor learning and cerebellar LTD and in refinement and maintenance of Purkinje cell synapses. GluR δ 2 mutant mice failed to learn in the delay paradigm of eyeblink conditioning, but not in the trace paradigm. On the other hand, GluR δ 1 mutant mice exhibited an impairment of learning in the trace paradigm, suggesting that neural substrates underlying eyeblink conditioning are different depending on the temporal overlap of the conditioned and unconditioned stimuli. Our molecular genetic studies on GluR channels imply that learning and memory may share a common molecular mechanism with synapse formation during brain development.

Thus, elucidation of how the neural network is formed and modulated is essential to understand how the brain functions. To address this issue, we developed a novel strategy that enables visualization and manipulation of developing neurons *in vivo*. Olfactory and retinotectal projection systems in transparent zebrafish embryos are suitable to analyze axonal pathfinding and synptogenesis *in vivo*. Microinjection of an olfactory marker protein gene promoter-driven double-cassette vector directed the expression of both the dominant form of PKA and green fluorescent protein fused with the microtubule-associated protein tau in the same olfactory neurons. The dominant-negative form of PKA enhanced the turning of olfactory neuron axons in the olfactory placode, whereas the disturbance effect of the constitutively active form on the axonal pathfinding was prominent in the olfactory bulb. These results suggest that the switching of PKA signaling in developing olfactory sensory neurons is important for axonal pathfinding through the boundary between the olfactory placode and the olfactory bulb *in vivo*. We thus propose that the regulation of PKA signaling plays a key role in the long-distance axonal pathfinding through intermediate guideposts. Microinjection of the nicotinic acetylcholine receptor β 3 (nAChR β 3) gene promoter-driven double-cassette vectors directed the expression of dominant-negative glycogen synthase kinase-3 β (dnGSK-3 β) and EGFP in the same neurons. We found that expression of the dominant negative form of zebrafish GSK-3 β suppressed the arborization field of RGC axon terminals in the tectum as estimated by the reduction of arbor branch length and arbor areas. Furthermore, suppression of GSK-3 β activity increased the size of vesicle-associated membrane protein 2-EGFP puncta in RGC axon terminals at the early stage of innervation to the tectum. These results suggest that GSK-3 β regulates the arborization field and maturation of RGC axon terminals *in vivo*.

ISN-3

Cdk5: A new player at the neuromuscular synapse

Nancy Y. Ip

Department of Biochemistry, Molecular Neuroscience Center, and Biotechnology Research Institute, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China

The development of the neuromuscular junction (NMJ) depends on reciprocal signaling between motor neurons and their target muscle fibers. A number of ligand-receptor interactions have been implicated to mediate these signaling cascades at the NMJ. For example, MuSK receptor is part of the receptor complex that mediates agrin-induced aggregation of AChRs while activation of ErbB receptors by neuregulin (NRG) regulates the synaptic transcription of acetylcholine receptors (AChR). My laboratory is interested in delineating the roles of receptor tyrosine kinases in the development and maintenance of the NMJ. During the course of our studies on the regulation of AChR expression, we have identified a new player, cyclin-dependent kinase 5 (Cdk5), which is involved in signaling at the NMJ.

Cdk5, a member of the Cdk family, is involved in cellular functions that are not related to the regulation of cell cycle progression. While other members of the family associate with cyclins, the serine/threonine kinase activity of Cdk5 depends on its regulatory partners, p35 or p39, which are prominently expressed in the CNS. To date, the best-characterized role for Cdk5 is in regulating neuronal migration and axonal guidance as well as cytoskeletal dynamics. There is also increasing evidence that links Cdk5 activity to the regulation of membrane transport and dopamine signaling. In addition to its known functions in neuronal development, recent evidence suggests a novel role for Cdk5 in the modulation of post-synaptic functions. For example, Cdk5 can regulate the expression of AChR in muscle and NMDA receptors in cultured hippocampal neurons. In adult muscle, Cdk5 and its activators are localized to the NMJ and mediate the actions of NRG by associating with the ErbB receptors. Treatment of cultured myotubes with NRG increases the p35-associated Cdk5 kinase activity. While inhibition of Cdk5 activity attenuates NRG-induced ErbB activation and AChR transcription in cultured myotubes, overexpression of p35 results in an increase in AChR promoter activity in muscle. To further understand the regulation of Cdk5 and p35 in rat skeletal muscle during the formation of NMJ, we have examined whether the expression of p35 and Cdk5 can be regulated after nerve injury. While the transcripts of cdk5 and p35 increase in muscle after denervation, they exhibit different temporal profiles suggesting that these changes in gene transcription might be regulated by different mechanisms. Furthermore, the dependence of p35 mRNA expression on both trophic factors and electrical activity is similar to that observed for AChR. Recent analysis of the Cdk5 null mice in our laboratory reveals an interesting abnormal phenotype at the developing NMJ. Moreover, our studies on the identification of p35-interacting proteins would delineate the signaling molecules that act in concert with the Cdk5 complex to affect the process of synapse formation.

ISN-4

Structure, regulation and function of PKB/Akt—a pleiotropic protein kinase involved in multiple signaling pathways

Brian A. Hemmings

Friedrich Miescher-Institute, Switzerland

ISN-5

EXCITATORY AMINO ACID TRANSPORTERS: NEW INSIGHTS INTO STRUCTURE, FUNCTION AND REGULATION

Amara, S. G.

Vollum Institute and Howard Hughes Medical Institute, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Portland OR USA 97201

Neurotransmitter transporters present on the plasma membrane contribute to the clearance and recycling of neurotransmitters and have a profound impact on the extent of receptor activation that occurs during neuronal signaling. Work in our laboratory has focused on the structure, regulation and cellular physiology of sodium-dependent transporters for the biogenic amines and another family of sodium-dependent carriers for excitatory amino acid neurotransmitters. Five different human subtypes of excitatory amino acid transporters (EAATs1-5) and their homologues in several species have been identified. The glial carriers (EAAT1 and EAAT2) are abundantly expressed and are the major regulators of extracellular glutamate concentrations in the CNS. The neuronal isoforms of glutamate transporters (EAAT3 and EAAT4) are located predominantly outside the synapse, in the perisynaptic membrane and/or in spine regions in contact with glial cells. Thus, rather than being poised to influence synaptic glutamate concentrations, the neuronal carriers are positioned to regulate the amount of glutamate that escapes the synapse. This unique distribution implies that glutamate carriers may indirectly control the amount of glutamate available to activate metabotropic glutamate receptors and thus links them to the feedback control of excitability and neurotransmitter release.

Although the EAATs are thought to regulate CNS concentrations of glutamate, several subtypes also possess a ligand-gated chloride channel activity that may regulate neuronal excitability and signaling in a different way. Structure-function studies support the notion that the binding sites for substrates, inhibitors, and co-transported ions, as well as, the transport pathway are formed from multiple domains that may or may not be adjacent to one another in the primary sequence. Efforts to link specific domains with various functional properties have demonstrated the importance of residues within highly conserved domains in the C-terminal half of the carrier for substrate binding and transport. Recently a cluster of residues in an extracellular helix has been identified where mutations or modifications disrupt transport, but preserve the substrate-activated anion current. These observations suggest that the substrate-gated chloride conductance is linked to a different pathway or conformation than that required for substrate transport. This lecture will consider some of the novel aspects of neurotransmitter transporter function and will present the results of molecular genetic, electrophysiological and cell biological approaches aimed at defining the relationships between neurotransmitter transporter structure, substrate transport, inhibitor binding and ion permeation.

OS-1

How important model animals are in research for psychiatric disorders!

○Keiji Wada¹, Yuko Santo-Yamada¹, Etsuko Wada^{1,2}, Hiroshi Maeno¹, Kazuyuki Yamada^{1,3}

¹Natl. Inst. Neurosci. NCNP, ²JSPS, ³BSI, RIKEN

One of the serious issues against the research for psychiatric disorders is a shortage of model animals. We have aimed to improve the situation by providing useful models. In this symposium, we are going to present our recent studies on bombesin (BN) receptor knockout mice. In mammals, BN-like peptides, gastrin releasing peptide (GRP) and neuromedin B (NMB), exert various biological activities including behavior through G protein coupled receptors, GRP-R and NMB-R. Besides the two receptors, bombesin receptor subtype 3 (BRS-3) is identified, but its natural ligand has not been identified yet. We generated three strains of BN-like peptide receptor knockout mice, and each strain of the knockout mice exhibited unique phenotypes. Non-aggressive social behavior was elevated in GRP-R deficient mice. In contrast, BRS-3 deficient mice showed reduced non-aggressive social behavior as well as anxiety-related behavior. The mice also exhibited increased risk assessment behavior. NMB-R knockout mice showed altered response to stress and reduced risk assessment behavior. These results indicate that BN-like peptides/receptors play an important role in emotional behavior. Since emotion and anxiety are important subjects in human life, the knockout mice we generated would be useful for investigating human psychiatric disorders.

OS-2

心・行動と細胞内情報伝達系

○齋藤 利和

札幌医大・医・神経精神

向精神薬の投与によって脳内の情報伝達系に変化を引き起こすことが広く知られている。その中でアデニル酸シクラーゼ(AC)系は神経精神疾患患者の脳においてよく検索されている情報伝達系の一つである。例えば、慢性的飲酒はアルコール依存症者の脳においてACとプロテインキナーゼA(PKA)活性を低下させることが証明されている。さらにうつ病者の脳におけるGs-ACカップリングの障害を抗うつ薬が補正することも知られている。このようにアルコール依存症者やうつ病患者の不安や抑うつ気分などの臨床症状はサイクリックAMP情報伝達系の変化によることが推察される。さらに我々は最近、しばしば認知や情緒障害がみられるアルツハイマー型痴呆(DAT)患者の脳におけるAC量と活性の低下を報告した。サイクリックAMP情報伝達系の下流に存在する転写因子であるリン酸化CREB量がDATの海馬で減少していることも報告し、これによりサイクリックAMP情報伝達系の変化が、この神経変性疾患の病態生理に関与していることが示唆された。これらの脳内サイクリックAMP情報伝達系の障害がCREBによる脳由来神経栄養因子(BDNF)の遺伝子転写の変化を介して、脳内神経ネットワークの変化を引き起こすことが考えられる。こうした脳内神経ネットワークの変化が不安、抑うつなどの情緒障害や認知の障害と深く関わっていると思われる。

OS-3

PET imaging on developmental disorder and learning formation

○Yasuyoshi Watanabe

Department of Physiology, Osaka City University
Graduate School of Medicine

Development of the brain and mind is controlled by genetic and epigenetic factors. In pervasive developmental disorders (PDD) such as infantile autism, both genetic and epigenetic defects are thought to be involved. The epigenetic factors, especially sensory inputs from the external world, communication (language), and learning, could produce synaptic rearrangement. The modification factors of synaptic rearrangement are neurotransmitters, neuromodulators, neurotrophic/growth factors, proteases, etc., which functionally make some specific synaptic communications stronger or weaker through the mechanisms of synaptic plasticity. We have been focusing on the neuronal and glial monoaminergic systems controlling synaptic plasticity and early postnatal development. Especially from our recent PET studies in autistic patients with tetrahydrobiopterin (BH4) treatment in a crossover double-blind manner, dopaminergic and serotonergic functions were found to be poorly developed. We here propose the role of monoaminergic precendently developing system in checking the brain development as a molecular and neural mechanisms of my original concept "Brain of the Brain". On the other hand, we could now unveil the mechanisms of entrance process of learning by use of PET on behaving monkeys (and of course infants). The PET activation studies on the new learning and acquisition of the learning by monkeys revealed the role of various brain regions in the formation of learning. We should integrate the ideas and knowledge obtained from such functional brain imaging studies on the subjects during normal and abnormal development.

OS-4

遺伝と環境は人の性格形成にいかに関わるか—進化・発達生物学の視点から—

○神庭 重信

山梨医科大学精神神経医学講座

講演では、人のこころの発達とその病理の理解をめざすとき、ヒトの脳の系統発生 (phylogeny) と個体発生 (ontogeny) ・発達の両者の観点からみていく重要性を強調する。講演では、3つの関連しながらも独立したテーマを扱う。第1のテーマ「脳とこころの来歴」では、主として、ヒトの脳とこころの来歴を生物進化の道を進むことで、“ヒトに共通した脳構造と脳機能は何か”を知り、“こころの本性は何か”という問いに対する答えを求めてみたい。第2のテーマ「こころの発達：遺伝子と環境はどう作用するか」は、人において顕著に見られる個性に着目し、その由来を脳の個体発生 (主として発達) の過程に求めた。人においては、他の種の動物にはみられないほどの長い年月をかけて、脳が念入りに作られる。それは無数の環境と遺伝との相互作用が繰り返される過程でもある。環境と遺伝がどのような綾をなしてこころを作っていくのか、その研究の歴史を古典的研究から近年の実証的フィールド・リサーチの結果にわたって駆け足でレビューする。第3のテーマ「養育環境と脳の発達、脳科学からのメッセージ」では、ある特殊な環境が、脳の機能と構造にさまざまなしかも永続的な影響を及ぼすことを紹介する。

OS-5

A novel pharmacological approach to the molecular basis of schizophrenia

○Toru Nishikawa¹, Yasushi Kajii^{1,2}, Shin-ichiro Muraoka¹, Asami Umino¹, Yasukazu Kuroda¹

¹Section of Psychiatry and Behavioral Sciences, Tokyo Medical and Dental University Graduate School,

²Mitsubishi Pharma Corporation

The onset of schizophrenia typically occurs after adolescence and the psychotomimetic effects of a dopamine (DA) agonist and a NMDA type glutamate receptor antagonist appear to be much more prominent in the adult period than in the childhood. The behavioral responses to these psychotomimetic drugs in experimental animals also depend upon the postnatal stages. These observations indicate that the neuron circuits and molecular pathways implicated in schizophrenia could mature during postnatal development.

To obtain insight into the molecular basis of schizophrenia from a developmental point of view, we investigated in the developing rats the effects of schizophrenomimetic drugs, methamphetamine (MAP, a DA agonist) and phencyclidine (PCP, a NMDA antagonist), on gene expression in the brain, using a histochemical and differential cloning technique (RNA arbitrarily primed PCR).

MAP and PCP induced tPA mRNA in a group of cortico-fugal neurons of the adult, but not the infant, rat in a D1 or D2 receptor antagonist-sensitive manner. The differential distribution patterns of MAP- and PCP-induced c-Fos-like immunoreactivity markedly changed in the neocortex, but not in other regions including pyriform cortex and septum, between postnatal days 8 and 50. The adult pattern was seen after postnatal days 21 - 23. From the neocortex, we isolated the transcripts designated as *mrt-1* (MAP-responsive transcript-1) and *prt-1* (PCP-responsive transcript-1) that exhibited a developmentally regulated response to MAP and PCP, respectively. A selective D1 antagonist SCH23390 antagonized a MAP-induced upregulation of neocortical *mrt-1* expression, while a D2-preferring antagonist haloperidol failed to inhibit the increasing effects of PCP on *prt-1* expression.

The present data suggest that *mrt-1* or *prt-1* could be involved in the molecular cascade related to antipsychotic-responsive or -resistant symptoms of schizophrenia, respectively.

OS-6

Antidepressants: New pharmacological strategies

○Tsukasa Koyama

Dept. Psychiatry, Hokkaido Univ. Grad. Sch. Med.

S1-1

New strategies using adult neural stem cells in defined media and the absence of gene transfer.

○T. Shingo, I. Date, T Ohmoto, and S. Weiss

Department of Neurological Surgery, Okayama University Medical School, and Genes & Development Research Group, University of Calgary Faculty of Medicine

Neural stem cells (NSCs) of the forebrain subventricular zone (SVZ) produce neuronal precursors that migrate to the olfactory bulb, replacing dopaminergic (DA) and GABAergic neurons through adulthood in all mammals. Thus, these NSCs are a potentially important source of *in vitro* generated neurons for transplantation and intrinsic mobilization and induction of tyrosine hydroxylase (TH) in newly-generated neurons *in vivo* in Parkinson's disease. Here we report that a cocktail of defined trophic and intracellular activating molecules produce a robust and sustained activation of TH in NSC-derived neurons *in vitro* and *in vivo*.

The neurospheres derived from the adult SVZ were plated in the presence of both BMP-2 and BDNF, and the TH induction cocktail. Although no TH-immunoreactive neurons were observed in control conditions, the presence of the induction cocktail induced TH in approximately 50% of the adult NSC-derived neurons up to seven days *in vitro*. Furthermore, when these factors were infused into the brain in the Parkinson's model animals new generated DA neurons emerged over the lesioned striatum. The animals infused the TH induction cocktail showed the functional recovery. Thus, in the absence of gene transfer and in defined conditions, a large number of TH-expressing neurons can be generated *in vitro* and *in vivo*.

S1-2

Recent advance in the adenovirus mediated gene transfer system for neuronal regeneration

○Hiroshi Kiyama, Kazuhiko Namikawa

Dept. Anatomy, Grad. Sch. Med., Osaka City Univ.

The adenovirus vector is one of the potent tools to transfer genes into neuronal cells. The major advantages of adenovirus are the higher infection efficacy to neuronal cells and the easiness of the manipulation. The adenovirus is particularly useful in expressing a certain gene transiently in an acute time window, and also in killing certain cells by expressing suicide gene, even though there are some disadvantages in the use of the adenovirus. The use of Cre-loxP system allows a wide range of application in the use of adenovirus vector. The Cre-loxP system readily regulates alternative expression of two genes. For instance the over expression of gene A can be switched off, and simultaneously another gene B expression is turned on. In addition, neural cell specie-specific expression becomes feasible by using the cell specie-specific promoter for the expression of Cre. To achieve neuronal cell-specific expression, a popular neuron-specific promoter such as that of SCG10 was modified by attaching additional NRSE sequences at specific positions. Similarly astrocyte- or Schwann cell-specific promoters were also designed. In this symposium, we introduce some types of adenovirus vectors we constructed, and demonstrate applications of the vectors. The exogenous gene expression using those vectors eventually succeeded in rescuing injured neurons and promoting neurite elongation in animal. We conclude that the adenovirus vectors provide a wide range of tools in the field of experimental neuroscience as well as gene therapy.

S1-3

AAV Vectors and Gene Therapy for Parkinson's Disease

○Keiya Ozawa¹, Shin-ichi Muramatsu², Lijun Wang^{1,2}, Kunihiro Ikeguchi², Ken-ichi Fujimoto², Takashi Okada¹, Hiroaki Mizukami¹, Keiji Terao³, Imaharu Nakano²

¹Div. Genet. Therapeut., ²Ctr. Mol. Med., Jichi Med. Sch., Dpt. Neurol., Jichi Med. Sch., ³Tsukuba Primate Ctr., Nat. Inst. Infect. Dis.

Parkinson's disease (PD) is a progressive neurodegenerative disorder that predominantly affects dopaminergic neurons in the substantia nigra. Since the affected lesion is localized, PD is considered to be a suitable candidate of gene therapy. As for the gene-delivery vehicles to neurons, adeno-associated virus (AAV) vectors are appropriate, because they are derived from non-pathogenic virus, efficiently transduce non-dividing cells, and cause long-term gene expression. In addition, multiple genes can be introduced by co-transduction using separate AAV vectors. A promising approach to the gene therapy of PD is the intrastriatal expression of dopamine (DA)-synthesizing enzyme genes. Local production of dopamine in the striatum caused long-term behavioral recovery in parkinsonian rats. To investigate whether this finding can be extrapolate into humans, preclinical study was conducted in a primate model of PD. AAV vectors expressing tyrosine hydroxylase (TH), aromatic l-amino acid decarboxylase (AADC), and GTP cyclohydrolase I (GCH), were stereotactically injected into the unilateral putamen of MPTP-induced parkinsonian monkeys. As a result, motor abnormalities on the contralateral side were ameliorated with robust transgene expression and elevated DA synthesis in the treated putamen. Another approach is the neuroprotective treatment using the glial cell line-derived neurotrophic factor (GDNF) gene to suppress the disease progression. AAV vector-mediated delivery of a GDNF gene prevented progressive degeneration of dopaminergic neurons in a rat model of PD. These gene therapy strategies would be feasible as novel treatments of PD patients in the near future.

S1-4

Transdifferentiation of bone marrow stromal cells into neurons and Schwann cells, and application to the CNS regeneration

○Mari Dezawa

Dept. Anat. Yokohama City Univ. Sch. Med.

Bone marrow stromal cells (MSCs) are well known as multipotential stem cells that under specific experimental conditions differentiate into several types of cells, for example, osteoblasts, adipocytes and chondrocytes. In the present symposium, MSCs are demonstrated to be induced into cells with neuronal and Schwann cell characteristics, which will be applicable to the treatment of CNS regeneration. MSCs differentiated to neuronal phenotype were immunopositive to neuronal markers of MAP-2, Tuj-1, beta-3-tubulin, neurofilament-M, and were positive to neurotransmitter related molecules such as vesicular acetylcholine transporter and tyrosine hydroxylase. By a certain procedure, percentage of tyrosine hydroxylase positive cells can be increased, and the effect of transplantation of these cells were investigated using model rats for Parkinson's disease, which resulted in the remarkable decrease of apomorphine induced rotation. When corpus striatum of the brain of these animals were investigated under a microscope, virtually all the GFP-labeled transplanted cells had differentiated into neurons expressing neurofilament. MSCs were also differentiated to Schwann cells which expressed p75, S-100, GFAP and O4. GFP-labeled MSCs were transplanted into the cut ends of sciatic and optic nerves, which then responded with vigorous nerve fiber regeneration. Myelination of regenerated fibers by GFP-expressing MSCs was recognized by confocal and immunoelectron microscopy, suggesting that MSCs derived Schwann cells are able to supporting nerve fiber re-growth, and they can therefore be applied to induce nerve regeneration. In conclusion, differentiated MSCs are considered to be one of the strongest candidates for the cell transplantation of CNS disorders.

S1-5

Strategies toward their potential therapeutic application in to spinal cord injury model

○Hideyuki Okano

Dept. Physiol., Keio Univ. Sch Med., CREST, JST

It had been long considered that functional regeneration of injured central nervous system (CNS) is impossible, as Santiago Ramon y Cajal described in the early 20th century "once the development was ended, the founts of growth and regeneration...dried up irrevocably" In the mammalian neural development, neuronal production (neurogenesis) is concentrated mainly in the embryonic stage. However, recent findings indicate that neurogenesis continues in the olfactory bulb, hippocampus, and dentate gyrus of adult mammalian animals from the neural stem cells (NSCs). It has become possible to isolate, culture, and grow pluripotent self-renewing NSCs not only from the embryonic brains but also from adults, and it is attracting attention that this may lead to regeneration and reconstruction therapy for the damaged CNS. In this review, recent topics of the stem cell biology of the CNS as well as strategies toward their potential therapeutic application will be discussed.

S2-1

Cross-modal plasticity in the deaf and blind: studies with non-invasive human neuroimaging techniques

○Norihito Sadato

Nat'l Inst. Physiol. Sci.

Recent advances in functional neuroimaging enable exploration of living human brain with high spatial resolution. The state of the art technique will be reviewed, and its application to the cross-modal plasticity in human will be presented. The brain is a dynamically changing structure in relation to learning, alterations of the peripheral input or brain injury. PET and functional MRI depicted functional reorganization due to early deafferentation. Studies of Braille reading by the blind revealed that the tactual information process is taken in the primary and association visual cortex. Combination of fMRI and transcranial magnetic stimulation proved that the primary visual cortex of the early blind is functionally relevant for the tactile discrimination task. Hence electrophysiological and hemodynamic imaging technique is complementary and essential to explore the human cortical plasticity. Functional MRI studies of sign languages also showed that the sign activated the auditory cortex of the deaf. These studies may provide biological basis for training in the handicapped, and also shed lights on how the brain organizes itself to integrate multimodal sensory input for communication.

S2-2

Quantification and Alteration of Dopamine Receptors in Living Human Brain

○Tetsuya Suhara

Natl Inst. Radiol. Sci., CREST

The symptoms of schizophrenia have been discussed with a functional impairment in dopaminergic transmission in the prefrontal cortex and mesolimbic system. Pharmacological evidence of antipsychotic drugs has focused on the role of dopamine D2 receptor in schizophrenia. However, quantitative analysis has mostly been carried out for the dopamine D2 receptors in the striatum, since the density in the cortical and limbic regions is too low. [^{11}C]FLB457, a substituted benzamide with a very high affinity for dopamine D2 receptors, shows significant specific binding in the cortical and limbic regions. Scatchard analysis revealed that the density was highest in the thalamus (2.3 ± 0.6 pmol/ml), followed by the temporal cortex (1.5 ± 0.5 pmol/ml), hippocampus (1.4 ± 0.5 pmol/ml), parietal cortex (0.9 ± 0.4 pmol/ml), frontal cortex (0.8 ± 0.2 pmol/ml) and occipital cortex (0.7 ± 0.3 pmol/ml). There was no significant difference in K_d values in these six regions. The present results demonstrate that dopamine D2 receptor densities in the extrastriatal regions were only 2-8 % of that in the striatum. Eleven drug-naïve male patients with schizophrenia were examined. Unexpectedly, the binding was significantly lower in the anterior cingulate cortex in drug naïve patients with schizophrenia and a significant negative correlation was observed between D2 receptor binding in the anterior cingulate cortex and the positive symptom score on BPRS. Alterations in D2 receptor function in the extrastriatal region may underlie the positive symptoms of schizophrenia whereas dysfunctions of D1 receptors underlie the negative symptoms.

S2-3

Imaging of cholinergic system of the brain

○Hiroki Namba

Dept. Neurosurg. Hamamatsu Univ. School of Med.

We have developed a method for mapping acetylcholinesterase (AChE) activity (imaging cholinergic system) in the brain using radiolabeled lipophilic acetylcholine analogs. These lipophilic acetylcholine analogs, unlike acetylcholine itself, enter the brain through blood-brain barrier and are hydrolyzed by the specific enzyme to produce a hydrophilic product which has limited permeability through blood-brain barrier and essentially trapped in the brain at the site of the hydrolytic reaction. The radioactivity distribution of the metabolite in the brain reflects AChE activity, which is a marker of the brain cholinergic system. For in vivo mapping of the human cerebral AChE with positron emission tomography (PET), we selected N-[^{11}C]methylpiperidin-4-yl acetate ([^{11}C]MP4A), as the tracer. Quantitative measurement of cortical AChE has been accomplished by fitting the time-course of cerebral radioactivity concentration measured by PET and the metabolite-corrected arterial plasma input function using a nonlinear least squares optimization. Normal control studies showed no effects of aging on k_3 value, an index of AChE activity, in the cerebral cortex ranging from 24 to 89 years of age. Significant reduction of k_3 value in the cerebral cortex was observed in patients with Alzheimer's disease with mild dementia compared with age-matched healthy controls. A study in patients with Parkinson's disease and progressive supranuclear palsy showed that ratio of thalamic to cortical k_3 determined by [^{11}C]MP4A/PET differentiate these two clinically similar disorders. The method has been also applied to other neurodegenerative disorders, such as dementia with Lewy body and frontotemporal dementia and proved useful for evaluation of central cholinergic functions.

S2-4

The application of PET imaging to clinical researches on the histaminergic neuron system: From neurochemical studies to drug development.

○Kazuhiko Yanai

Dept. Med. Grad. Sch. Pharmacopathol. Sci., Tohoku Univ.

Positron emission tomography (PET) is a unique method to examine brain chemistry non-invasively in humans. We have been developing and utilizing the PET techniques to understand the functions of histaminergic neuronal system in the living human brain. For histamine receptors, several radiotracers for selective labeling of H1, H2, and H3 receptors have been synthesized. We have been utilizing two carbon-11 labeled H1-receptor ligands, [^{11}C]pyrilamine and [^{11}C]doxepin for human PET studies. In this paper, we mainly describe the human PET studies of [^{11}C]doxepin. Four topics of our studies on H1 receptor imaging will be presented: (1) the effects of normal and abnormal aging on H1 receptors, (2) application to psychoses, (3) pain perception through H1 receptors and (4) sedative mechanisms caused by antihistamines. A role for histamine was mainly obtained by deducing from classical pharmacological experiments and the studies of gene knockout mice. Although knockout mice provide an ideal opportunity to analyze the specific functions of individual mammalian genes, their roles equivalent to humans are not always deduced from the phenotypic changes observed in the null-mutant mice. For this reason, PET is a very useful method because we can obtain the chemical and physiological information non-invasively from humans.

S2-5

PET imaging of neural substrates for fatigue sensation

○Yasuyoshi Watanabe

Dept. Physiol. Osaka City Univ. Grad. Sch. Med.

Fatigue is an indispensable sense for ordering rest. However, the neuronal and molecular mechanisms of fatigue remain unclear. Chronic fatigue syndrome (CFS) with long-lasting fatigue sensation seems to be a good model for studying these mechanisms. Recently, we found that most of these patients showed a low level of serum acetylcarnitine, which well correlated with the rating score of fatigue, and that a considerable amount of acetyl moiety of serum acetylcarnitine is taken up into the brain. Here we show by metabolite analysis of the mouse brain that an acetyl moiety taken up into the brain through acetylcarnitine is mainly utilized for the biosynthesis of glutamate. When we performed PET study on the cerebral uptake of acetylcarnitine by using [^{11}C]acetyl-L-carnitine in 8 patients with CFS and in 8 normal age- and sex-matched controls, a significant decrease was found in several regions of the patients group, in the prefrontal (Brodmann's area 9/46d), temporal (BA21 and 41), and anterior cingulate (BA24 and 33) cortices, and cerebellum. There was no close relationship in the decrease between cerebral uptake of acetylcarnitine and regional cerebral blood flow. These findings suggest that the levels of biosynthesis of neurotransmitters through acetylcarnitine might be reduced in some brain regions of chronic fatigue patients. Furthermore, to explore the brain regions and neural circuits responsible for fatigue sense of healthy human subjects, we performed PET scans during mental load (Advanced Trail Making Test, ATMT) tasks. Almost two-hour challenge of different kinds of ATMT tasks, volunteers' fatigue sense evaluated both by visual analogue scale and by ATMT performance increased stepwisely and reached their maximum fatigue state. SPM99 analysis revealed that rCBF during ATMT tasks increased and decreased in several brain regions, their pattern being characteristic of two different types of tasks. A part of BA 11 was delineated by correlation analysis with the subjective fatigue.

S3-1

The voltage-sensitive sodium channel has a bell-shaped structure with multiple cavities; Structure revealed by single particle analysis

○Chikara Sato¹, Yutaka Ueno¹, Toshihiko Ogura¹, Masahiko Sato², Yoshinori Fujiyoshi³

¹N.I.A.I.S.T., ²Itoham Foods Inc., ³Dept. Biophy. Facul. Sci. Kyoto Univ.

Sodium channels, glycosylated proteins of approximately 300 kDa are responsible for signal transduction and amplification and are major targets of anesthetic drugs and neurotoxins. The structure was not known because it was difficult to form crystals. Single particle analysis is a method which does not require crystals. Here we present the three-dimensional structure of the voltage sensitive sodium channel from the eel *Electrophorus electricus*. The 19 structure was determined by helium cooled cryo-electron microscopy and single particle image analysis of the solubilized sodium channel. This has a bell shaped outer surface of 135 height, 100 side length at the square-shaped bottom, and a spherical top of 65 diameter. Multiple inner cavities are connected to four small holes and eight orifices close to the extracellular and cytoplasmic membrane surfaces. Homologous voltage sensitive calcium and tetrameric potassium channels, which regulate secretory processes and the membrane potential, may possess a related structure. (1) The voltage-sensitive Sodium Channel is a bell-shaped molecule with several cavities. C. Sato, et al. Nature 409, 1047-1051 (2001).

S3-2

Conformation of a peptide bound to a G-protein coupled receptor

○Masahiro Shirakawa

Grad. Sch. Integ. Sci., Yokohama City Univ.

G-protein coupled receptors (GPCRs) mediate important cellular events by responding to a variety of ligands. Of them, members of the glucagon /secretin receptor family are specific receptors for a group of peptide hormones, including pituitary adenylate cyclase-activating polypeptide (PACAP), vasoactive intestinal polypeptide (VIP), secretin, and glucagon. By binding to their specific GPCR, these peptide ligands elicit a wide array of physiological effects. Much work has been directed towards understanding the mechanism of receptor recognition and activation. However, conformations of peptides bound to their specific GPCRs had not been reported. PACAP is distributed in the central nervous system and its peripheral organs, and elicits a biological events, such as secretion of pituitary hormones, catecholamines and insulin. It also mediate a neuroprotective action against gp120-induced cell death. We have determined the conformation of a truncated form of PACAP, PACAP(1-21)NH₂ (termed PACAP21), bound to a PACAP-specific receptor by NMR spectroscopy. We employed proton two-dimensional transferred nuclear Overhauser effect (TRNOE) spectroscopy for the structure determination. Although this method has been widely used for structure determination of small ligands bound to larger molecular systems, its application to peptide-GPCR systems had been limited due to technical difficulty. After overcoming the problems, we collected 387 distance restraints for the GPCR-bound peptide, and calculated its conformation by a simulated annealing protocol. We also determined the conformation of PACAP27 bound to dodecylphosphocholine (DPC) micelles. Residues 3-7 of the receptor-bound PACAP21 form a unique β -coil structure, while a C-terminal region (residues 8-21) adopts an amphipathic α helix. While the C-terminal helix resembles to that of the micelle-bound PACAP, the β -coil structure of the receptor-bound PACAP21 is unique. This β -coil conformation of the receptor-bound PACAP21 produces a hydrophobic patch, and an associated hydrophobic pocket. Importance of residues that form the patch was examined by single site-directed Ala substitutions. Binding abilities of the substituted peptides to the receptor indicate that the patch and local conformation are crucial for the receptor binding. The limitation of the conformational difference between the receptor- and micelle-bound PACAPs to the N-terminal residues including the β -coil may supports the two-step ligand transportation model that a ligand binds nonspecifically to the membrane surface first, and then is transferred to a target receptor. Reference: Inooka, H., Ohtaki, T., Kitahara, O., Ikegami, T., Endo, S., Kitada, C., Ogi, K., Onda, H., Fujino, M., and Shirakawa, M. (2001). Conformation of a peptide ligand bound to its G-protein coupled receptor. Nature Structural Biology, 8, 161-165.

S3-3

Molecular mechanism of signal transduction mediated by Grb2

○Fuyuhiko Inagaki

Graduate School, CREST, Hokkaido Univ.

Grb2 is an adaptor protein containing a SH2 domain flanked by two SH3 domains. Grb2 mediates signal transduction from growth factor receptors to Ras, which is recognized as a major signal transduction pathway for cell proliferation. Grb2 also plays important roles for controlling cytoskeletons in response to the extracellular signals. We determined the structure of individual domains complexed with target peptides by NMR and elucidated the molecular mechanism for specific and general recognition of the target peptides.

Here, we report the structure of intact Grb2 in solution by NMR and small angle X-ray scattering. We found that intact Grb2 in solution is flexible around the linker connecting the SH2 and the C-terminal SH3 domains, while each domain retains its domain structure. Using consolidated SH3 binding peptides connected by linkers with different number of amino acid residues, we showed that Grb2 binds to these peptides with much higher affinity than those of individual SH3 domains. This clearly shows that Grb2 binds to the consolidated peptides bivalently and its flexibility is important for high affinity binding. Two SH3 domains could adapt their relative disposition and orientation for the recognition of the target peptides. The flexible nature of Grb2 also could assemble their target proteins efficiently.

Vav is a guanine nucleotide exchange factor exclusively expressed in hematopoietic cells and regulates its proliferation and differentiation. Vav contains SH3-SH2-SH3 at the C-terminus and the binding of Vav nSH3 to Grb2 cSH3 is essential for exertion of its biological function. Vav nSH3 is unique in that it contains proline rich region (PRR) in the RT-loop (PPPPG, 607-611). From NMR analysis, we found that Vav nSH3 exists in two conformations due to cis-trans isomerization at P615. We prepared the P615G mutant and found that the major form takes trans around G614-P615. The structure of the trans form was subsequently determined by NMR. Interestingly, the PPPPG is configured to PPII helix and P609 located at the apex of the prism is tightly embedded in the hydrophobic pocket of Vav nSH3, while the basement seems to be presented ready for binding to the target proteins. The structure of Vav nSH3 complexed with Grb2 cSH3 was studied by X-ray crystallography. Vav nSH3 has two binding surfaces with Grb2 cSH3 molecules; one binding site involves the PPPPG region of Vav nSH3, while the other contains the concave hydrophobic surface located at the opposite side of the PPPPG region. From NMR and mutagenesis studies, we elucidated the novel recognition mechanism between Vav nSH3 and Grb2 cSH3.

S3-4

Structure and function of signaling proteins controlling cell adhesion and cytoskeleton

○Toshio Hakoshima

Nara Institute of Science and Technology/CREST

Cell-cell and cell-ECM adhesion events accompany recruitment of several cytoplasmic proteins at the beneath of plasma membranes. These proteins contain scaffolding proteins linking actin cytoskeleton to adhesion molecules, as well as signaling proteins such as Rho GTPases with their regulators and effectors. Recent biochemical studies have suggested that some adhesion molecules associate with membrane receptors to modify the receptor activity. These adhesion molecules playing as co-receptors include CD44 co-localized with erbB, syndecans with heparan sulfate-binding growth factor receptors, and L1-CAM with neuropilin for Sema3A. ERM (ezrin/radixin/moesin) proteins play a key role in the formation of the membrane-associated cytoskeleton by linking actin filaments and adhesion molecules such as CD44, CD43 and immunoglobulin-family adhesion molecules such as ICAMs. ERM proteins also bind sodium and hydrogen ion exchanger regulatory factors (NHERFs), which interact with the ion channel NHE to modify the channel activity. These binding activities of ERM proteins are initiated by binding to phosphatidylinositol 4,5-bisphosphate (PIP2) in the downstream of the Rho signaling pathway. Interestingly, the N-terminal conserved domain of ERM proteins, the FERM (4.1 and ERM) domain, mediates the multiple interactions with IP3, ICAMs, CD44, CD43, NHERF, and RhoGDI. Here I will describe our recent crystal structures of the radixin FERM domain complexed with these binding partners and discuss the molecular mechanisms by which ERM proteins accomplish the multiple molecular recognition. Based on the three-dimensional structures of the complexes, I will address possible ERM-binding partners including L1-CAM.

S3-5

Mechanism of Processive Movement of Monomeric KIF1A Motor Revealed with Biophysics, Cryo EM and X ray Crystallography.

○Nobutaka Hirokawa

Dept. Cell Biol. and Anat. Grad. Sch. Med., Univ. of Tokyo

To elucidate the mechanism of intracellular transport in neurons we have identified all members of Kinesin superfamily proteins, KIFs in mammals. Among them KIF1A is a unique monomeric motor transporting synaptic vesicle precursors anterogradely from cell body to the nerve terminal. In this symposium I will present data how KIF1A can move processively along microtubules by hydrolysing ATP using molecular biophysics, cryoEM and X ray crystallography.

S4-1

Aspects and applications of organotypic brain slice cultures

○Takuya Sakaguchi

Development. Res. Lab., Shionogi & Co., Ltd.

The cultivation of brain tissues preserving organotypic cellular architecture has been performed since the middle 20th century. Basic characterization of this preparation was achieved in detail following the establishment of the "roller-tube" culture technique in the 1980' s. Furthermore, in the past decade, some different laboratories independently developed "interface" culture techniques using porous membranes. The invention of such simple techniques facilitated the use of slice cultures in the brain research. The spread use revealed aspects of this preparation in further detail and led new strategies of applications in conjunction with molecular biology. Here I review some aspects of organotypic brain slices cultured mainly from hippocampal tissues, focusing on neuronal similarities and differences between organotypic cultures and *in vivo* brain tissues. Following points are discussed for clarifying advantages and limitations of the preparation for possible applications: Expressions of neuronal receptors, modes of synaptic plasticity and developments of neuronal circuits.

S4-2

Development of hippocampal mossy fibers in organotypic cocultures

○Yuji Ikegaya, Norio Matsuki

Lab. Chem. Pharmacol., Grad. Sch. Pharmaceut. Sci., Univ. Tokyo

The mossy fiber (MF) is the only axon projecting from the dentate gyrus to the hippocampus and its pathway is highly stereotyped and topographically restricted; the axon runs through the stratum lucidum and made giant synapses with a proximal, narrow-segment of the apical dendrites of hippocampal CA3 pyramidal cells. Using explant or overlay cocultures of the fascia dentata and hippocampal Ammon's horn tissues, we have investigated the cellular basis for the specific MF pathfinding. In this symposium, we present the usefulness of these coculture systems through introducing our recent work. In cultured entorhino-hippocampal slices, MF development can be easily assessed by membrane tracer labeling, Timm staining, electrophysiological recording of synaptic responses, and optical analyses using a voltage-sensitive dye. In addition, tissue preparations from green fluorescent protein (GFP)-overexpressed rats allowed us a time-lapse observation of lamina-specific MF extension under confocal microscopic controls. This new method uncovered that the mechanisms underlying MF guidance undergo a developmental switch from a diffusible factor-dependent manner to a membrane-bound-factor-dependent manner. Slice overlay assay, in which DiI-labeled or GFP-transfected dentate granule cells are dispersed over hippocampal slice cultures, provides a unique opportunity to examine endogenous local factors supporting cell survival, neuronal polarity, and axon and dendrite guidance. This assay revealed that MF growth is tightly regulated by positional factors in the environment. Thus, the cellular basis for MF pathfinding is being clarifying by the organotypic coculture systems.

S4-3

Activity-dependent branch formation of cortical axons: A morphological and electrophysiological study using organotypic slice cultures

○Nobuhiko Yamamoto, Naofumi Uesaka, Satoshi Hirai, Soichiro Ohnami

Lab. Neurosci., Grad. Sch. Frontier Biosci., Osaka Univ.

During development axons form branches on reaching their target zones. In later stages, some arbors are complicated but others are diminished. A developmental program, which governs spatio-temporal patterns of expression of guidance molecules, is likely to regulate the early process, whereas neuronal activity including spontaneous firing is thought to play an important role in the later process. To understand how neuronal activity is involved in branch formation in cellular and molecular levels, we focus on neocortical neurons in the upper layers. It has been shown that branch formation of these neurons contains additive and retractive processes. Evidence further suggests the involvement of neuronal activity in axonal branching in this system. Here we studied a relation between branch formation of individual upper cortical neurons and their neuronal firing by using organotypic slice cultures, in which cortical cellular organization is preserved. The following results were obtained. 1) Upper cortical axons form terminal arbors in a manner that is similar to that found in vivo. 2) Axonal branching begins to take place when spontaneous firing is increased. 3) Long-term blockade of neuronal activity with pharmacological manipulations causes the decrease of branching, while enhanced neuronal activity increases branch-length. These findings suggest that firing activity is one of the components that regulate branch formation of cortical axons.

S4-4

Analysis of neuron-glia interaction involved in the dendritic morphogenesis of cerebellar Purkinje cells using an organotypic slice culture system

○Masahiko Tanaka

Div. Cell Biol., Inst. Comprehens. Med. Sci., Fujita Hlth. Univ.

During postnatal cerebellar development, Purkinje cells form the specific morphology of dendritic trees. Our organotypic slice culture system of postnatal rat cerebellum (Tanaka et al., 1994) reproduces cerebellar cortical development including the morphogenesis of Purkinje cell dendrites, and thus can be a useful model system for the study of molecular mechanisms underlying this morphogenesis. PTP ζ /RPTP β , a receptor-type protein tyrosine phosphatase synthesized as a chondroitin sulfate (CS) proteoglycan, uses a heparin-binding growth factor pleiotrophin (PTN) as a ligand. PTP ζ and PTN are expressed by Bergmann glia and distributed around the differentiating dendrites of Purkinje cells in the postnatal cerebellum. Using the slice culture system, we tested the hypothesis that Bergmann glia-Purkinje cell interaction through PTN-PTP ζ signaling is involved in the morphogenesis of Purkinje cell dendrites. An aberrant morphology of Purkinje cell dendrites such as multiple and disoriented primary dendrites was induced by 1) addition of an anti-PTP ζ antibody, 2) inhibition of protein tyrosine phosphatase activity, 3) enzymatic removal of the CS chains, 4) addition of exogenous CS chains, and 5) addition of exogenous PTN, all of which inhibit PTN-PTP ζ signaling. These findings showed that PTN-PTP ζ signaling regulates the morphogenesis of Purkinje cell dendrites. Furthermore, the perturbation of PTN-PTP ζ signaling reduced the immunoreactivity to GLAST, a glial glutamate transporter, on Bergmann glial processes. Altogether, it is suggested that PTN-PTP ζ signaling acts on the Bergmann glial processes and controls the formation and/or maintenance of the GLAST-positive processes of Bergmann glia, which regulate the morphogenesis of Purkinje cell dendrites.

S4-5

哺乳類胚長期培養系を用いた嗅球発生におけるPax6遺伝子の機能解析

○野村 真, 大隅 典子

東北大学・院医・器官構築

脊椎動物の終脳前端部に存在する嗅球は、嗅覚系の一次中枢として機能する重要な領域である。興味深いことに、転写因子の一つであるPax6の機能欠損変異マウス及びラットでは、嗅球様の構造物が終脳の前端ではなく側面に存在する。この事実はPax6が発生期の終脳において、嗅球を正しい位置に構築する機構に関わっている可能性を示唆している。我々はPax6の嗅球発生における役割を解析するために、哺乳類全胚培養系と終脳器官培養系を組み合わせた長期培養系を構築し、この培養系で嗅球の発生が進行させられることを確認した。この長期培養系を用いて終脳の細胞標識を行ったところ、胎生12.5日の野生型ラット胚では、終脳前端部の神経上皮細胞が将来の嗅球の僧帽細胞を生み出すことが判った。ところがPax6変異ラット胚では、終脳前端部に由来する細胞が終脳の後側へと移動し、異所的な嗅球様構造を構築することが判明した。この細胞移動様式の異常が細胞自律的な変化であるかを確認するため、正常ラット胚とPax6変異体との間で、終脳前端部の細胞の交換移植を行った。その結果、正常胚由来の細胞は変異体の終脳前端に移植されると終脳後側に移動し、異所的な嗅球を構築した。ところが変異体由来の細胞は正常な終脳に移植されると、終脳前端に留まることが確認された。これらの結果からPax6変異体の細胞移動の異常には、細胞非自律的な変化が関与していることが示唆された。現在我々はPax6の下流で実際に細胞移動を制御している分子の検索を行っている。これら一連の嗅球発生機構の解析において強力なツールとなっている長期培養系と遺伝子導入技術についても、本シンポジウムで詳しく紹介する予定である。

S5-1

A Possible mechanism for attaching synaptic vesicles to microtubules

○Teruo Abe

Dept. Cell. Neurobiol. Brain Res. Inst., Niigata Univ.

The synaptic vesicle membrane protein synaptotagmin I (syt I) plays multiple roles in the synaptic vesicle cycle. However, the mechanisms of its action remain unclear. We searched for cytosolic proteins that interact with recombinant syt I. We have found that a direct, stoichiometric binding of the cytoskeletal protein tubulin to syt I. The binding was dependent on mM Ca. Both of the C2 domains of syt I, and the C-terminal region of beta-tubulin were involved in the binding. Tubulin/syt I and tubulin/syt I/SNARE complexes were immunoprecipitated by monoclonal antibodies to tubulin and SNAP-25. Moreover, syt I promoted tubulin polymerization and bundling of microtubules in the presence of Ca. Liposomes containing full-length syt I could attach to microtubules. In contrast, no significant attachment of liposomes was observed, when liposomes containing full-length VAMP-2 instead of syt I was used. These results suggest that direct interaction between syt I and tubulin provides a bridge linking the synaptic vesicle to the microtubule. To gain insight into the role of the tubulin/syt I binding in the nerve terminal, we have examined dynamic behavior of tubulins and synaptic vesicles using the neuromuscular junction of the *Drosophila shibirets1* mutant whose vesicle recycling can be manipulated in a temperature-sensitive manner. Some structure containing tubulin was transiently formed near the presynaptic membrane after high K⁺ stimulation of the nerve terminal. The Ca²⁺-dependent tubulin/syt I binding may underlie such transient structure and be involved in transient retention of synaptic vesicles near the presynaptic membrane.

S5-2

Diversity of Ca²⁺ channel subtypes in presynaptic terminals

○Hiromu Yawo

Dept. Develop. Biol. Neurosci., Tohoku Univ. Grad. Sch. Life Sci., CRST, JST

Transmitter release from a nerve terminal is primary regulated by a Ca²⁺ influx through voltage-dependent Ca²⁺ channels. Among the large molecular family of Ca²⁺ channels, Cav1.2(α1C), 1.3(α1D), 2.1(α1A), 2.2(α1B), 2.3(α1E), 3.1(α1G), 3.2(α1H) and 3.3(α1I) are distributed in the central nervous system. The distributions and functions of Ca²⁺ channel subtypes remain unrevealed in presynaptic terminals. We applied Ca²⁺/Sr²⁺ fluorometry on mossy fiber terminals at CA3 regions of hippocampal slices and measured Ca²⁺/Sr²⁺ inflows during action potentials. The Ca²⁺/Sr²⁺ inflow was blocked by ω-conotoxin GVIA, ω-agatoxin IVA and nimodipine, respectively. The residual component was almost completely blocked by the Cav2.3-specific toxin SNX-482, being homogeneous pharmacologically. In summary, we identified four subtypes in hippocampal mossy fiber terminals; N (Cav2.2), P/Q (Cav2.1), L (Cav1.2/1.3) and Cav2.3-based R. We compared the subtype's efficacy of Ca²⁺ inflow to evoke transmitter releases. Although N and P/Q subtypes tightly coupled to transmitter release, the Cav2.3-based R did less. The subtype expression was variable among individual mossy fiber terminals. It is suggested that transmitter releases from the individual presynaptic terminal are uniquely regulated through Ca²⁺ channel subtypes.

S5-3

Synaptic Targeting of PSD Scaffold Proteins Containing Leucine-zipper Motifs; PSD-Zip45 and PSD-Zip70

○Kenji Sobue, Daijiro Konno, Shinichi Usui, Kei Hori, Hisato Maruoka
Department of Neuroscience (D13), Osaka University Graduate School of Medicine

The postsynaptic density (PSD) is a dense structure in the excitatory synapses of the central nervous system. Recent studies have focused on the identification of PSD scaffold proteins and their involvement in synaptic functions. Using PSD-specific monoclonal antibodies, we recently isolated PSD scaffold proteins containing leucine-zipper motifs; PSD-Zip45 and PSD-Zip70. PSD-Zip45 (a member of the Homer family) is composed of an enabled/VASP homology 1 (EVH1) domain at its N terminus and C-terminal leucine-zipper motifs. The EVH1 domain interacts with the PPxxF motif found in group I metabotropic glutamate receptors (mGluRs), shank family members, inositol triphosphate (IP₃) receptors, and ryanodine receptors. The extreme C-terminal leucine-zipper motif is involved in self-multimerization. We analyzed the dynamic behavior of PSD-Zip45 within the dendritic spines using time-lapse confocal microscopy and demonstrated its high steady-state turnover rate and assembly/disassembly dynamics depending upon the Ca²⁺ signal from different sources. We further studied the molecular mechanism underlying the postsynaptic organization of PSD scaffold proteins and demonstrated that PSD-Zip45/shank and PSD-95/GKAP interactions form different compartments within the postsynaptic structure. PSD-Zip70 contains an N-myristoylation consensus sequence and a polybasic cluster at its N terminus region and four leucine zipper motifs in the C-terminal region. Light and electron microscopy revealed the localization of this protein in the dendritic spines, especially in the PSD and the postsynaptic membrane. Fractionation of the synaptic plasma membrane demonstrated the localization of PSD-Zip70 to the PSD and the dendritic raft. In hippocampal neurons, N-myristoylation is also required for the membrane localization and its C-terminal region is critical for the synaptic targeting. Thus, these two PSD scaffold proteins might be involved in the synaptic function.

S5-4

Differential dynamic behavior of postsynaptic density proteins and its regulation by neuronal activity

○Shigeo Okabe
Dept. Cell Biol. Grad. Sch. Med., Tokyo Medical and Dental Univ.

Recent technical advances in our ability to visualize protein molecules in living neurons have revealed dynamic properties of synaptic structures. Individual anchoring/scaffolding proteins in the postsynaptic density (PSD) are composed of several protein-interacting domains that can support the association and cross-linking of other postsynaptic proteins. PSD-95 family of proteins is a predominant component of the PSD and has been shown to interact with NMDA-type glutamate receptors. Cytoplasmic proteins that interact with non-NMDA-type glutamate receptors have also been identified. Among them, the Homer/Vesl family proteins, including PSD-Zip45 (also known as Homer 1c/Vesl-1L) specifically interact with group I metabotropic glutamate receptors. PSD-95 and PSD-Zip45 have distinct protein-interacting domains and interact with different glutamate receptors. Therefore, the dynamic properties of these two PSD proteins might be quite different. We tested this hypothesis by using confocal imaging of GFP-tagged PSD-95 and PSD-Zip45 molecules in living hippocampal neurons in culture. PSD-95-GFP clusters were stable on the time scale of several hours, a property consistent with the biochemical evidence for a tight association between PSD-95 molecules and the core PSD structure. In contrast, a fraction of PSD-Zip45 clusters changed their distribution rapidly and the direction of assembly/disassembly was regulated by the combination of calcium influx through NMDA receptors and voltage-dependent calcium channels (VDCCs). Thus, neuronal activity differentially redistributes a specific subset of PSD proteins, which are important for localization of both surface receptors and intracellular signaling complexes.

S5-5

Role of drebrin in signal transmission within the dendritic spine

○Tomoaki Shirao, Satoshi Tanaka, Hiroyuki Yamazaki, Yuko Sekino
Dept. Neurobiol. & Behav. Gunma Univ. Sch. Med.

Drebrins are actin-binding proteins, which expression is closely related to dendritic spine morphology. It has been raised the possibility that drebrin plays as an adaptor protein in the postsynaptic signal transmission from membrane receptor to actin cytoskeleton. In the previous study, we established the primary culture of cortical neurons, in which neurons could survive more than four weeks and their dendritic spines could be fully matured. Using this culture systems, we inhibited the drebrin expression with antisense-oligonucleotides, and demonstrated that activity-dependent accumulation of synaptic functional proteins were changed. We have previously shown that drebrin composed of unique actin cytoskeletal complex in the dendritic spine. This complex contained gelsolin, non-muscle type myosins in addition to actin filaments. Further, it has been reported that drebrin binds to profilin and Homer. However the molecular basiss of drebrin function has not been clarified. Therefore, we performed the yeast two hybrid screen of a rat brain cDNA library that used drebrin fragments as baits. DNA sequence analysis of the positive-interactin clones revelaed that about 70% of clones encodeed cDNA related with an identical protein. We designated this protein as DRAP1. Then we performed its biochemical characterization and found its N-terminal half bound to the N-terminal region of a drebrin molecule.

S6-1

MOLECULAR CONTROL OF NEURAL STEM CELL FATE

○Masato Nakafuku, Motoshi Nagao
Dept. Neurobiol. Graduate Sch. Med., Univ. Tokyo

Multipotential neural stem cells serve as the origin of diverse cell types in the mammalian central nervous system (CNS). During early development, stem cells continue proliferation and increase their total cell number without overt differentiation. At subsequent stages, they withdraw from this self-renewal mode, and commit to generating multiple neuronal and glial cell types. This lineage commitment and differentiation of stem cells proceeds with distinct kinetics at defined times and positions, control of which is a critical part of the complex morphogenesis of the CNS. The molecular and cellular details of these events, however, still remain poorly understood. In this presentation, molecular mechanisms underlying those cell fate determination events of stem cells will be discussed with emphasis on crosstalks between cell-intrinsic determinants and environmental signals. References: Torii M. et al., Development 126, 443-456, 1999; Mizuguchi R. et al., Neuron 31, 757-771, 2001; Yamamoto S. et al., J. Neurosci. 21, 9814-9823, 2001; Yamamoto N. et al., J.B.C. 276, 45031-45040, 2001; Yamamoto S. et al., Exp. Neurol. 172, 115-127, 2001; Kobayashi D. et al., Development 129, 83-93, 2002; Fu H. et al., Development 129, 681-693, 2002; Heins N. et al., Nat. Neurosci. in press.

S6-2

Neurogenin 1 as a positive bHLH regulator of neuronal differentiation

So-Yeon Kim¹, Yong-Soo Yoon¹, Yong-Jin Jeon¹, Mi-Young Jung¹, Seong-Soo Kim¹, Jiwon Kim¹, Seung-Up Kim², Young-Don Lee¹, ○Haeyoung Suh-Kim^{1,2}

¹Department of Anatomy, Ajou University, School of Medicine, ²BDRC, Ajou University, School of Medicine

Basic helix-loop helix (bHLH) proteins play critical roles in determination of neuronal lineage during mammalian neurogenesis. Several bHLH proteins appear to have proneural function including neurogenin1 and 2 and MASH1. In this report, we showed that *ngn1* could substitute for retinoic acid (RA) in directing neuronal differentiation in P19 cells.

P19 cells are pluripotent embryonic carcinoma cells which can differentiate into neurons in the presence of RA. We investigated a role of a basic helix-loop-helix cascade including neurogenin1 (*ngn1*) in neuronal differentiation using P19 cells. Treatment of P19 cells with RA during embryoid body formation caused sequential expression of *ngn1*, *neuroD* and other neuronal markers. Western analysis showed that P19 cells contained E47, a ubiquitous heterodimeric partner of *ngn1*. Therefore, overexpression of *ngn1* alone was sufficient to elicit E-box mediated gene expression. Overexpression of *ngn1* increased EGFP expression derived by the nestin promoter/enhancer as well as the mRNA level of the endogenous nestin. *Ngn1* also enhanced the luciferase activity driven by the 2.4 kb *NeuroD* promoter. Sustained expression of *ngn1* was sufficient to caused expression of neuronal markers, such as neuron-specific enolase and βIII-tubulin in the absence of RA. These results suggest that the RA induce neuronal differentiation by activating a bHLH cascade including *ngn1* in P19 cells.

We also investigated the negative partners of bHLH factors, Id proteins, during neuronal differentiation. Overexpression of Id inhibits neurite outgrowth of F11 neuroblastoma cells. Delivery of Id genes into the developing mouse embryos enhances nestin gene expression. The result suggests that Id proteins may be important for maintenance of neural stem cell properties.

S6-3

Cell-external cues and cell-intrinsic programs regulating lineage-determination in the developing brain.

○ Tetsuya Taga, Takumi Takizawa, Kinichi Nakashima

Dept. Cell Fate Modulation, Inst. Molecular Embryology and Genetics, Kumamoto Univ.

Three major cell lineages in the central nervous system, neurons, astrocytes and oligodendrocytes, are differentiated from a common multipotent progenitor cell called neural stem cell. In the developing brain, lineage specification is thought to be regulated by various determinants. We found that two different types of cytokines, LIF and BMP2, act in dramatic synergy on neural progenitors to induce astrocyte differentiation. The synergy between LIF and BMP2 can be explained by a complex formation of their respective downstream transcription factors STAT3 and Smad1 bridged by a coactivator p300. We also found that BMP2 induces expression of negative helix-loop-helix (HLH) proteins, leading to inhibition of transcriptional activity of neurogenic basic-HLH transcription factors, which eventually changes the fate of neural progenitor cells from neurogenesis to astrocytogenesis. During brain development, astrocyte differentiation does not occur until the late gestational stage but becomes apparent just before birth. As we have shown, astrocyte differentiation is largely dependent on STAT3 activation. We found that there exists a STAT3 binding element in the promoter region of the gene for an astrocyte marker, GFAP, and that a cytosine residue in this element is highly methylated in neuroepithelial cells in mid gestation but becomes demethylated as brain develops. Based on the result that this methylation inhibits STAT3 binding, we suggest that DNA methylation is a critical determinant in the developmental stage-dependent regulation of astrocytogenesis. In conclusion, fate of neural stem cells during development is regulated by cell-external cues like cytokine signalings and cell-intrinsic programs such as epigenetic modification including DNA methylation.

S6-4

Molecular Mechanisms Underlying Oligodendrocyte Development

○Tetsushi Kagawa, Kazuhiro Ikenaka
Neuralinfo. Lab., Natl. Inst. Physiol. Sci.

Oligodendrocyte (OL) progenitors in the mouse spinal cord are generated from strictly restricted regions in the ventricular zone of the ventral cord as early as embryonic day 11 (E11). One reason for this ventral specific appearance of OLs may be caused by inhibitory activity for OL development secreted from the dorsal spinal cord, as we previously reported. To identify the unknown dorsal factors, we characterized developmental change of the inhibitory activities. They were very strong at E11, gradually reduced, and disappeared by E14. This pattern seemed to be well correlated with developmental profile of Wnt3a expression in/near the roof plate. A conditioned medium of L cells that stably expressed Wnt3a showed significant reduction of O4 positive OLs in the ventral spinal cord explants, indicating that Wnt3a is one of the dorsal factors that inhibit OL development. Addition of Wnt3a supernatant to CG4 cells, an OL progenitor strain, and to the dissociated primary cultured cells suggested that Wnt3a directly acts on OL lineage cells and inhibits a differentiation step from OL progenitor to O4-positive stage. Misexpression of LEF- β -Catenin, a down stream target of Wnt3a signaling, inhibited OL differentiation of CG4 cells, confirming this scenario. As O4-positive OLs cannot migrate, preventing premature differentiation of OL progenitors might be necessary for the OL lineage cells to migrate dorsally. Thus, Wnt3a may directly control the timing of OL differentiation and the motility of OL lineage cells. A population of myelinating OLs in the dorsal area was further demonstrated to be ventral origin by the newly established cell marking system using *in utero* DNA electroporation.

S6-5

Genetic dissection of differentiation and outgrowth of the cranial motor and sensory neurons in zebrafish

○Hitoshi Okamoto
Lab. Dev. Gene Reg., BSI, RIKEN, CREST, JST

Islet-1 is a LIM/homeodomain-type transcription factor which is expressed in the motor and sensory neurons. Using zebrafish as a model system, we generated a series of constructs that partially lack the motor- or sensory-specific enhancer regions of the Islet-1 gene, microinjected them into the one-cell stage zebrafish embryos, and analyzed their activities by GFP expression *in vivo*. We found that the enhancer activity for expression in the cranial motor neurons lies within about 600-bp region 10 kbp downstream of the transcription initiation site, and that the one for expression in the sensory neurons lies within about 500-bp region 55kbp downstream of the transcription initiation site, and that the sequences of both the motor- and sensory-specific elements are highly conserved among zebrafish, mouse and human. Using the transgenic line expressing GFP under control of the Islet-1 enhancer, we analyzed the mechanisms by which the cranial motor axons exit from the hindbrain. We found that the trigeminal motor axons extend along the afferent trigeminal axons from the placode-derived trigeminal sensory neurons which also play a role of the guide rail for the afferent axons from the later recruited sensory neurons. We have been undertaking mutant screening by using an Isl1-GFP transgenic fish to facilitate observations of neuronal differentiation in embryonic zebrafish brain. We have isolated one mutant in which the axonal outgrowth of both the facial and vagus motor nerves is severely and selectively impaired, and that this mutant lacks facial and vagus sensory ganglia, further supporting the roles of the cells in the sensory ganglia as guide posts for motor nerve extension.

S7-1

Function and regulation of the Src family tyrosine kinases

○Masato Okada

Department of Oncogene Research, Research Institute for Microbial Diseases, Osaka University

The Src family tyrosine kinases (SFKs), benign relatives of the oncogenic v-src gene product, are non-receptor type of protein tyrosine kinases (PTKs) that are associated with the plasma membrane. The SFKs serve as molecular switches involved in the initiation of a variety of cellular events, including cell growth, attachment and movement, differentiation, survival and death. In response to an external stimulus, an SFK becomes activated through dephosphorylation of a critical C-terminal tyrosine or through binding to another protein that interferes with the inactive conformation. The phosphorylation of the regulatory tyrosine of SFK is strictly controlled by another PTK, the C-terminal Src Kinase (Csk). The loss-of-function of Csk lead to constitutive activation of SFKs, accompanied by severe defects in embryonic neural tissues. Therefore, it is believed that SFKs, under control of Csk, play essential roles in the neural development, although their primitive functions still remain elusive. The regulation mechanism of Csk itself has also remained unknown. Recently, we isolated a membrane phosphoprotein Cbp (Csk binding protein) that specifically binds to the SH2 domain of Csk. A line of evidence revealed that Cbp serves as a membrane anchor of Csk required for an efficient interaction between cytoplasmic Csk and the membrane bound SFKs. It was also found that Cbp is a substrate of SFKs, implicating a role of Cbp as a mediator in a negative feedback loop of the SFK regulation. More interestingly, Cbp is localized exclusively in a membrane microdomain, so-called lipid Rafts, in which SFKs are enriched. This suggests that Rafts is a critical platform where the SFK-mediated signaling is initiated and terminated. To further understand the molecular basis of the regulation of SFKs, we have started structural analyses of the SFK-related molecules. The molecular mechanism of the SFK regulation will be discussed based on a piece of structural information.

S7-2

Regulatory roles of protein tyrosine phosphatases in Ca^{2+} -dependent neurotransmitter release from neuronal cells.

○Makoto Itakura¹, Saori Yamamori¹, Hiroshi Ohnishi², Masami Takahashi^{1,3}

¹Dept. Biochem. Sch. Med., Kitasato Univ., ²Biosignal Research Center Inst. Molecular and Cellular Regulation, Gunma Univ., ³CREST, JST

Neurotransmitter release is regulated by various protein kinases and we have already shown that the activation of PKA and PKC enhance, and those of nonreceptor type tyrosine kinases including Src kinase and JAK2 suppress neurotransmitter release from clonal rat pheochromocytoma PC12 cells. Since the phosphorylation status is determined not only by protein kinases but also by protein phosphatases, it is quite important to study the roles of various protein phosphatases to elucidate the regulatory mechanisms of neurotransmitter release. Treatment of PC12 cells with phenylarsine oxide (PAO), a non-selective protein tyrosine phosphatase (PTP) inhibitor, induced tyrosine phosphorylation of many cellular proteins and suppression of Ca^{2+} -dependent dopamine release. The tyrosine phosphorylation was markedly reduced by pretreating the cells with PP2, a specific inhibitor of Src family kinases, prior to PAO treatment, indicating that Src family kinases were constitutively activated and made a major contribution of tyrosine phosphorylation in PC12 cells. Alpha-bromo-4-hydroxyacetophenone (PTP inhibitor I), a more specific PTP inhibitor, also inhibited dopamine release, however, much less proteins were tyrosine phosphorylated. These results suggest that various PTP are involved in the regulation of multiple cellular functions and a tyrosine phosphorylation regulated by PTP inhibitor I-sensitive PTP is essential for the negative regulation of neurotransmitter release.

S7-3

Modulation of the NMDA receptor by a protein tyrosine kinase and PSD-95

○Makoto Inui

Dept. Pharmacol. Yamaguchi Univ. Sch. Med.

The function of the NMDA receptor is modulated by protein phosphorylation at serine or threonine and at tyrosine. Modulation of NMDA receptor channels has been extensively studied using heterologously expressed recombinant NMDA receptors. The activation of protein kinase C potentiates the channel activity of recombinant both NR1/NR2A and NR1/NR2B receptors. The protein tyrosine kinase Src potentiates the channel activity of the NR1/NR2A receptor, but not NR1/NR2B. The NR2 subunits of NMDA receptors have long cytoplasmic C-terminal domains which contain sites for interaction with other proteins. The C-terminus of NR2 subunits binds to PDZ domains of the postsynaptic density protein PSD-95. The interaction of these proteins is implicated in clustering and targeting of the receptors. We have been studying the channel properties of PSD-95-coupled NMDA receptors using the *Xenopus* oocyte expression system, and found that PSD-95 inhibits the protein kinase C-mediated potentiation of NR1/NR2A and NR1/NR2B channels. Here we demonstrate that PSD-95-coupled NR1/NR2A receptor channels are not potentiated by Src. In addition, the high-affinity, voltage-independent Zn^{2+} inhibition is significantly reduced in these channels. It has been shown that Src-induced potentiation of NR1/NR2A channels is caused by the removal of tonic Zn^{2+} inhibition, although the Src-induced potentiation of NMDA receptor currents in hippocampus neurons is not mediated by a reduction in Zn^{2+} inhibition. Our results indicate that the absence of Src-induced potentiation of PSD-95-coupled NR1/NR2A channels is not due to the reduced sensitivity of the channels to Zn^{2+} . These two effects induced by PSD-95 are independent. Thus, PSD-95-coupled channels exhibit different properties than those without PSD-95. Our results well explain why Src-induced potentiation of NMDA receptor currents in hippocampus neurons is not mediated by a reduction in Zn^{2+} inhibition and suggest that a novel mechanism may be involved in the Src-induced potentiation of NMDA receptor channels in neurons.

S7-4

Positive and negative regulations of synaptic function by receptor tyrosine kinases.

○Nobuyuki Takei, Hisaaki Namba, Mako Narisawa-Saito, Tadasato Nagano, Hiroyuki Nawa

Dept. Mol. Neurobiol., Brain Res. Inst., Niigata Univ.

Brain-derived neurotrophic factor (BDNF) and its cognate receptor tyrosine kinase TrkB are implicated as a positive regulator of synaptic development and plasticity. In developing CNS neurons, TrkB signaling increased and stabilized the surface expression of AMPA receptors. Accordingly, TrkB activation increased the levels of AMPA receptor-associated PDZ proteins such as SAP97, GRIP and Pick1. Conversely, AMPA receptors and those PDZ proteins were down-regulated in BDNF knockout brain. In addition to the biochemical evidences, electrophysiological studies showed that the activation of TrkB increased AMPA-mediated currents in cortical neurons, which means the increased AMPA receptors and presumably PDZ proteins were functional for excitatory synaptic transmission. Furthermore, the amplitude of miniature EPSCs were reduced in these neurons of BDNF knockout mice. These results indicate that TrkB signaling positively regulates the development of functional AMPA synapses. In contrast, epidermal growth factor (EGF) family ligands and their receptor tyrosine kinases, ErbBs are found to be involved in down-regulation of excitatory synaptic development and functions. Stimulation of ErbB receptors by EGF, transforming growth factor α (TGF α) and heparin binding-EGF like growth factor HB-EGF caused the decrease of GluR1 AMPA receptor subunit in developing cortical neurons. ErbB signaling also decreased the levels of above-mentioned PDZ proteins. ErbB activation reduced AMPA currents in cortical neurons, indicating that the stimulation of these receptor kinases negatively regulates these synaptic function. Positive and negative regulation of synaptic functions by different receptor tyrosine kinases will be further discussed.

S7-5

ErbBレセプター型チロシンキナーゼによる
シナプス機能の制御機構

○尾崎 美和子, 端川 勉

理研・脳センター・神経構築

神経細胞のもつ最も大きな特徴は、細胞自体が神経活動と呼ばれる電気的な活動を有することである。その活動は発生の初期の段階では観察されず、発生分化が進むにつれより強い、様々なパターンの活動へと変化する。その過程で、神経細胞は神経伝達物質や神経栄養因子といった刺激に反応し、イオンチャンネルや伝達物質受容体などを発現し、神経細胞固有の伝達物質感受性や興奮性（固有の神経活動）を獲得していくものと考えられる。これまで我々は、特定の神経活動のパターンが神経の発生・分化に重要であり、また特定のパターンは積極的に様々な分子の挙動を制御できることも報告してきた。

今回我々は、ErbBレセプター型チロシンキナーゼとそのリガンドであるニューレグリン (NRG) と呼ばれる多機能性神経栄養因子に着目し、蛋白のプロセッシングが特定の神経活動のパターンにより制御されていることを明らかにした。また、NRGは小脳顆粒細胞と苔状線維間のシナプス形成と発達に重要な分子であり、ErbBレセプターの活性化を通し、興奮性伝達物質グルタミン酸の受容体NMDA受容体NR2Cサブユニットと抑制性伝達物質GABA受容体の $\beta 2$ サブユニットの発現を制御している因子でもあった。我々は、顆粒細胞にとって興奮性または抑制性シグナルの入力に関与している伝達物質受容体サブユニットの発現バランスが、異なる神経活動のパターンを介し、NRGの蛋白分解の効率と伝達受容体やイオンチャンネルの活性化とのコンビネーションにより制御されていることを明らかにした。また、異なる神経活動のパターンは異なる分子の挙動を制御しているとも考えられ、神経活動のパターンのもつ役割に関して報告する。

S8-1

Glial cells in development, function, degeneration
and regeneration of the nervous system

○Shun Shimohama

Dept. Neurol. Grad. Sch. Med., Kyoto Univ.

Glial cells have long been recognized as a passive framework for neurons but recently were shown to play much more active roles in the brain. The focus of this symposium is the role of glial cells in the development, function, degeneration and regeneration of the normal and diseased nervous system. In the development of the brain, glial cells guide incoming axons acting as intermediate targets. Glia are the part of the blood-brain barrier, provide nourishment for neurons, and regulate the extracellular concentration of ions and neurotransmitters. Glia interact with each other and with neurons, thus influencing neural activity. In addition, reactive glial cell properties could contribute to pathomechanisms underlying neurological diseases such as stroke and Alzheimer disease. The main role of glia in the adult nervous system might be to respond to disruption of the homeostasis of the brain, whether that disruption comes from direct damage to neurons, neuronal degeneration or through disease. Control of glia is likely to be just as important in regeneration as providing a favorable environment for neurons grow. For example, our experimental results, together with recent findings that beta adrenergic receptor plays an important role in glial scar formation in vivo, raise the possibility that beta adrenergic receptor activation modulates small heat shock protein expression after focal ischemia and is involved in the transformation of astrocytes to their reactive forms. Clarification of what regulates the transformation of astrocytes to their reactive form will provide the chance for eventual therapeutic target after brain injury. Glia must be regarded as a concept that shapes the approaches taken to development, degeneration, disease and regeneration of the nervous system.

S8-2

Unique responses of astrocytes to Alzheimer's amyloid β protein

○Kazuho Abe, Miwa Misawa
Dept. Pharmacol., Sch. Pharm., Hoshi Univ.

Amyloid β protein ($A\beta$) is a 40-42 amino acid peptide that principally constitutes senile plaques in the brain of patients with Alzheimer's disease. Although neurotoxicity of $A\beta$ has been extensively studied, little is known about the action of $A\beta$ on glial cells. Therefore, we compared the effects of $A\beta$ on cultured neurons and astrocytes. Primary cultures of neurons were prepared from the cerebral cortices and hippocampi of 18-day-old embryos of Wistar rats, and astrocyte cultures were from the cerebral cortices of 2-day-old neonates of Wistar rats. Exposure of cultured rat cortical or hippocampal neurons to $A\beta$ (1-20 μ M) resulted in a concentration- and time-dependent decrease in cell survival. The $A\beta$ neurotoxicity was significantly attenuated by the presence of antioxidants (catalase, Trolox or propyl gallate). On the other hand, cultured rat cortical astrocytes were resistant to $A\beta$ toxicity, and exhibited morphological and functional changes in response to $A\beta$. Following exposure to $A\beta$ (1-20 μ M), astrocytes changed from flattened, polygonal cells to process-bearing stellate cells, resembling reactive astrocytes *in vivo*. The L-glutamate uptake of astrocytes was enhanced after treatment with $A\beta$. These responses of astrocytes to $A\beta$ were not affected by antioxidants. Furthermore, $A\beta$ induced phosphorylation of extracellular signal regulated kinase (ERK) in cultured astrocytes, but not in neurons. The $A\beta$ -induced ERK phosphorylation in astrocytes was blocked by U0126 and PD98059, specific inhibitors of the ERK-activating kinase MEK. The differential responses of neurons and astrocytes to $A\beta$ will give new insight into therapeutic strategy for Alzheimer's disease.

S8-3

Regulation of amyloid- β clearance in microglia

○Yoshihisa Kitamura, Jun-ichi Kakimura, Takashi Taniguchi
Dept. Neurobiol., Kyoto Pharm. Univ.

Alzheimer's disease (AD) is characterized by the accumulation of fibrillar amyloid- β peptides ($A\beta$) to form amyloid plaques. Understanding the balance of production and clearance of $A\beta$ is the key to elucidating amyloid plaque homeostasis. Microglia in the brain, associated with senile plaques, are likely to play a major role in maintaining this balance. Here we show that heat-shock proteins (HSPs), such as Hsp90, Hsp70 and Hsp32, induce the production of interleukin (IL)-6 and tumor necrosis factor (TNF)- α and increase the phagocytosis and clearance of $A\beta$. This suggests that microglial interaction with $A\beta$ is highly regulated by HSPs. The mechanism of microglial activation by exogenous HSPs involves the NF- κ B and p38 MAP kinase pathways mediated by Toll-like receptor (TLR)-4 activation. In AD brains, levels of Hsp90 were increased in both the cytosolic and membranous fractions, and Hsp90 was co-localized with amyloid plaques. These observations suggest that HSP-induced microglial activation may serve a neuroprotective role by facilitating $A\beta$ clearance and cytokine production.

S8-4

Therapeutic application of glia-neuron interaction on neurodegeneration

○Keiji Wada¹, Takayuki Harada¹, Chikako Harada¹, Yu-Lai Wang¹, Mami Noda², Hitoshi Osaka^{1,3}

¹Dept. Degene. Neurol. Dis., Natl. Inst. Neurosci. NCNP, ²Lab. Pathophysiol. Grad. Sch. Pharm. Sci., Kyushu Univ., ³PRESTO, JST

We present here our new approaches to treat neurodegenerative disorders. We used light-induced or ischemia-induced retinal degeneration as models to develop therapeutic strategies. We found that retinal degeneration activates microglia, which results in the production of various ligands including NGF, CNTF and BDNF. Exogenous NGF decreased bFGF production in Muller glial cells. Reduction of bFGF level likely contributed to photoreceptor apoptosis. Blockade of the NGF pathway was able to rescue photoreceptor apoptosis. In contrast to NGF, CNTF and BDNF increased bFGF production from Muller cells. Thus, our data implicate that modification of glia-glia and glia-neuron interaction as new therapeutic targets for neurodegeneration. Besides the neurotrophic interactions, we also found that the ubiquitin system plays an important role in retinal degeneration. We studied ischemia-degenerated retina in the gracile axonal dystrophy (gad) mutant mouse that lacks functional expression of ubiquitin C-terminal hydrolase 1 (UCH-L1). Unexpectedly, photoreceptor apoptosis was reduced in the gad mouse. Our result indicates that modification of the ubiquitin-proteasome pathway could be another therapeutic target for neurodegeneration. Since UCH-L1 is expressed selectively in neuron, studies of functional difference in the ubiquitin system between glia and neuron should be an important subject to prevent neurodegeneration.

S8-5

Reg family; new members of neuron-Schwann cell mediator during nerve regeneration.

○Kazuhiko Namikawa, Munehisa Fukushima, Koichi Murakami, Hiroshi Kiyama

Dept. of Anatomy, Osaka City University, Graduate School of Medicine

Axons of PNS neurons are myelinated by Schwann cells. This could be a major reason why the PNS neurons can readily survive and regenerate after nerve injury. Schwann cells can proliferate to give a path for regenerating axons, and also produce a number of neurotrophic factors and cytokines, which have potent survival and regeneration promoting activities for injured neurons. Recently, a pancreatic beta-cell regeneration factor, PAP/Reg-2, was ascribed as a chemical mediator between neurons and Schwann cells. PAP is synthesized and released from motor neurons in response to nerve injury, and works as Schwann cell mitogen as well as motor neuron survival factor. PAP together with other family members such as RegI, RegIII and PAPIII comprise a new family of trophic factor, which is distinct from the conventional neurotrophins and cytokines. Among Reg family members, PAP is the only one whose function in nerve regeneration is rather explored. To ascertain roles of Reg family during nerve regeneration in vivo, we examined the mRNA expressions of rat Reg family members (RegI, PAP, RegIII, PAPIII), and their receptor (RegR) in injured hypoglossal motoneurons and Schwann cells. A substantial up-regulation of PAP expression in injured motor neurons was evident. In addition, marked expression of PAPIII and RegR was also observed in the injured nerve, suggesting PAPIII might be an autocrine mitogenic factor for injured Schwann cells. Collectively, the cooperative inductions of Reg family ligands and their receptor might play crucial role in neuron-Schwann cell interaction during nerve regeneration.

S8-6

Changes of axonal protein organization under abnormal myelin conditions

○Hiroko Baba

Dept. Mol. Neurobiol. Tokyo Univ. Pharm. Life Sci.

Ion fluxes in mammalian myelinated axons are restricted to nodes of Ranvier, where, in particular, voltage-gated Na⁺ channels are clustered at a high density. The node of Ranvier is separated from the internode by two distinct domains of axolemma, paranode and juxtaparanode. Each axonal domain is characterized by the presence of specific protein complex. Although oligodendrocytes and/or myelin membranes are believed to play some instructive roles in organization of axonal domains, the mechanisms leading to their distributional changes are not well understood. In this symposium, we focus on the involvement of myelin sheaths in this domain organization. We have examined the distribution of axonal components in various pathological conditions of myelin, including demyelination, dysmyelination and paranodal disruption, and showed that compact myelin and paranodal junction of myelin had individual influences on the axonal protein organization. Recently we identified three myelin molecules playing key roles in the formation of paranodal axo-glial junction as well as the organization and maintenance of axonal specific domains. These results may provide not only new functional aspects of myelin but also a potential therapeutic approach to human disorders of myelin, such as multiple sclerosis.

S9-1

A circadian output in *Drosophila* mediated by fragile X mental retardation gene

Shunsuke Ben Inoue¹, Masami Shimoda², Izumi Nishinokubi³, Mikiko Chihara Siomi¹, Norio Ishida^{3,4,5},
○Haruhiko Siomi¹

¹Inst. Genome Res., Univ. Tokushima, ²Nat. Inst. Agrobiol. Sci., ³Clock Cell Biol., Nat. Inst. AIST., ⁴Dept. Biomole. Engineering., Tokyo Inst. Tech., ⁵Center Interdis. Res.

Mutations that abolish expression of an X-linked gene, FMR1, result in the pathogenesis of fragile X syndrome, the most common form of inherited mental retardation. To understand how the absence of the RNA binding FMR1 protein leads to the syndrome, we have created a model system for the disease by producing a fly strain bearing a deletion in a *Drosophila* homolog of FMR1 (*dfmr1*). We show that under constant darkness a lack of *dfmr1* expression causes arrhythmic locomotor activity in flies. However, since their behavioral rhythms in light-dark cycles (LD), and their biochemical rhythms of expression of *per* and *tim* under LD conditions appear normal, the *dfmr1* mutant flies are rhythmic, indicating that they have an active circadian pacemaker that can be entrained by light. In addition, the clock-controlled eclosion rhythm is normal in *dfmr1* mutant flies. Overexpression of *dfmr1* in clock-gene-expressing neurons alone also disturbs locomotor activity in adult flies, suggesting that DFMR1 functions in clock cells. We conclude that a lack or high levels of *dfmr1* expression blocks or interferes with elements in the flow of temporal information from circadian pacemaker cells to selective behaviors.

S9-2

The mutations in the neural sex-determinant gene fruitless in *Drosophila* enhances male-to-male courtship

○Daisuke Yamamoto

School of Human Sciences, Waseda University

The mutations in the fruitless (*fru*) locus of *Drosophila* are characterized by two discrete phenotypes, enhanced male-to-male courtship and loss of the male-specific muscle of Lawrence (MOL). The *fru* gene encodes a series of proteins with a BTB domain and two Zn-finger motifs. These structural features implicate their role as transcription factors. The *fru* gene primary transcript is subjected to sexually dimorphic splicing in response to binding of Transformer (Tra), a splicing accelerating protein, which is a female determinant present only in the female. The Tra-binding sequence is conserved in the female-type mRNA while it is removed from the male-type mRNA by the sex-specific splicing. The *fru* gene is therefore a direct target of Tra. A well-known target of Tra is doublesex (*dsx*), which produces sex-specific transcription factors that regulate development of most sexually dimorphic structures. In contrast, *fru* mRNAs are predominantly expressed in a subset of neurons, suggesting that *fru* is a neural sex determinant. Although *fru* mRNAs are expressed in both sexes, the Fru protein is detectable histochemically only in the male CNS. Luciferase reporter assays with transfected cells have shown that Tra represses translation by binding to the reporter mRNA. Tra is thus considered to be a bifunctional protein that acts as a translational repressor and also as a splicing factor. Since the Tra-binding sequence is present in the female-type *fru* mRNA and not in the male-type, the Fru protein would be translated only in the male CNS. Ectopic expression of the Fru protein in females through a *fru* transgene without the Tra-binding sequence results in formation of the male-specific MOL in the female, demonstrating that the presence of Fru is sufficient for masculinization.

S9-3

Ribosomal protein L38 is essential for early embryogenesis and neural tube formation of mice

○Toshihiko Shiroishi

Mammalian Genetics Laboratory, Genetic Strains Research Center, National Institute of Genetics

Three mouse skeletal mutations, Tail-short (Ts), Tail-short shionogi (Tss) and Rabo torcido (Rbt), have been mapped to the same distal region of the chromosome 11. All three mutations exhibit very similar phenotypes: homozygotes are morphologically abnormal at the morula stage and are lethal around the implantation stage, and the heterozygotes show a shortened kinky tail, neural tube defect (NTD) characterized by exencephaly and skeletal abnormalities. The causative genes of these mutations have been thought to play indispensable roles in early embryogenesis, neural tube formation and skeletal patterning. Using positional cloning approach, we found that a gene encoding Rpl38 protein, a small 8.1kD component of the 60S large subunit of ribosome, is mutated in all three Ts, Tss, and Rbt mutations. Furthermore, we demonstrated that transgene of the wild-type Rpl38 rescued all the phenotypes of Ts mutation. All data present direct evidence that defect of a small ribosomal protein causes embryonic lethality and abnormal morphogenesis including neural tube defect.

S9-4

The RNA-binding protein causes aberrant splicing of presenilin-2 pre-mRNA in sporadic Alzheimer's disease

○Taiichi Katayama^{1,3}, Takayuki Manabe^{1,3}, Shinsuke Matsuzaki^{1,3}, Kazunori Imaizumi^{2,3}, Masaya Tohyama^{1,3}

¹Department of Anatomy and Neuroscience, Osaka University Graduate School of Medicine, ²Division of Structural Cell Biology, Nara Institute of Science and Technology (NAIST), ³CREST/JST

The splicing variant (isoform PS2V) of Presenilin-2 (PS2) gene, which is a marked diagnostic of sporadic Alzheimer's disease (SAD), is generated by exon 5 skipping through the action of the high mobility group protein I (HMG-I). This protein was purified and identified as PS2 pre-mRNA binding factor in nuclear extracts from hypoxia-exposed human neuroblastoma SK-N-SH cells. HMG-I bound to a specific sequence on exon 5 of the PS2 pre-mRNA, nearby the downstream 5' splice site, and this sequence-specific binding was required for exon 5 skipping. Under hypoxia, HMG-I was reorganized into the nuclear speckles with the endogenous splicing factor SC35. Since HMG-I has a strong binding affinity to the 70K protein of U1 snRNP, the HMG-I induced exon skipping of PS2 pre-mRNA is probably caused by interference of the functional U1 snRNP allocation to the 5' splice site. HMG-I levels were significantly increased in the nuclei of SAD brain cells, suggesting direct involvement of HMG-I-mediated aberrant splicing of PS2 gene transcript in SAD. Moreover, transient transfections of oligo RNA, including the binding site of HMG-I, dramatically inhibited the expression of PS2V underlying hypoxia in SK-N-SH cells. Further, PS2V-induced cell deaths were also suppressed by the addition of oligo RNA. Here, in this presentation, we will report a potential treatment and/or prevention of SAD using oligo RNA including the specific binding site of HMG-I.

S9-5

Aberrant mRNA splicing in neurodegenerative disorders

○Kazunori Imaizumi

Div. Structural Cell Biol., Nara Inst. Sci.&Tech. (NAIST)

Alternative splicing represents a typical mechanism underlying regulation of gene expression in eukaryotic cells. Exon selection results in the production of different protein isoforms from the same gene, isoforms that may share functions with the original form. Alternatively, variant protein isoforms may either lack function or confer novel characteristics on their cellular environment. In fact, tau splicing mutations which increase 4-repeat isoforms containing exon 10 of tau were found in frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17). In addition, it has been reported that aberrant splicing causes some neurodegenerative disorders such as sporadic Amyotrophic Lateral Sclerosis (ALS) and spinal muscular atrophy (SMA). In this symposium, I will introduce our attempts to elucidate molecular mechanisms responsible for aberrant splicing in SMA and FTDP-17. SMA results from the loss of functional survival motor neuron (SMN1) alleles. Two nearly identical copies of SMN exist and differ only by a single non-polymorphic C to T transition in exon 7. This transition leads to alteration of exon 7 splicing, that is, SMN1 produces a full-length transcript whereas SMN2 expresses a low level of full-length transcript and predominantly an isoform lacking exon 7. The truncated transcript of SMN encodes a less stable protein with reduced self-oligomerization activity that fails to compensate for the loss of SMN1. Recently, we identified the cis-acting elements in intron 6 responsible for the regulation of SMN exon 7 splicing. Mutation in this cis-element caused an increase in exon 7 inclusion. I will show the roles of the cis-acting element in splicing regulation of SMN exon 7. Missense, silent, and intronic tau mutations increase or decrease inclusion of tau exon 10 by splicing defects. I will also present the regulation of tau exon 10 splicing and an attempt to identify splicing factors that induce aberrant splicing of tau exon 10.

S10-1

Expression and possible function of versican/Bral1 complex at the nodes of Ranvier in the CNS

○Toshitaka Oohashi

Dept. Mol. Biol.&Biochem. Grad. Sch. Med. Dent., Okayama Univ.

The central nervous system (CNS) is composed of not only cellular components of neurons and glial cells but also an extracellular matrix that serves to maintain a structural and functional environment for these cells. The extracellular matrix of the CNS is abundant in hyaluronan (HA) and molecules that have the ability to bind to HA. Lecticans are a family of chondroitin sulfate proteoglycans (CSPGs) encompassing aggrecan, versican/PG-M, neurocan, and brevican molecules. All members of the lectican family are present in neuronal tissues during certain phases of development. We have cloned Bral1, a brain-specific link protein, recently. Single link protein (cartilage link protein-1; Crtl1) gene has been known so far. The major function of link protein found to date is the stabilization of HA/aggrecan aggregates by binding to both molecules, the proteoglycan monomer and HA. To gain insight into the role of Bral1, we generated specific antibody against this protein. We have examined the detailed localization pattern of Bral1 protein and compared it with that of other members of the lectican proteoglycan family such as brevican and versican with which Bral1 is predicted to interact. The immunoreactivity of Bral1 antibody was predominantly observed in myelinated fiber tracts in the adult brain and could be detected at P20 in the white matter of the developing cerebellum, suggesting that the expression start when axonal myelination takes place. Furthermore, immunostaining demonstrated that the Bral1 colocalized with versican V2 isoform at the nodes of Ranvier. The data suggest that the Bral1 may play a pivotal role in the formation of the hyaluronan-associated matrix in the CNS that facilitates neuronal conduction by forming an ion diffusion barrier at the nodes.

S10-2

Signal transduction of PTP ζ and morphogenesis of Purkinje cells

○Nobuaki Maeda¹, Masahiko Tanaka²

¹Dept. Dev. Neurosci., Tokyo. Metro. Inst. Neurosci.,

²Inst. Compre. Med. Sci., Fujita Health Univ.

PTP ζ (RPTP β) is a receptor-type protein tyrosine phosphatase, which is abundantly expressed in the developing brain as a chondroitin sulfate proteoglycan. PTP ζ binds with pleiotrophin and midkine with high affinity. Pleiotrophin shows 45% amino acid sequence identity to midkine, forming a new family of heparin-binding growth factors. The chondroitin sulfate portion of PTP ζ plays essential roles in the binding to these growth factors. Removal of chondroitin sulfate chains from PTP ζ by chondroitinase ABC digestion resulted in the marked decrease of the binding affinity to pleiotrophin and midkine and the loss of signal transduction. The fine structure of chondroitin sulfate chains of PTP ζ regulates its binding to these growth factors. Chondroitin sulfate chains of PTP ζ contain about 1% D-unit (D-glucuronic acid 2-sulfate(β 1 $\rightarrow$ 3)N-acetylgalactosamine 6-sulfate disaccharide unit), which seems to be the essential structure for the high affinity binding to pleiotrophin and midkine. Immunohistochemical analysis of PTP ζ , pleiotrophin and chondroitin sulfate D-unit demonstrated that the expression of these molecules are highly correlated with the dendrite formation of cerebellar Purkinje cells. So, we examined the roles of the signal transduction of PTP ζ in the morphogenesis of Purkinje cells using organotypic slice culture system of rat cerebellum. Abnormal Purkinje cells with disoriented and/or multiple primary dendrites markedly increased by the additions of (1) polyclonal antibodies against PTP ζ , (2) sodium vanadate, a protein tyrosine phosphatase inhibitor, (3) chondroitinase ABC, (4) chondroitin sulfate D, and (5) pleiotrophin. These results suggest that pleiotrophin-PTP ζ signaling regulates the morphogenesis of Purkinje cell dendrites.

S10-3

Signal Transduction of Phosphacan via TAG-1/Lyn in Lipid Rafts

○Kohji Kasahara, Yutaka Sanai
Tokyo Metro Inst Med Sci

Chondroitin sulfate proteoglycans, which are constituents of the extracellular matrix, play a significant role in cell migration, neurite outgrowth, pathfinding and synaptogenesis in the developing brain. In this study, we found that phosphacan, a neural tissue-specific chondroitin sulfate proteoglycan, adsorbed onto culture dish exerts repulsive effects on rat primary cerebellar cultures. Sequential treatment of cerebellar cultures with phosphacan and anti-phosphacan antibody induced transient tyrosine phosphorylation of 80-kDa protein. Phosphacan is a high affinity ligand of TAG-1, a GPI-anchored neuronal cell adhesion molecule of the immunoglobulin superfamily, which is involved in neurite outgrowth. We previously demonstrated that the antibody-mediated crosslinking of TAG-1 induced activation of src-family tyrosine kinase Lyn and rapid tyrosine phosphorylation of an 80-kDa protein, a putative substrate for Lyn, in the lipid raft fraction of primary cerebellar cultures (Kasahara et al. J.Biol.Chem. 275, 34701-34709, 2000). The tyrosine phosphorylation of 80-kDa protein was inhibited by pretreatment with methylcyclodextrin. Immunocytochemical study showed that both TAG-1 and Lyn are present in cerebellar granule cells, which have small, phase dark somata and fine dark processes. These observations suggest that phosphacan transduces signal via TAG-1/Lyn in lipid rafts of cerebellar granule cells.

S10-4

Structure and expression pattern of neuroglycan C, a brain-specific transmembrane chondroitin sulfate proteoglycan, in the developing brain

○Sachiko Aono, Yoshihito Tokita, Shinobu Yamauchi, Takuya Shuo, Atsuhiko Oohira
Dept. Perinatol., Inst. Dev. Res., Aichi Human Service Ctr.

Neuroglycan C (NGC), a membrane-spanning chondroitin sulfate (CS) proteoglycan with a single epidermal growth factor-like module, is exclusively expressed in the brain, especially in the developing brain. Three distinct splicing variants (NGC-I, -II, and -III) were expressed in the brain, and NGC-I was the major one. Most NGC molecules were localized to the dendrites of primary cultured neurons. NGC-III, which has a peptide insertion in the cytoplasmic domain of NGC-I, was localized to the axons of a subpopulation of neurons. NGC was post-translationally modified in its ectodomain; glycosylated and phosphorylated. Site-directed mutagenesis revealed that NGC had a single CS chain at Ser-123. CS of NGC contained a higher proportion of Δ Di-diSE, a highly-sulfated CS disaccharide, than those of other brain-specific proteoglycans such as neurocan and phosphacan. NGC changed its structure from a proteoglycan form to a non-proteoglycan form without CS in the cerebellum and the retina as the tissues mature. In addition to CS chain, NGC had both N- and O-linked oligosaccharides on its ectodomain. Reactivities of NGC with various lectins and the HNK-1 antibody also changed during the brain development. The carbohydrate moieties would regulate molecular interactions between NGC and its ligands in the brain development. Unexpectedly, the ectodomain of NGC was phosphorylated in primary cultured brain cells. The phosphorylation by a casein kinase II-like kinase may also be implicated in the modification of molecular interactions in which NGC is involved. Finally, homologous targeting studies proceeding in our laboratory would produce more direct clues to clarify the physiological roles of NGC in the brain development.

S10-5

Specific Heparan Sulfate Structures Involved in Retinal Axon Targeting

○Atsushi Irie

Dep. Biochem. Cell Res., Tokyo Metropolitan Inst. Med. Sci.

Heparan sulfate (HS), a structurally diverse molecule comprising distinct sequences of sulfated disaccharide units, is abundant in the developing brain and binds to several axon guidance molecules. However, how structural diversity of HS relates to its putative role in axon guidance is not known. Using a live brain assay in *Xenopus*, we examined the structural characteristics of HS that affected retinal axon targeting to the optic tectum in vivo. Inhibiting sulfation of endogenous HS with chlorate causes retinal axons to bypass their target, the tectum. Exogenous addition of HS and heparin to brains also induces the bypass phenotype, presumably owing to competitive inhibition of endogenous HS function. Treatment with chemically modified heparins reveals that 2-O- and 6-O-sulfate groups have potent bypass-inducing activity. Experiments with purified heparin saccharides show that bypass-inducing activity correlates with distinct structures, particularly those containing a combination of 2-O- and 6-O-sulfate groups. Taken together the results indicate that specific sequences, rather than gross structural composition, are critical for activity. In situ hybridisation revealed that HS 6-O-sulfotransferase is regionally expressed along the border of the dorsal optic tract whereas HS 2-O-sulfotransferase is widely distributed in the developing brain. Our results demonstrate that specific HS sequences are essential for regulating retinotectal axon targeting and suggest that regionalised biosynthesis of specific HS structures is important for guiding axons into tectum.

S11-1

Brain cytokine expression mediated by the vagus nerve and leptin

○Yasunobu Okuma, Toru Hosoi, Yasuyuki Nomura

Dept. Pharmacol. Grad. Sch. Pharm. Sci., Hokkaido Univ.

Peripheral immune system can signal the brain during inflammation. Then, possible roles of the afferent vagus nerve in regulation of interleukin (IL)-1 β expression in the brain were examined. Direct electrical stimulation of the afferent vagus nerves itself can induce production of IL-1 β in the brain and activate the HPA axis. Therefore, the afferent vagus nerve may play an important role in transmitting peripheral signals to the brain in the acute phase of infection and inflammation. Increasing evidence has suggested that leptin, the product of the ob gene may interact with cytokines in immune system. Peripherally applied leptin increased IL-1 β transcripts in the hypothalamus, the hippocampus, the cortex, the cerebellum and the brainstem. Leptin increased the expression of IL-1 β mRNA in mouse primary cultured glial cells, indicating that one of the target cells of the leptin-induced IL-1 β transcript may be a glial cells. It was reported that leptin and CCK activate the afferent vagus nerve. Increasing evidence has suggested that leptin may interact with cytokines in the immune system. Peripherally applied leptin increased IL-1 β transcripts in the hypothalamus in mice. On the other hand, CCK did not affect IL-1 β transcripts in the hypothalamus. Then, we investigated whether leptin-induced IL-1 β expression in the hypothalamus is mediated via afferent vagus nerve. Subdiaphragmatic vagotomy did not significantly modify leptin-induced IL-1 β expression in the hypothalamus, indicating circulating leptin directly induce IL-1 β transcript in the brain independent of afferent vagus nerve. Therefore, it is suggested that there exist an afferent vagus nerve-dependent and -independent mechanisms leading to IL-1 β expression in the brain during the infection and inflammation.

S11-2

Functional regulation of primary afferent neurons by interleukin-1 β

○Norimitsu Morioka¹, Atsuko Inoue², Yoshihiro Nakata²

¹Dept. Dental Pharmacol., Grad. Sch. Biomed. Sci., Hiroshima Univ., ²Dept. Pharmacol., Grad. Sch. Biomed. Sci., Hiroshima Univ.

Substance P (SP) is a neuropeptide which has been shown to be released from nerve terminals of primary afferent neurons. This molecule relays nociceptive information to the spinal cord, and also induces inflammatory responses in the peripheral tissues. Interleukin-1 β (IL-1 β) is a proinflammatory cytokine that is known to play important roles in the inflammatory responses. In this study, to understand the interaction between SP and IL-1 β , we investigated the effects of the IL-1 β on the SP release in the primary cultured rat dorsal root ganglion (DRG) cells. In cultured DRG cells, treatment with IL-1 β increased the release of SP after incubation for more than 6 hours. This increase of SP release was significantly suppressed by not only IL-1 receptor antagonist but also the inhibitors of secretory phospholipase A₂(sPLA₂), cytosolic PLA₂ (cPLA₂) or cyclooxygenase (COX)-2. In non-treated DRG cells, the expression of sPLA₂-IIA and COX-2 mRNA was undetectable and low levels, respectively, while in DRG cells incubated with IL-1 β , the mRNA levels of these enzymes was dramatically increased. cPLA₂ mRNA was constitutively expressed in non-treated DRG cells, and treatment with IL-1 β significantly increased the levels of cPLA₂ mRNA. These observations suggested that the transcriptional regulation of distinct three types of enzymes involved in the pathway of arachidonic acid metabolism might be essential events for the IL-1 β -induced SP release from DRG cells. These studies might provide a new insight in the neural-immune intercommunication involved in nociceptive regulation system observed in the inflammation-induced hyperalgesia.

S11-3

Mechanism by which endogenous glucocorticoid controls lymphocyte trafficking

○Ryoichi Nagatomi

Dept. Med. Sci. Sports & Exerc., Tohoku Univ. Grad. Sch. Med.

Alteration in lymphocyte trafficking and distribution affects immune response. Glucocorticoid is known to alter lymphocyte trafficking and distribution, which leads to altered immune response. Synthetic glucocorticoid, dexamethasone, is known to up regulate CXCR4 expression on T cells. CXCR4 is a chemokine receptor supposedly important for tissue distribution of T cells, since its unique ligand CXCL12 is expressed constitutively in various tissues. We asked whether endogenous cortisol elicited during stress or exercise could affect T cell trafficking by modifying CXCR4 expression on T cells. We demonstrated that *in vitro* treatment of human peripheral blood mononuclear cells with cortisol lead to augmentation of CXCR4 expression on T cells in a dose-dependent manner within a range of physiological concentration. Cortisol treatment also lead to increased mobilization of T cells towards CXCL12 *in vitro*. We then tested if endogenous cortisol elicited after acute exercise could affect CXCR4 expression on circulating T cells. Contrary to our expectation, CXCR4 on T cells obtained immediately after exercise was down-regulated with less migratory activity. Incubation of T cells obtained immediately after exercise lead to CXCR4 augmentation without extra cortisol *in vitro*. Incubation of T cells obtained before exercise with post-exercise plasma also augmented CXCR4 expression. These facts suggest that T cells with enhanced CXCR4 quickly migrated out of circulation leaving T cells with less CXCR4 in the blood stream. We propose that regulation of chemokine-receptor expression by glucocorticoid is an important cross-talk between neuroendocrine and immune system possibly regulating immune response to an appropriate extent under stress or during exercise.

S11-4

Presence and involvement of non-neuronal lymphoid cholinergic system in regulation of immune function

○Takeshi Fujii, Koichiro Kawashima
Dept. Pharmacol., Kyoritsu Col. Pharmacy

Accumulating evidence has now convincingly shown that acetylcholine (ACh), a classical neurotransmitter, is present in the blood of various mammalian species, including humans. Furthermore, T-lymphocytes contain a substantial amount of ACh, and express both mRNA and protein for choline acetyltransferase (ChAT), an ACh-synthesizing enzyme. Stimulation of ACh receptors (AChRs) on lymphocytes by muscarinic and nicotinic AChR (mAChRs and nAChRs, respectively) agonists has numerous functional and biochemical effects. These findings support the notion that lymphocytes constitute an independent non-neuronal cholinergic system. Activation of T-lymphocytes by phytohemagglutinin, a T-cell activator, modulates the function of the lymphoid cholinergic system, as demonstrated by increased synthesis and release of ACh, increased acetylcholinesterase (AChE) activity, and increased expression of mRNAs encoding ChAT and AChRs. Because T-lymphocytes can make direct contact with their target cells, even a small amount of ACh released from activated T-lymphocytes should interact with ACh receptors on target cells before hydrolysis by AChE. The presence of both mAChRs and nAChRs on lymphocytes, together with the observations of mAChR- and nAChR-mediated modulation of intracellular Ca^{2+} signaling and c-fos gene expression, strongly suggests that ACh synthesized and released from T-lymphocytes acts as an autocrine and/or paracrine factor regulating immune function. In summary, immune function is not only regulated by the cytokine system, but is also under the control of an independent, lymphoid cholinergic system.

S11-5

The effects of nicotine on Th1/Th2 balance in murine model of both Th1- and Th2-mediated inflammatory bowel disease

○Yuhji Murata¹, Jugoh Itoh²

¹Dept. Gastroenterol., Aomori Gen. Hosp., ²First Dept. Int. Med., Hirosaki Univ.

Aim: Epidemiological studies have demonstrated that smoking appears to be beneficial in ulcerative colitis (UC) but deleterious in Crohn disease (CD), and nicotine enema/patch are effective in active UC. However, the underlying mechanisms are still obscure. Recently, distinct immunological behaviours between CD and UC have been focused, and so we hypothesized that CD may be Th1 type- and UC be Th2 type-disease, respectively based on the previous reports, and that nicotine may modulate differently each disease through nicotinic acetylcholine receptors (nAChR) on T cells. To address this question, we utilized animal model of colitis, dextran sulphate sodium (DSS)-induced colitis for CD and oxazolone-induced colitis for UC, showing mainly Th1 type and Th2 type of immune response, respectively. **Methods:** Mice were treated subcutaneously with nicotine for 3 weeks. For DSS-induced colitis, BALB/c mice were fed with 5% DSS for last 7 days and to develop oxazolone-induced colitis, SJL/J mice were administered per rectum with 6 mg of oxazolone 3 days before the endpoint. Colonic mucosal damage was histologically evaluated and cytokine productions by affected colon were examined by ELISA. **Results:** 1) DSS-induced colitis: The histological damage of nicotine-treated mice became more severe than that of saline-treated mice. In cytokine production, both Th1-associated cytokine (IL-12, IFN- γ) and Th2-associated cytokine (IL-4 and 10), and IFN- γ /IL-4 ratio were significantly increased in DSS-treated mice compared with control. In nicotine-treated mice, IL-12 and IFN- γ production were increased, but IL-4 and IL-10 decreased, resulting in an elevated ratio of IFN- γ /IL-4 or IL-12/IL-4 compared with saline-treated mice. 2) Oxazolone-induced colitis: Histological damage score was significantly decreased in nicotine-treated mice compared with saline-treated mice. Decreased IFN- γ production, increased both IL-4 and IL-10 production and lower level of IFN- γ /IL-4 ratio were noticed in oxazolone-induced colitis compared with control. In nicotine-treated mice, IFN- γ production was increased, but both IL-4 and IL-10 production decreased, resulting in an elevated level of IFN- γ /IL-4 ratio compared with saline-treated mice. **Conclusion:** These results suggest that Th1 and Th2 response is predominant in DSS- and oxazolone-induced colitis, respectively, and nicotine may deteriorate DSS-induced colitis by enhancement of Th1 response and improve oxazolone-induced colitis by inhibition of Th2 response through nAChR on lymphocytes. The diverse effects of nicotine on CD and UC might be explained by such mechanism.

P01-E-1 (MS6-2)

Functional characterization of gabapentin transport and its interaction with voltage-gated Ca^{2+} channel in human primarily cultured astrocytes

○Takuya Fujita¹, Satoru Yamanaka¹, Kenji Shinomiya¹, Miyuki Wada¹, Miyuki Fukui¹, Akira Yamamoto¹, Michiko Oka²

¹Dept. Biochem. Pharmacol., Kyoto Pharmaceutical Univ., ²Nippon Shinyaku Co., Ltd.

Gabapentin (GBR) is an efficacious drug in the treatment of epilepsy, neurophatic pain, and anxiety states. GBR is orally active and can readily cross the blood-brain barrier because GBR is demonstrated to be a substrate for Na^+ -independent amino acid transport system L. On the other hand, GBR is considered to bind to the $\alpha 2 \delta$ subunit of voltage-gated Ca^{2+} channels (VGCC) with nanomolar affinity. In the present study, we investigated the functional characteristics of gabapentin transport and its interaction with VGCC in human primarily cultured astrocytes.

The uptake of leucine (Leu) and phenylalanine (Phe) in these cells was predominantly Na^+ -independent, was stimulated by lowering the extracellular pH, and was inhibited by hydrophobic neutral amino acids. These transport processes were saturable with Michaelis constants (K_i) of $46 \pm 7 \mu\text{M}$ and $39 \pm 10 \mu\text{M}$ for Leu and Phe uptake, respectively. GBR inhibited the Na^+ -independent uptake of Leu and Phe in a concentration-dependent manner, and its K_i values were $533 \mu\text{M}$ and $93 \mu\text{M}$ for Leu and Phe uptake, respectively. These K_i values were consisted with a previous report by Su et al. (J. Neurochem. 64, 2125, 1995) and were comparable to the K_i value for GBR in rat astrocytes.

GBR decreased K^+ (50 mM)-stimulated $[\text{Ca}^{2+}]_i$ increase and cyclic GMP formation, which reflects the activation of nNOS and/or eNOS, in a concentration-dependent manner ($\text{IC}_{50} \sim 300 \mu\text{M}$). BCH, a specific inhibitor of system L transporter, and Leu were found to recover the inhibitory action of GBR on K^+ -stimulated $[\text{Ca}^{2+}]_i$ in human astrocytes. However, both compounds *per se* were failed to decrease the K^+ -stimulated $[\text{Ca}^{2+}]_i$.

P01-E-2 (MS6-1)

NEUROSPHERES ISOLATED FROM HIPPOCAMPUS OF ADULT MICE

○Tomoya Kitayama, Masanori Yoneyama, Yukio Yoneda

Dept. Mol. Pharmacol., kanazawa Univ. Facul. Pharm. Sci.

We have previously shown that the systemic administration of N-methyl-D-aspartate (NMDA) inhibited the incorporation of 5-bromo-2'-deoxyuridine in the hippocampal dentate granular cells of adult mice 2 days after administration. As adult neurogenesis is shown to undergo in the dentate granular layers, we have attempted to isolate progenitor cells from hippocampus of adult mice. Hippocampus was dissected from adult mouse brain, followed by treatment with enzyme cocktail containing papain, neural protease and DNAase I for cell trituration, and subsequent washing before suspension with Percoll. Suspensions were centrifuged at 20,000g for 30 min at 18° C, followed by collection of the lower layer and subsequent plating on appropriate culture dishes. Under these conditions, cells were immunoreactive with an antibody against nestin, but not with those against MAP-2 and GFAP. Culturing in DMEM/F12 supplemented with FGF led to formation of typical neurospheres within 14 days in vitro (DIV), while brief exposure to NMDA for 5 min resulted in marked expression of both c-Jun and c-Fos proteins 2 h later in a manner sensitive to the NMDA antagonist MK-801 in these neurospheres. NMDA was also effective in inhibiting the formation of neurospheres in hippocampal preparations cultured for 14 DIV when exposed to cultured cells at 4 to 12 DIV. These results suggest that NMDA receptors may be expressed in progenitor cells to inhibit the formation of neurospheres through the AP1 complex in hippocampal dentate neurons of adult mice.

P01-E-3

FUNCTIONAL EXPRESSION OF GABA_B RECEPTORS IN CULTURED CALVARIAL OSTEOBLASTS

○Sayumi Fujimori, Eiichi Hinoi, Yukio Yoneda
Dept. Mol. Pharmacol., Kanazawa Univ. Pharm. Sci.

γ -Aminobutyric acid (GABA) plays a neurotransmitter role through ionotropic GABA_A and GABA_C receptors as well as metabotropic GABA_B receptors (GABA_BR) in the mammalian nervous systems. An RT-PCR analysis revealed constitutive expression of mRNA for the 2 splice variants of GABA_BR-1 subunit GABA_BR-1a and -1b, but not for GABA_BR-2 subunit, in rat calvarial osteoblasts cultured for 7 and 21 days in vitro (DIV). Expression of mRNA was also seen for α -1, α -2 and α -4 subunits of GABA_A receptors in immature but not mature cultured osteoblasts, while no mRNA was expressed for GABA_C receptor subunits in osteoblasts cultured from 7 to 21 DIV. Immunoblotting analysis confirmed expression of both GABA_BR-1a and -1b splice variants with the corresponding molecular weights. The agonist for GABA_BR baclofen significantly inhibited cAMP formation induced by forskolin in a manner sensitive to the antagonist 2-hydroxysaclofen in osteoblasts cultured for 7 DIV. Similar expression was seen with mRNA for GABA_BR-1a and -1b splice variants in the murine calvarial osteoblast cell line MC3TC-E1 cells cultured for 7 and 21 DIV. In MC3T3-E1 cells, baclofen not only inhibited the maturation-dependent increase in the activity of alkaline phosphatase, but also exacerbated that in Ca²⁺ accumulation. These results suggest that GABA may play a hitherto unidentified role in mechanisms associated with cellular proliferation, differentiation and/or development through functional GABA_BR expressed in cultured osteoblasts.

P01-E-4 (MS6-3)

M5 muscarinic receptors mediate the vasodilator actions of acetylcholine in cerebral arterioles and arteries.

○Yamada Masahisa¹, Noguchi Takanori², Lamping G Kathryn³, Duttaroy Alokesh⁴, Zhang Weillie⁴, Faraci M Frank³, Ogawa Masaharu¹, Wess Jügen⁴

¹Laboratory for Cell Culture Development, RIKEN Brain Science Institute, Saitama 351-0198, Japan,

²Laboratory for Behavioral Genetics, RIKEN Brain Science Institute, Saitama 351-0198, Japan.,

³Departments of Internal Medicine and Pharmacology, Cardiovascular Center, University of Iowa, Iowa City, IA, 52242, ⁴Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland 20892, USA.

The physiological functions of the M5 muscarinic acetylcholine receptor (mAChR) subtype are not well understood, primarily due to the lack of M5 receptor selective ligands. In addition, M5 receptors are expressed only at very low levels, representing less than 2% of the total mAChR population (M1-M5) expressed in the brain. To reveal the potential physiological roles of the M5 receptors, we generated and analyzed M5 receptor deficient mice. It is well known that ACh is a powerful dilator of most vascular beds and that this activity is mediated by endothelial mAChRs triggering the release of NO, the actual vasorelaxing agent. Since M5 receptor expression has been detected in vascular tissues, we tested the hypothesis that M5 receptors are involved in mediating ACh-induced vasodilation responses. Application of ACh produced concentration-dependent vasodilator responses in several in vitro and in vitro vascular preparations in WT mice. However, ACh was no longer able to dilate cerebral arterioles and arteries in M5 receptor deficient mice. These results demonstrate that M5 receptors mediate the vasodilator actions of ACh in cerebral arterioles and arteries. The novel phenotypes displayed by M5 receptor deficient mice will be discussed in detail.

P01-E-5

Expression pattern of oncostatin M receptor β in the murine central nervous system

○Shinobu Tamura¹, Yoshihiro Morikawa¹, Minoru Tanaka², Atsushi Miyajima², Emiko Senba¹

¹Dept. Anat. & Neurobiol., Wakayama Med. Sch., ²Lab. Cell. Biosyn., Inst. Mol. Cell. Biosci., Tokyo Univ.

Oncostatin M (OSM) is a member of the interleukin-6 family of cytokines, and appears to play important roles in hematopoiesis and the development of the liver. OSM receptor β (OSMR β) mRNA was shown to be expressed in many tissues, including lung, heart, thymus, spleen, and brain. However, little is known about the role of OSMR β in the murine brain. Using Northern blot analysis, we examined the regional distribution of OSMR β mRNA in the adult brain. Signals for OSMR β mRNA were observed with low levels in various regions. In situ hybridization histochemistry showed that signals for OSMR β mRNA were found mainly in epithelial cells of choroid plexus and pia mater. In addition, we studied the gene expression of OSMR β in the developing central nervous system (CNS) at different time points. Its gene expression was first observed in large neurons of the hypoglossal nuclei at embryonic day 14.5, which was sustained until postnatal day 7 (P7). At P14, OSMR β mRNA signals were not found in this region. Our results suggest that OSM contributes to the developmental processes, including the differentiation and maturation of specific neuronal populations, in the murine CNS.

P01-E-6

Involvement of mitochondria in Ca^{2+} response to ATP in PC12 cells

Kouji Maruyama, Toshio Ohta, ○Shigeo Ito
Lab. Pharmacol., Grad. Sch. Vet. Med., Hokkaido Univ.

The involvement of mitochondria in the Ca^{2+} response to ATP was examined in rat pheochromocytoma PC12 cells. Intracellular Ca^{2+} and Na^+ concentrations ($[\text{Ca}^{2+}]$ and $[\text{Na}^+]$) were measured using fura-2 and SBFI methods, respectively. ATP caused concentration-dependent increases in $[\text{Ca}^{2+}]$ and $[\text{Na}^+]$. High concentrations of ATP elicited a transient increase and then slow recovery of $[\text{Ca}^{2+}]$ to the base line (shoulder phase). The shoulder phase appeared when the peak $[\text{Ca}^{2+}]$ exceeded 500nM. The rise in the shoulder $[\text{Ca}^{2+}]$ was linearly correlated with that in the peak $[\text{Ca}^{2+}]$. The Ca^{2+} response to ATP was abolished by removal of extracellular Ca^{2+} . The shoulder $[\text{Ca}^{2+}]$ gradually declined on Ca^{2+} removal during the shoulder phase, and was not affected by suramin, thapsigargin, nifedipine or ω -conotoxin GVIIA. FCCP greatly enhanced the Ca^{2+} response to ATP in cells with a shoulder phase. Clonazepam and CGP37157, mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange inhibitors, or removal of extracellular Na^+ inhibited the shoulder phase of the response to ATP. In the absence of extracellular Na^+ , 210 s after ATP stimulation, the reintroduction of NaCl at various concentrations caused increases in $[\text{Ca}^{2+}]$ and $[\text{Na}^+]$. The rise in $[\text{Ca}^{2+}]$ was linearly correlated with that in $[\text{Na}^+]$. FCCP caused an increase in $[\text{Ca}^{2+}]$ when applied during the shoulder phase at 150 s but not 270 s after ATP stimulation in the presence of extracellular Na^+ , and it caused a great increase in $[\text{Ca}^{2+}]$ even at 390 s after ATP stimulation in the absence of extracellular Na^+ . These results suggest that the rapid decline and subsequent shoulder phases of the Ca^{2+} response to ATP are mainly due to mitochondrial Ca^{2+} accumulation and Ca^{2+} release through mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchangers in PC12 cells, respectively.

P01-E-7

Comparison of the effects of PSD-95 and MALS-2 on the channel activity of the NR1/NR2B-subtype NMDA receptor

○Takashi Iwamoto^{1,2}, Yasue Yamada¹, Takamasa Oda¹, Yoshifumi Watanabe², Kenji Sobue³, Makoto Inui¹

¹Dept. Pharmacol. Sch. Med., Yamaguchi Univ., ²Dept. Neuropsychiat. Sch. Med., Yamaguchi Univ., ³Dept. Neurochem. & Neuropharmacol. Grad. Sch. Med., Osaka Univ.

Proteins containing PDZ domains play an important role in recruiting ion channels and receptors to appropriate sites in neurons. We previously demonstrated using *Xenopus* oocyte expression system that PSD-95 functionally modulates the channel activity of the NR1/NR2B-subtype NMDA receptor. PSD-95 decreases the sensitivity of the channels for L-glutamate and inhibits the protein kinase C (PKC)- and insulin-mediated potentiation of the NR1/NR2B channels. It has been shown that MALS-2 binds to the C-terminal of the NR2 subunits through the PDZ domain. In this study, we compared the effects of MALS-2 with those of PSD-95 on the NR1/NR2B receptor channels. When co-expressed with the receptor in oocytes, MALS-2 increased the current responses of the channels, and decreased the sensitivity of the channels for L-glutamate. However, MALS-2 did not inhibit the PKC- and insulin-mediated potentiation of the channels. To clarify these differences between PSD-95 and MALS-2, we construct two chimeras. In chimera 1, the N-terminal region of PSD-95 was replaced by that of MALS-2. In chimera 2, three PDZ domains of PSD-95 were replaced by the PDZ domain of MALS-2. Chimera 1 only slightly inhibited the PKC- and insulin-mediated potentiation of the channels, whereas chimera 2 completely inhibited it as PSD-95. When two cysteine residues near the N-terminus at positions 3 and 5 in PSD-95 were substituted by serine, the mutated PSD-95 revealed the same effects on the PKC- and insulin-mediated potentiation as chimera 1. These results indicated that PSD-95 and MALS-2 differently modulate the NR1/NR2B receptor channels and that the difference is caused by the N-terminal region of the two proteins.

P01-E-8

ACCUMULATION OF [³H]L-SERINE IN RAT BRAIN SYNAPTOSOMAL FRACTIONS

○Takeshi Takarada, Vladimir J. Balcar, Katsuhiro Baba, Akiko Takamoto, Yukio Yoneda

Dept. Mol. Pharmacol., Kanazawa Univ. Pharm. Sci.

L-Serine is shown to be a neurotrophic factor released from astroglia, in addition to holding the possibilities to be an inhibitory neurotransmitter and a substrate for serine racemase responsible for the formation of D-serine. Crude synaptosomal fractions were freshly prepared from rat whole brain, followed by incubation with radiolabeled neuroactive amino acids at 10 nM under isotonic conditions. Accumulation of [³H]L-serine was found to be temperature-sensitive and to consist of both Na⁺-dependent and Na⁺-independent components. Accumulation of [³H]L-serine exhibited substrate selectivity different from that of the uptake of [³H]L-proline, [³H]L-glutamate and [³H]GABA. Accumulation of [³H]L-serine was fully inhibited by L-glutamine, L-asparagine, L-cysteine, L-alanine, L-leucine, L-isoleucine, L-threonine and by the synthetic inhibitor for the large neutral amino acid transport system 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid, but not by D-alanine, taurine, glycine nor by the inhibitor for the A system, L-2-methylaminoisobutyric acid. Kinetic analysis revealed the presence of at least two distinct transport systems for [³H]L-serine: a Na⁺-dependent system of lower affinity and a Na⁺-independent system of higher affinity. These results suggest that L-serine transport may be mediated neither by the typical "A", nor by the "large neutral" amino acid transport systems, but predominantly by transporters belonging to the recently identified LAT family in rat brain.

P01-E-9

FUNCTIONAL HETEROMERIC NMDA RECEPTORS EXPRESSED IN CULTURED RAT CALVARIAL OSTEOBLASTS

○Yukio Yoneda, Eiichi Hinoi, Sayumi Fujimori
Dept. Mol. pharmacol., Kanazawa Univ. Pharm. Sci.

We have previously shown the constitutive expression of particular subtypes of receptors for the central neurotransmitter L-glutamate in several mammalian peripheral tissues such as adrenal and pituitary glands. Calvaria were isolated from neonate rats 1 to 2 days after birth, followed by sequential enzymatic digestions with collagenase and trypsin. Osteoblasts were cultured for 1 to 28 days in vitro (DIV) with medium change at intervals of every 2 to 3 days. Alkaline phosphatase activity increased in proportion to the culture period from 3 to 28 DIV, while calcium content only increased in cultured osteoblasts after 18 up to 28 DIV. An RT-PCR analysis revealed selective expression of both the N-methyl-D-aspartate (NMDA) receptor NR1 and NR2D subunits in osteoblasts cultured for 7 and 28 DIV. Immunostaining assays confirmed the expression of NR1 and NR2D subunits in osteoblasts cultured for 7 DIV. Both alkaline phosphatase activity and calcium accumulation were significantly reduced by sustained exposure to the NMDA channel blocker MK-801 throughout the culture period up to 28 DIV. The mature osteoblastic marker protein osteocalcin was highly expressed at nodules and clusters seen in osteoblasts cultured for 28 DIV on morphological immunostaining analysis, which was completely abolished by sustained exposure to MK-801. These results suggest that NMDA receptors may be involved in mechanisms underlying cell proliferation and/or differentiation in cultured rat calvarial osteoblasts.

P01-E-10

FUNCTIONAL METABOTROPIC GLUTAMATE RECEPTOR GROUP III SUBTYPE EXPRESSED IN PRIMARY CULTURES OF RAT CALVARIAL OSTEOBLASTS

○Maki Inoue, Eiichi Hinoi, Sayumi Fujimori, Akihiro Takemori, Yukio Yoneda
Dept. Mol. Pharmacol., Kanazawa Univ. Pharm. Sci.

L-Glutamate (Glu) has been believed to play an excitatory amino acid neurotransmitter role through ionotropic (iGluR) and metabotropic (mGluR) receptors in the mammalian central nervous system. In this study, we have attempted to demonstrate the possible functional expression of particular subtypes of these Glu receptors (GluR) in putative non-excitatory tissues such as bone. Reverse transcription polymerase chain reaction analysis revealed expression of mRNA for particular GluR in primary cultures of rat calvarial osteoblasts under premature to mature states according to the duration of days in vitro (DIV). These included mRNA for mGluR4 and mGluR8, in addition to several iGluR subunits such as NR1 and NR2D. The agonist for group III mGluR L-(+)-2-amino-4-phosphonobutyric acid (L-AP4) significantly inhibited the formation of cAMP induced by forskolin and parathyroid hormone in both premature and mature cultured osteoblasts, which occurred in a manner sensitive to prevention by a group III mGluR antagonist. Moreover, pertussis toxin markedly prevented the inhibition by L-AP4 of forskolin-induced cAMP formation in calvarial osteoblasts cultured for 7 DIV. These results suggest that Glu may at least in part play a role in mechanisms associated with cellular proliferation, differentiation and/or maturation through group III mGluR functionally expressed in rat calvarial osteoblasts.

P01-E-11

Reguration of dopamine D2-receptor mediated endocytosis by endophilin-3 in the olfactory glomeruli.

○Hiroko Sugiura¹, Ken Iwata², Masato Matsuoka³, Masao Norita³, Takashi Yamauchi⁴, Masumi Ichikawa⁵, Tatsuya Haga², Kanato Yamagata¹

¹Dept.Neuropharm.,Tokyo Metropol.Inst.Neurosci.,

²Inst.Biomolecul.Sci.,Facul.Sci.,Gakushuin Univ.,

³Neurobiol.& Anat.,Niigata Univ.Grad.Sch.Med.&

Den., ⁴Dept.Biochem.,Grad.Sch.Pharm.,Tokushima

Univ., ⁵Dept.Develop.Morphol.,Tokyo Metropol.Inst.Neurosci.

Endophilin-1 is proposed to participate in synaptic vesicle endocytosis through SH3 domain-mediated interactions with the IP3 phosphatase synaptojanin and the GTPase dynamin. However, other endophilin family members have not been analyzed as a regulator of endocytosis. Here, we show that endophilin-3 is highly expressed in the olfactory glomeruli where dopamine D2 receptor is concentrated, and suppresses the D2 receptor mediated endocytosis. This property of endophilin-3 is independent of its putative lysophosphatidic acid acyl transferase (LPA-AT) activity that is mediated by its NH2-terminal region, and requires the activation of dynamin GTPase. Co-expression of endophilin-3 and dynamin in HEK293T cells induced the formation of large intracellular vesicles, indicating an inhibition of rapid dynamin's GTP-dependent vesiculating activity. These data strongly suggest that the receptor-mediated endocytosis may be regulated by GTP hydrolysis of dynamin rather than by the LPA-AT activity of endophilin.

P01-E-12

Expression of dominant negative isoform of rat norepinephrine transporter

○Shigeo Kitayama¹, Kei Kumagai², Katsuya Morita², Toshihiro Dohi²

¹Dept. Dent. Pharmacol., Okayama Univ. Grad. Sch. Med. Dent., ²Dept. Dent. Pharmacol., Hiroshima Univ.

Grad. Sch. Biomed. Sci.

Norepinephrine transporter (NET) acts to terminate noradrenergic synaptic transmission by reaccumulating released norepinephrine (NE). We have recently identified splicing variants of rat NET mRNAs which encode different carboxy terminal tails. One of these isoforms, rNETb, a rat NET isoform having a unique carboxy terminus, revealed a dominant negative effect when co-expressed with another functional isoform, rNETa, suggesting that carboxy terminal tail of NET plays an important role in the functional expression of NET. To evaluate the importance of NET splicing regulation and expression of the dominant negative isoform, we isolated rat NET gene at 3'-region and determined its nucleotide sequence to compare with human NET gene. Both alternative splicing variants, rNETa-S and rNETb, were predicted to be produced by aberrant splicing not relating to the GT-AG rule. Therefore, to confirm further the occurrence of these variants, we examined their expression in different ways including RT-PCR and Northern blot analysis for variant mRNA expression and Western blot analysis using rNETb-specific antisera in rat brain, different clones of PC12 cells, and reconstitution system in mammalian cell lines transfected with minigene construct of rat NET gene 3'-region. All results confirmed the presence of alternative splicing of rat NET pre-mRNA resulting in the production of dominant negative isoform, rNETb.

P01-E-13

Effects of atypical antipsychotics on 5-HT_{1A} receptor-mediated [³⁵S]GTP γ S binding in native brain membranes

○Yuji Odagaki, Toshio Yamauchi
Dept. Neuro-psychiat., Saitama Med. Sch.

Re-evaluation of clozapine as an antipsychotic drug that is efficacious not only for positive symptoms but also for negative and cognitive symptoms of schizophrenia with no extrapyramidal adverse effects has facilitated the development of several novel clozapine-like or atypical antipsychotic drugs. The molecular mechanisms underlying the atypicality of these antipsychotics have not been fully understood. There have been several reports showing that some atypical antipsychotics act as partial agonists at 5-HT_{1A} receptors. However, these results are based chiefly on the experiments using cultured cells expressing 5-HT_{1A} receptors and it is not known whether this phenomenon takes place also in the native brain membranes. In the present study, we utilized [³⁵S]GTP γ S binding in rat brain membranes in order to elucidate whether atypical antipsychotics such as clozapine behave as 5-HT_{1A} receptor agonists even in native brain membranes. 5-HT stimulated [³⁵S]GTP γ S binding in a concentration-dependent manner in rat hippocampal and cerebral cortical membranes. This response was mimicked by the selective 5-HT_{1A} receptor agonist 8-OH-DPAT, but not by the selective 5-HT₂ receptor agonist ($\pm$)-DOI. Clozapine, a prototype of the atypical antipsychotics, failed to stimulate the 5-HT_{1A} receptor-mediated [³⁵S]GTP γ S binding up to 100 μ M. Perospirone did not behave as an agonist at 5-HT_{1A} receptors, either. On the other hand, another atypical antipsychotic drug, quetiapine, stimulated the 5-HT_{1A} receptor-mediated [³⁵S]GTP γ S binding at the higher concentrations investigated ($\sim$ 100 μ M) with low intrinsic activity at least in the hippocampal membranes. These results provide important information concerning the involvement of the 5-HT_{1A} receptor agonist action in the clinical characteristics of the atypical antipsychotics.

P01-E-14

Delphilin: a novel PDZ protein that synaptically colocalizes and interacts with glutamate receptor δ 2

○SUSumu Kawamoto¹, Yohei Miyagi², Tetsuji Yamashita¹, Masahiro Fukaya³, Tomoko Sonoda¹, Toshiaki Okuno⁴, Kazuyuki Yamada³, Masahiko Watanabe³, Kenji Okuda¹, Masayoshi Mishina⁴
¹Yokohama City University, ²Kanagawa Cancer Center, ³Hokkaido University, ⁴University of Tokyo/CREST

The glutamate receptor δ 2 (GluR δ 2) subunit is selectively expressed in cerebellar Purkinje cells, and plays an important role in cerebellar long-term depression, motor learning, motor coordination, and synapse development. We identified a novel GluR δ 2-interacting protein, named Delphilin, containing a single PDZ domain and formin homology (FH) domains FH1 and FH2 plus coiled-coil structure. Yeast two-hybrid and surface plasmon resonance (SPR) analyses indicated that Delphilin interacts with the GluR δ 2 C-terminus via its PDZ domain. This was also supported by coimmunoprecipitation experiments using heterologous expression system in mammalian cells. Yeast cell and SPR analyses also demonstrated the possibility that the FH1 proline-rich region of Delphilin interacts with profilin, an actin-binding protein, and with the SH3 domain of neuronal Src protein tyrosine kinase. In situ hybridization demonstrated the highest expression of Delphilin mRNA in Purkinje cells. Delphilin polypeptide was highly enriched in the synaptosomal membrane fraction of the cerebellum, and coimmunoprecipitated with the GluR δ 2 subunit. Post-embedding immunogold technique demonstrated that Delphilin is selectively localized at the postsynaptic junction site of the parallel fiber-Purkinje cell synapse, and colocalized with GluR δ 2. Delphilin is a postsynaptic scaffolding protein at the parallel fiber-Purkinje cell synapse, where it may serve to link GluR δ 2 with actin cytoskeleton and various signaling molecules. Thus, the discovery of Delphilin, although the ligand of the GluR δ 2 protein is still unknown, should help to understand the GluR δ 2 function and the induction mechanism of cerebellar LTD.

P01-E-15

Molecular cloning of human p40BBP and its distribution in human brain

○Tokiko Hama, Yukiko Honda
Mitsubishi Kagaku Inst. of Life Sci.

We have been studying neuronal survival using a 13-mer brain injury-derived neurotrophic peptide (BINP). BINP enhances the survival of septal cholinergic neurons and mesencephalic dopaminergic neurons in cultures from P14 rats. BINP also rescues hippocampal neurons from cell death caused by glutamate in cultures from E18 rats. And we characterized BINP-binding sites in rat brain. Cross-linking experiments with a ^{125}I -labeled BINP analogue showed bands corresponding to about 40, 50 and 100 kDa on autoradiography after SDS-PAGE. To study further about BINP-binding protein, we have developed a monoclonal antibody against BINP-binding protein and obtained monoclonal antibody (mAb) named 6A22. The mAb 6A22 recognizes a 40 kDa protein and inhibits BINP-binding of rat brain synaptosomes. Then we have cloned the cDNA by expression cloning using mAb 6A22 from rat hippocampus cDNA library. The cDNA named rBBP codes a novel membrane protein named p40BBP. BINP binding was observed when the cDNA rBBP was transfected into COS7 cells and cell surface was stained by mAb 6A22. From northern blotting analysis, the mRNA of this cloned cDNA was specific in brain and increased after birth in rat. In this study we have cloned the cDNA of human p40BBP from human hippocampus cDNA library and investigate human brain distribution. Human p40BBP consists of 300 amino acids. The amino acid sequence of p40BBP is conserved between human and rat though a few human specific sequences exist. The expression of human p40BBP gene is not in all region of human brain. It was abundant in thalamus and some were in cerebral cortex, medulla, caudate nucleus and hippocampus.

P01-E-16

Expression of ASCT2 neutral amino acid transporter in cultured astrocytes and neurons

○Shigeki Furuya¹, Yoko Shinoda¹, Syogo Endo², Thomas Launey², Kazuyuki Yoshida^{1,3}, Masahiko Watanabe⁴, Jyunya Mitoma⁵, Yoshio Hirabayashi¹
¹Neuronal Circuit Mech. Res. G., RIKEN BSI, ²Lab. for Memory and Learning, RIKEN BSI, ³United Grad. Sch. Agri. Sci., Tokyo Univ. Agri. Tech., ⁴Dept. Anatomy. Hokkaido Univ. Med. Sch., ⁵Glycobiology Program, The Burnham Institute

We demonstrated that L-Ser serves as a major astrocyte-derived neurotrophic factor (Mitoma et al., J. Biol. Chem., 273, 19363, 1998; Furuya et al., PNAS, 97, 11528, 2000). In astrocytes, System ASC-like transporters seem to mediate transport of L-Ser (Broer et al, J. Neurochem., 73, 2184, 1999; Ribeiro et al, Brain Res., 929, 2002). To gain an understanding of the L-Ser transport mechanism in astrocytes, we cloned ASCT2 transporter from mixed cerebellar cultures by RT-PCR, determined the structure, and characterized its L-Ser transport activity and expression. The rat cerebellar ASCT2 and mouse ASCT2 share 84.5% of identity in their amino acid sequence. When expressed HEK cells, we observed Na⁺-dependent high-affinity uptake of ^{14}C -L-Ser. This uptake was inhibited strongly by unlabeled L-Ser, L-Ala, L-Thr, and L-Cys, and moderately by D-Ser and Gly. When HEK cells were preloaded with ^{14}C -L-Ser, enhanced L-Ser efflux was detected in the presence of unlabeled L-Ser. Northern blot analysis showed that the ASCT2 transcript was expressed mainly in astrocyte-enriched primary cultures. We raised polyclonal antibodies against C-terminal portion of the ASCT2 and examined the cellular localization in cerebellar glial and neuronal cultures by immunocytochemistry. The ASCT2 immunoreactivity was detectable in fine processes and soma of GFAP-positive astrocytes. In cerebellar neuronal cultures, Purkinje cell dendrites were labeled with patchy patterns. These data suggest a role of ASCT2 in the efflux of L-Ser from astrocytes and the uptake in Purkinje neurons in the cerebellum.

P01-E-17

パーキンソン病モデル線条体グリア細胞におけるドパミントランスポーターの発現誘導

○浅沼 幹人, 宮崎 育子, 東 洋一郎, 小川 紀雄
岡山大学・院医歯・神経情報学

パーキンソン病およびそのモデル動物においてドパミン神経が著しく変性脱落しているにもかかわらず、L-DOPAの治療効果が認められる。われわれはパーキンソン病モデルにL-DOPAを連日投与すると、障害側線条体でドパミン代謝回転が著明に亢進することを既に報告している。また、グリア細胞において多くの膜レセプター、チャネル、トランスポーターの発現が報告されていることから、パーキンソン病線条体ではL-DOPA・ドパミンがグリア細胞において取込まれ、保持される可能性が想定される。とくにアストロサイトにおけるL-DOPA・ドパミンの取込みを明らかにするために、6-OHDA片側黒質-線条体路注入片側パーキンソン病モデルラットを用いて、L-DOPA/carbidopaを1週間連日投与し、線条体を含む脳凍結切片上でのアストロサイトでのドパミン、ドパミントランスポーター発現を二重免疫染色で検出した。片側パーキンソン病モデルにL-DOPA連日投与を行うと、障害側線条体で増殖したアストロサイトにおいてドパミントランスポーターの発現増加とドパミン陽性シグナルの増加がみられた。われわれは、障害を受けた初代培養ニューロン+アストロサイト共培養系にドパミンを添加すると、アストロサイトにおいてドパミントランスポーター蛋白が発現し、ドパミンが取込まれることを確認している(宮崎ら, 本学会)。これらより、ドパミン神経障害時にアストロサイトはドパミントランスポーターを介してドパミンを取込みうる事が示唆された。このようなドパミン神経障害時のグリア細胞におけるドパミン取込み機構は、パーキンソン病でのL-DOPA長期投与による副作用発現において重要な役割を果たしているのかもしれない。

P01-E-18

Differential regulation of aquaporin 5 and 9 expression in astrocytes by protein kinase A

○Kazuya Sobue¹, Naoki Yamamoto², Masaaki Inagaki¹, Hiroaki Itho¹, Yoshihito Fujita¹, Hirotada Katsuya¹, Kiyofumi Asai²

¹Dept. Anesthesiol. Med. Crisis Management, Nagoya City Univ. Grad. Sch. Med. Sci., ²Dept. Mol. Neurobiol., Nagoya City Univ. Grad. Sch. Med. Sci.

Aquaporins (AQPs) are a family of water-selective transporting proteins that increase plasma membrane water permeability in secretory and absorptive cells. In astrocytes, using the reverse transcription-PCR (RT-PCR), we previously detected AQP3, AQP5, and AQP8 mRNAs in addition to the reported AQP4 and AQP9. However, the molecular mechanisms for regulating water balance in brain are unknown. In this study, we investigated the effects of protein kinase A (PKA) activators on the expression of AQP4, 5, and 9 in cultured rat astrocytes. Treatment of the cells with dbcAMP caused decreases in AQP5 mRNA and protein and increases in AQP9 mRNA and protein in time- and concentration-dependent (0-3.0 μ M) manners. The dbcAMP-induced effects on AQP5 and AQP9 mRNAs were inhibited by PKA inhibitors, H8 and H89. In addition, pretreating the cells with an inhibitor of protein synthesis, cycloheximide, inhibited the increase in AQP9 mRNA induced by dbcAMP, but not the decrease in AQP5 mRNA. Likewise, forskolin induced the same reaction of AQP5 mRNA and 9 mRNA in astrocytes. These results suggest that signal transduction via PKA may play important roles in regulating the expression of AQP5 and AQP9, and the effect on AQP9 may be regulated by some factors induced by dbcAMP.

P01-E-19

アストロサイトにおけるドパミントランスポーターを介したドパミン取込み機構

○宮崎 育子, 浅沼 幹人, 東 洋一郎, 小川 紀雄
岡山大・院医歯・神経情報学

近年, グリア細胞特にアストロサイトが多くの膜レセプター, チャンネル, トランスポーターを発現していることが報告されている。われわれは, 昨年, 初代培養アストロサイトにおいて, ドパミンレセプターのうちD1, D4, D5 mRNAおよびドパミントランスポーターmRNAが発現していることを報告し, アストロサイトがドパミンニューロンの投射するシナプス後ニューロンと同様にドパミンに反応している可能性を示唆した。そこで, われわれは, ラット胎児中脳の初代培養アストロサイトにおけるドパミンレセプターおよびドパミントランスポーターの蛋白発現をWestern blot法により検討した。初代培養アストロサイトにおいて, mRNA発現と同様に, ドパミンレセプターD1, D4, D5およびドパミントランスポーターが発現していた。さらに, 6-OHDAおよび過酸化水素添加で障害させた初代培養ニューロン+アストロサイト共培養系にドパミン添加を行い, ドパミンあるいはドパミントランスポーターとアストロサイトのマーカーであるGFAPとの蛍光二重染色を行った。初代培養ニューロン+アストロサイト共培養系にあらかじめ神経毒や酸化ストレスで障害を与えると, ドパミン添加によりアストロサイトにドパミントランスポーター蛋白が発現しドパミンが取込まれることが確認できた。これらのことから, ドパミン神経障害時にアストロサイトはドパミントランスポーターを介してドパミンを取込み得ることが示唆された。さらに, ラット胎児線条体の初代培養アストロサイトにおけるドパミン取込みについて同様の検討を現在行っている。

P01-E-20

Critical regions for inositol 1,4,5-trisphosphate receptor gating

○Keiko Uchida^{1,2,3}, Hiroshi Miyauchi¹, Teiichi Furuichi⁴, Takayuki Michikawa^{1,5}, Katsuhiko Mikoshiba^{1,2,5}

¹Dept. Basic Med. Sci., Div. Mol. Neurobiol., Inst. Med. Sci., Univ. Tokyo, ²Lab. Dev. Neurobiol., BSI, RIKEN, ³Dept. Pediatrics, KEIO Univ. Sch. Med., ⁴Lab. Mol. Neurogenesis, BSI, RIKEN, ⁵Calcium Oscillation Project, ICORP, JST

To know the molecular mechanism of the ligand-dependent gating of the inositol 1,4,5-trisphosphate (IP₃) receptor (IP₃R)/Ca²⁺ release channel, we have analyzed channel properties of seven internal deletion mutants that retain both ligand-binding domain and channel-forming domain intact. We have introduced mutated cDNA of the mouse IP₃R1 into the DT40 cells in which all three intrinsic IP₃R genes had been disrupted, and have established stable cell lines that express the mutant IP₃Rs. All of these wild type or mutant IP₃Rs expressed in the DT40 cells exhibited similar binding affinities for IP₃ as the native IP₃R expressed in mouse cerebellum. By measuring IP₃-induced Ca²⁺ release (IICR) from membrane fractions prepared from stable cell lines, we found that deletion of either three regions including amino acid residues 650-1130, 1845-2042 or 2610-2748 abolished the IICR activity. We also performed site-directed mutagenesis on three cysteine residues (1976, 2610 and 2613) that are conserved among both the IP₃R and the ryanodine receptor families, located in the regions described above, and found that either cysteine 2610 or 2613 is critical for IICR. A mutant D1131-1379, defecting amino acid residues 1131-1379, retained a small, but definite IICR activity among the deletion mutants. D1131-1379 showed a similar sensitivity for IP₃ to that of the wild type IP₃R1 but some gating properties were different; no cooperativity ($nH = 1.0 \pm 0.1$), smaller time constants of the fast phase of IICR, and no steep IP₃-dependent increase of the relative amount of the fast component of Ca²⁺ release. In spite of these differences, D1131-1379 showed a quantal Ca²⁺ release as the wild type IP₃R does. These results indicated that (1) amino acid residues 650-1130, 1845-2042 and 2610-2748 contain regions which are crucial for the conformational change during IP₃-dependent gating, (2) cysteine residues 2610 and 2613 are involved in the IP₃-dependent gating process, (3) neither positive IP₃-dependency of fast component of Ca²⁺ release nor cooperativity is responsible for quantal release property.

P01-E-21

Conformational Changes in Inositol 1, 4, 5-Trisphosphate Receptor Regulated by Calcium

○Kozo Hamada¹, Katsuhiko Mikoshiba^{1,2,3}

¹Lab. Dev. Neurobiol., BSI, RIKEN, ²Div. Mol. Neurobiol., Inst. Med. Sci., Tokyo Univ., ³ICORP, JST

Inositol 1, 4, 5-trisphosphate receptor (IP₃R) is a highly controlled calcium (Ca²⁺) channel gated by inositol 1, 4, 5-trisphosphate (IP₃). The most characterized type 1 IP₃R (IP₃R1), a predominant type in rodent cerebellar endoplasmic reticulum and spine apparatus, plays an integral role in the Ca²⁺ signaling and neural plasticity. The protomer of IP₃R1 contains the IP₃-binding core, membrane-spanning domains, and widespread allosteric sites for intracellular effector molecules; Ca²⁺, calmodulin, ATP, and for phosphorylation by protein kinase; cAMP-dependent protein kinase, protein kinase C, cGMP-dependent protein kinase, Ca²⁺/calmodulin-dependent protein kinase II, tyrosine kinase. These cumulative allosteric regulations imply a structural paradigm for global conformational changes within the higher ordered structure of IP₃R1. However, no structural evidence has been available. Here we show that Ca²⁺, the most pivotal regulator, induces marked structural changes in the tetrameric IP₃R1 purified from mouse cerebella. Electron microscopy and image analysis of the IP₃R1 particles reveal two distinct structures with fourfold symmetry: a windmill structure and a square structure. Ca²⁺ reversibly promotes a transition from the square to the windmill with relocations of four peripheral IP₃-binding domains, assigned by binding to heparin-gold. Ca²⁺-dependent susceptibilities to limited digestion strongly support the notion that these alterations exist. Thus, Ca²⁺ appeared to regulate IP₃-gating activity through the rearrangement of functional domains. Our novel model for structural rearrangements and the methodology presented here should be useful for understanding of the further biological significance of structural plasticity within IP₃R1 in the neural systems.

P01-E-22

Agonist-specific translocation of μ -opioid receptor and protein kinase C in living cells

○Akira Yoshida¹, Makoto Inoue¹, Naoaki Saito², Hiroshi Ueda¹

¹Dept. Mol. Pharmacol. Nagasaki Uni. Sch. Pharmac. Sci., ²Lab. Mol. Pharmacol. Biosignal Res. Centr. Kobe Univ.

We have studied the molecular mechanism for opioid tolerance, and particularly focused on the protein kinase C (PKC) involvement in such mechanisms (JPET 293, 662-669, 2000; J. Neurosci. 20, 7640-7647, 2000; J. Neurosci. 21, 2967-2973, 2001). In the series of reports, we showed that morphine pretreatment markedly reduced peripheral morphine analgesia, while DAMGO pretreatment did not. Moreover, we suggested that inhibitory mechanisms through PKC of μ -opioid receptor (MOPR) internalization were involved in morphine acute tolerance *in vivo*. We previously observed immunocytochemically that morphine caused the MOPR internalization when Chinese hamster ovary cells expressing MOPR were pretreated with calphostin C, a protein kinase C (PKC) inhibitor. However, the immunocytochemical observation has disadvantage not to detect MOPR internalization or PKC translocation (activation) in the same cells. In the present study, we analyzed these events occurring in the same cells by real time imaging of GFP-tagged proteins. MOPR or γ - and δ -subspecies of PKC fused with green fluorescent protein (MOPR-GFP or PKC-GFPs) was transiently expressed in the cells, and its fluorescence was observed by using confocal laser microscopy. The MOPR-GFP internalization occurred by the incubation with DAMGO, while not with morphine. Pretreatment of PKC inhibitor led to morphine-induced receptor internalization. On the other hand, morphine and DAMGO showed PKC-GFPs translocation to plasma membrane in the similar time course. These results suggested that the agonist-specific and PKC-modulating MOPR-internalization is not due to the difference of PKC activation, but to other mechanisms including changes in the PKC-sensitivity of MOPR.

P01-E-23

Tissue expression and identification of coding single nucleotide polymorphisms (cSNP) in human T2R receptors.

○Takashi Ueda¹, Shinya Ugawa¹, Yusuke Ishida², Yasuhiro Shibata¹, Shingo Murakami², Shoichi Shimada¹

¹Dept. Mol. Morphol. Grad. Sch. Med. Sci., Nagoya City Univ., ²Dept. Otolaryngol., Nagoya City Univ.

T2Rs comprise a G protein-coupled receptor superfamily mainly expressed in taste receptor cells in mouse and rat. They are thought to be involved in bitter tasting, because the mouse receptors localize on chromosome 6, a genetic loci that control sensitivity to bitter compounds in mice. In human, T2R are organized in the genome in clusters, but their functions are not yet known. Here, we report the tissue expressions and coding single-nucleotide polymorphisms (cSNPs) in human T2R genes (hT2R3, hT2R4 and hT2R5) on chromosome 7q31. We demonstrated that hT2R3, hT2R4 and hT2R5 are actually expressed in the circumvallate papillae of the human tongue by reverse transcription-polymerase chain reaction (RT-PCR). We identified six cSNPs within the T2R receptor genes. The hT2R4 and hT2R5 contained four and one cSNPs that cause missense mutations, respectively, while hT2R3 included one silent nucleotide mutation. However, we could not find any nonsense mutations that resulted in a frameshift or a premature stop codon within the open reading frames. Genotype frequencies of each cSNP were in Hardy-Weinberg equilibrium. To examine the receptor function, we next built up a calcium imaging system, and analyzed the hT2R4 receptor, which contains amino acid variations in higher frequencies. However, hT2R4 failed to respond to denatonium previously suggested as the ligand on condition that mouse T2R5 responded to cycloheximide at a concentration comparable to the murine threshold for aversion. We further try to determine the ligand for hT2R4 and examine the functional differences between a couple of the alleles on the ligand binding, receptor trafficking to plasma membrane, and receptor interactions with signaling and regulatory proteins.

P01-E-24

Developmental expression pattern of TROY in mouse central nervous system

○Tomoko Hisaoka¹, Yoshihiro Morikawa¹, Toshio Kitamura², Emiko Senba¹

¹Dept. Anat. & Neurobiol., Wakayama Med. Sch., ²Div. Cell. Thera., Advan. Clin. Res. Cent., Inst. Med. Sci., Tokyo Univ.

TROY is a recently identified member of the tumor necrosis factor (TNF) receptor superfamily. Previously, we reported that TROY induces the activation of nuclear factor kappa B via TNF receptor-associated factor 2, 5, and 6, and is strongly expressed in mouse embryos and the adult brain. In this study, we have performed in situ hybridization technique to explore the expression pattern of TROY in the central nervous system during mouse embryogenesis and in the postnatal development. At 11.5 days postcoitum, TROY mRNA was detected with high levels in the neuroepithelia, from which hippocampus, cortex, thalamus, and hypothalamus develop. During late embryogenesis, its gene expression was observed in the developing hippocampus, cortex, and olfactory bulb, and persisted to the adult. Furthermore, in the postnatal mice, its gene expression was also detected in the ventricular/subventricular zones and rostral migratory stream, which are known to contain neural progenitor cells. These results suggest that TROY plays some important roles in neurogenesis not only in embryos but also in postnatal mice.

P01-E-25

Identification and characterization of molecules that interact with melanin-concentrating hormone (MCH) receptor 1

○Yumiko Saito, Yuuki Kawamura, Mitsue Tetsuka, Li Yue, Kei Maruyama
Dept.Pharmacol. Saitama Medical School

Obesity is a major health problem that is plagued by the absence of effective treatment options. Over the last few years several neuropeptide systems have been shown to centrally mediate important aspects of feeding behavior. One recently characterized and highly promising peptide, melanin-concentrating hormone (MCH), has been implicated in feeding regulation. The orphan G protein-coupled receptors MCHR1 and MCHR2 were found to bind MCH with high affinity. Very recently, MCHR1 knock out mice were generated and it is showed that MCHR1 is a physiologically relevant MCH receptor in mice. Several recent reports have suggested that additional proteins may interact with certain GPCRs *in vivo*. However, for the large majority of GPCRs, few intracellular-associated proteins have been identified beyond those proteins that are necessary for signal transduction and functional regulation of the receptors. Therefore, we initiated a search for proteins that may interact with MCHR1 and might thus modify signal transduction properties of MCHR1. By using the yeast two-hybrid system we identified five positive clones from the mice brain interacting protein with the C-terminus of MCHR1. Two clones are novel, one of them (64-1) is specifically expressed in brain, and the other (34A) is exclusively expressed in kidney and brain. Now we are confirming an association of the candidate protein with MCHR1 by coimmunoprecipitation in 293T cells. This study will enable us to formulate a novel and physiological intracellular signaling pathway in the brain, and to establish a base to elucidate the effect of MCH on feeding behavior.

P01-E-26

Coupling with ADP-ribosyl cyclase of group III metabotropic glutamate receptors in retina

○Haruhiro Higashida¹, Xiao-Liang Chen¹, Jia-Sheng Zhang¹, Mami Noda², Naoto Hoshi¹, Minako Hashii¹, Shigeru Yokoyama¹

¹Depart. Biophys. Kanazawa Univ Grad. Sch. Med.,

²Depart. Pathophysiol. Kyushu Univ. Grad. SchPharm. Sci.

To examine the role of cyclic ADP-ribose (cADP-ribose) as a downstream signaling of metabotropic glutamate receptor (mGluR) activation, ADP-ribosyl cyclase activity was measured. ADP-ribosyl cyclase activity in crude membranes isolated from neuronal tissues was fluorometrically measured by the formation rate of nonhydrolyzable cyclic GDP-ribose (cGDP-ribose) from nicotinamide guanine dinucleotide⁺ (NGD⁺). ADP-ribosyl cyclase activity was high in the olfactory bulb, cerebral cortex, hippocampus, and cerebellum of 1- to 12-week-old rats, and was not much influenced by glutamate (1 nM to 100 μ M). Membranes prepared from retina from 3-week-old rats also exhibited ADP-ribosyl cyclase activity, albeit at much lower levels. Interestingly, cGDP-ribose fluorescence increased upon exposure to glutamate (1 μ M) in retina. The increase in ADP-ribosyl cyclase to 193 and 229 % of the pre-exposure level was obtained by 10 μ M and 100 μ M glutamate in retina, respectively. This glutamate-induced increase was reproduced by an mGluR group III-selective agonist, but not by group I- and II-selective agonists, respectively. Exposure to 10 μ M L-AP4 increased the ADP-ribosyl cyclase level to 170.2 % of the control level. In the presence of 10 μ M (RS)- α -cyclopropyl-4-phosphonophenylglycine (CPPG), a group III-selective antagonist, a subsequent application of glutamate had no further effect. In contrast, application of group I- and II-selective antagonists, (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA) and (2S)- α -ethylglutamic acid (EGLU), did not lead to an increase in the ADP-ribosyl cyclase level by themselves, but subsequent glutamate (10 nM - 1 μ M) did, suggesting that this glutamate effect in retina is mediated via the group III mGluRs.

P01-E-27

Hippocampal α_{2A} -adrenergic receptors are involved in methamphetamine-induced ambulatory activity in mouse

○Masahiro Nishio, Yasunari Kanda, Katsushige Mizuno, Yasuhiro Wawanabe
Dept. Pharmacol., National Defense Med. Col.

The administration of methamphetamine (MAP) was reported to activate the dopaminergic signaling in the CNS. It was also reported that noradrenaline was released in the hippocampi. However, the molecular mechanism of the formation of MAP-induced reverse tolerance is still unclear. We have studied that the repeated administration of MAP increased the α_{2A} -adrenergic receptors (α_{2A} -AR) expression in mice hippocampi. Moreover, the ambulatory activity by acute MAP administration was decreased in the mice which were received the α_{2A} -AR anti-sense oligo DNA into the hippocampi. In the present study, we focused on the involvement of hippocampal α_{2A} -AR on the formation of MAP reverse tolerance. The increase of hippocampal α_{2A} -AR was potentiated by repeated administration of MAP in the ddY mice (2 mg/kg, every three days, five-times s.c.). Micro-infusion of the α_{2A} -AR anti-sense oligo DNA into bilateral hippocampi before the six-time administration of MAP suppressed the hippocampal α_{2A} -AR. The treatment decreased ambulatory activity by the six-time administration of MAP. These results suggest that the α_{2A} -AR in hippocampus has some roles on the ambulatory activity by MAP.

P01-E-28

Regulation of P2X receptor by ubiquitin C-terminal hydrolase L1

○Mami Noda¹, Yoshiko Kanahori¹, Aki Shimada¹, Shunsuke Aoki^{2,4}, Yu-Lai Wang², Hitoshi Osaka^{2,3}, Keiji Wada²

¹Lab. Pathophysiol. Grad. Sch. Pharm. Sic. Kyushu Univ., ²Dept. Degene. Neurol. Dis., Natl. Inst. Neurosci., NCNP, ³PRESTO, JST, ⁴JSPS

Mammalian neuronal cells abundantly express a de-ubiquitinating isozyme ubiquitin carboxy-terminal hydrolase 1 (UCHL1). Loss of UCHL1 function causes the neuronal degeneration observed in the gracile axonal dystrophy (*gad*) mouse¹. The *gad* mouse displays reduced ubiquitin levels at neuronal neurites. However, the function of UCHL1 in a neuronal cell remains unclear. Here we show that UCHL1 has modulatory effect on a neurotransmitter receptor. Among neurotransmitter receptors, we analyzed ATP-activated receptors which are widely distributed including neuronal cells and involved in functions such as neurosecretion. ATP-induced inward currents due to the activation of P2X receptors were measured in untransfected or UCHL1-transfected PC12 cells at the holding potential of -70 mV under the conventional whole-cell patch clamp configuration. The amplitudes of ATP-induced inward currents were significantly greater in UCHL1-transfected PC12 cells. The increased ATP-induced inward currents were attenuated significantly by H-89, an inhibitor of cyclic AMP-dependent protein kinase (PKA), but not by MAPK inhibitor. These results suggest that UCHL1 modulates P2X receptors by activation of PKA and has further influences on various down-stream responses.

1. Saigoh K. et al. (1999) Intragenic deletion in the gene encoding ubiquitin carboxy-terminal hydrolase in *gad* mouse. *Nat Genet.* 23: 47-51.

P01-E-29

Protein kinase C regulates aquaporin expression in astrocytes

○Naoki Yamamoto¹, Kazuya Sobue², Yoko Kawai³, Yutaka Miura¹, Hirotsada Katsuya², Kiyofumi Asai¹

¹Dept. Mol. Neurobiol., Nagoya City Univ. Grad. Sch. Med. Sci., ²Anesthesiol. Med. Crisis Management, Nagoya City Univ. Grad. Sch. Med. Sci., ³Nagoya City Univ. Sch. Nursing

Aquaporins (AQPs) are water-selective channels that function to increase plasma membrane water permeability in secretory and absorptive cells that require rapid or regulated water movement. Eleven subtypes of aquaporins have been identified in mammals, aquaporin1-10 and the intrinsic protein of lens (also referred as AQP0). In astrocytes of the central nervous system (CNS), using the reverse transcription-polymerase chain reaction (RT-PCR), we previously detected AQP3, 5 and 8 mRNAs in addition to the reported AQP4 and 9. However the mechanisms regulating the expression of these AQPs are not known. In this research, we investigated the effects of a protein kinase C (PKC) activator on the expression of AQP4, 5 and 9 in cultured rat astrocytes. Treatment of the cells with TPA caused decreases in AQP4 and 9 mRNAs and proteins in time- and concentration-dependent manners. PKC inhibitors, H-7 and chelerythrine, inhibited the TPA-induced decreases in AQP4 and 9 mRNAs. Moreover, pretreatment of cells with an inhibitor of protein synthesis, cycloheximide, did not inhibit the decreases in AQP4 and 9 mRNAs induced by TPA. These results suggest that signal transduction via PKC may play important roles in regulating the expression of AQP4 and 9.

P01-E-30

cyclic AMP-induced up-regulation of Na⁺-dependent vitamin C transporter in C6 glioma

○Satoru Yamanaka¹, Kohei Mori¹, Akira Yamanaka¹, Michiko Oka², Takuya Fujita¹

¹Dept. Biochem. Pharmacol., Kyoto Pharmaceutical Univ., ²Nippon Shinyaku Co., Ltd.

Na⁺-dependent vitamin C transport system and its cyclic AMP (cAMP)-induced up-regulation in glial cells have been characterized using the rat glioma cell line C6 as an experimental model. These cells were found to express the Na⁺-dependent vitamin C transporter SVCT2, but not SVCT1, by RT-PCR analysis. The uptake of the reduced form of vitamin C (ascorbate), which was maintained by the addition of dithiothreitol in the uptake buffer, was Na⁺-dependent and substitution of Na⁺ by choline abolished its uptake completely. Kinetic analysis indicates the presence of a single saturable transport system for ascorbate with a Michaelis constant (*K_i*) of $23.5 \pm 5.7 \mu\text{M}$. The dependence of the uptake rate of ascorbate on Na⁺ concentration exhibited sigmoidal kinetics, and the Na⁺:ascorbate stoichiometry is 2:1 as deduced from the Na⁺-activation kinetics. These results were consistent with the previous reports using SVCT2-expression systems. SVCT2 expression in C6 glioma increased in response to 8-bromo cAMP, a cell permeable analog of cAMP, forskolin, an activator of adenylyl cyclase. The cAMP-induced effects were time- and concentration-dependent. Exposure of C6 glioma to 1mM 8-bromo cAMP for 16 hr increased the maximal velocity of Na⁺-dependent ascorbate uptake (*V_{max}*) without affecting *K_i* value, *K_{0.5}* for Na⁺ (i.e. the concentration of Na⁺ necessary for half-maximal activation), and Hill Coefficient. The cAMP-induced up-regulation of Na⁺-dependent ascorbate uptake mediated by SVCT2 was inhibited by actinomycin D and cycloheximide. It is concluded that cAMP regulates Na⁺-dependent transport of ascorbate via SVCT2 and that this regulation involves *de novo* protein synthesis.

P01-E-31

Functional characterization of MPP⁺ uptake mediated by norepinephrine transporter in PC-12 cells

○ Kenji Shinomiya, Satoru Yamanaka, Akira Yamamoto, Takuya Fujita
Dept. Biochem. Pharmacol., Kyoto Pharmaceutical Univ.

We investigated functional characterization of 1-methyl-4-phenylpyridinium (MPP⁺) transport in rat pheochromocytoma PC-12 cells. The transport is electrogenic and is energized by an inwardly directed NaCl gradient and a negative membrane potential. The transport process is saturable with a Michaelis-Menten constant (K_i) of 330 ± 20 nM. The Na⁺:Cl⁻:MPP⁺ stoichiometry is 1:1:1 as deduced from the Na⁺ and Cl⁻ activation kinetics.

Tricyclic antidepressants, imipramine and desipramine, inhibit the transporter markedly. In addition, norepinephrine and dopamine competitively inhibited the uptake of MPP⁺ with an inhibitory constant (K_i of 163 ± 21 nM and 527 ± 85 nM, respectively. Nisoxetine, a specific inhibitor of norepinephrine transporter, inhibited the uptake of MPP⁺ in a concentration-dependent manner, whereas GBR 12909, a specific inhibitor of dopamine transporter, did not affect the MPP⁺ uptake in PC-12 cells. These data indicate that it is the norepinephrine transporter, not the dopamine transporter, that is responsible for the MPP⁺ uptake in PC-12 cells. These results are confirmed by a RT-PCR analysis using a specific primer set for monoamine transporters.

P01-E-32

Intracellular dynamics of IP3 receptor as revealed by tagging with GFP

○Yoko Tateishi¹, Mitsuharu Hattori¹, Tomohiro Nakayama^{1,2}, Takeshi Nakamura⁴, Hiroko Bannai^{1,3}, Miwako Iwai¹, Keiko Uchida^{1,5}, Takayuki Michikawa^{1,4}, Takafumi Inoue^{1,4}, Katsuhiko Mikoshiba^{1,3,4}

¹Div. Molecular Neuroscience, Institute of Med. Sci., Univ. of Tokyo., ²Dept. Pediatrics, Tokyo Women's Med. Univ., ³Lab. Dev. Neurosci., BSI, RIKEN, ⁴Calcium Oscillation project, ICORP, JST, ⁵Dept. Pediatrics, Keio Univ. School of Med.

Inositol 1,4,5-trisphosphate receptor (IP3R) is an intracellular Ca²⁺ channel that plays crucial roles in controlling cellular functions. In some cases IP3R is distributed non-uniformly on the endoplasmic reticulum (ER) and supposedly changes its localization. However, mechanism underlying localization and dynamics of IP3R is largely unknown. In this study, green fluorescent protein (GFP) was fused to full-length or deleted mutants of IP3R type I (IP3R1) to monitor their intracellular dynamics. After agonist stimulation, GFP-tagged full-length IP3R1 formed large clusters, which was distinct from the ER vesiculation. There was significant delay between the peak of intracellular Ca²⁺ concentration and formation of these large clusters, and these clusters remained for prolonged time even after intracellular Ca²⁺ concentration returned to the basal level. The ligand-binding domain of IP3R1 was necessary for this large cluster formation. Although GFP-tagging to full-length IP3R had little effect on its channel properties, replacing the whole N-terminal cytoplasmic domain with GFP made a constitutively open channel. GFP-IP3R1 expressed in cultured hippocampal neurons rarely localized to dendritic spines. Our results indicated that localization of IP3R1 is highly regulated and that it changes according to circumstances, which may be important for regulating intracellular Ca²⁺ patterns and cellular function.

P01-E-33 (MS5-1)

Characterization of D-serine-regulated genes in rat cerebral cortex

○Naoki Yamamoto¹, Hideto Tsuchida¹, Asami Umino¹, Dai Shimazu^{1,2}, Shinichiro Sakurai^{1,2}, Go Taniguchi^{1,2}, Toru Nishikawa^{1,2}

¹Dept. Mental Disorder Res., National Institute of Neuroscience, NCNP, ²Section of Psychiatry and Behavioral Science, Tokyo Medical and Dental Univ. Grad. School

D-Serine is an endogenous neuromodulator of NMDA receptor-mediated glutamatergic signaling in the mammalian central nervous system. To obtain insight into the molecular pathways for D-serine metabolism and its physiological functions, we have explored transcripts that are responsive to D-serine in the rat cerebral neocortex. RNA arbitrarily-primed PCR (RAP-PCR), a differential cloning technique, was utilized in this study. We have isolated two D-serine responsive transcripts, named as *dsr-1* and *dsr-2*. The expression of *dsr-1* mRNA was predominant in the brain, lung, and testis. The vacuolar type proton ATPase subunit M9.2, a structurally related molecule to *dsr-1*, did not show any response to D-serine administration while the expression of *dsr-1* was significantly enhanced by D-serine in the cerebral cortex both at 3 h and 15 h. The *dsr-2* was also significantly induced by D-serine at 3 h. Expression of *dsr-2* was limited to central nervous system. Furthermore, the distribution pattern of *dsr-2* mRNA and its ontogenic change are closely correlated to those of free D-serine content and of NMDA receptor R2B subtype. These data suggest that *dsr-1* and *dsr-2* gene products could play crucial roles for D-serine metabolism and/or function in the mammalian central nervous system. The genomic organization and transcriptional regulation mechanism of the *dsr-1* and *dsr-2* are currently under investigation.

P01-E-34 (MS5-2)

Biochemical properties and partial purification of pChAT, a novel splice variant of choline acetyltransferase.

○Jean-Pierre Bellier, Yoshinari Aimi, Masami Park, Tadashi Hisano, Petra Minnasch, Akinori Matsuo, Osamu Yasuhara, Ikuo Tooyama, Hiroshi Kimura
Mol. Neurosci. Res. Center, Shiga Univ. Med. Sci.

Previously known choline acetyltransferase (ChAT) is a 67kDa protein that catalyzes the biosynthesis of the neurotransmitter acetylcholine. It has however well been documented that the ChAT gene generates multiple mRNA splice variants. Recently, our laboratory has reported the presence of a variant mRNA originated by alternative splicing that lacks the coding exons from 6 to 9. Such a variant codes for a 50 kDa protein was termed pChAT because of its dominant expression in peripheral nervous tissues. Our immunohistochemical studies using an antiserum raised against pChAT have confirmed its preferential localization in peripheral cholinergic cells. Here, we first report some biochemical properties of pChAT in extracts of the dorsal root, which contains only pChAT as shown by our preliminary RNA and protein analysis. Knowledge concerning the tissue localization of pChAT, its solubility, isoelectric point, substrate binding order, and its behavior in the presence of pChAT antibody, all allowed us to establish a scheme for the its purification from bovine intestine, using techniques including affinity chromatography. The analysis of our final preparation on SDS-PAGE revealed a major band at about 50 kDa. Western-blot demonstrated that the 50 kDa band was immunoreactive for pChAT. The specific activity of ChAT in our preparation was 250 pmol/min/mg. These results indicate that pChAT is capable of producing acetylcholine, having biochemical properties different from the 67kDa ChAT. The low enzyme activity of pChAT will be discussed in relation to functional roles for acetylcholine other than a neurotransmitter.

P01-E-35

Suppressive Effects of Trihexyphenidyl on Methamphetamine-Induced Dopamine Release in the Nucleus Accumbens: Relevance to Pharmacotherapy

○Kazuaki Shimosato¹, Satoru Watanabe¹, Shigeo Kitayama²

¹Dept. Pharmacol., Kawasaki Med. School, ²Dept. Dent. Pharmacol., Okayama Univ. Grad. Sch. Med. Dentist.

Methamphetamine (MAP) and cocaine, widely abused throughout the world, cause severe medical and social problems. Our previous study found that a muscarinic cholinergic receptor antagonist trihexyphenidyl (THP) specifically suppressed rewarding properties of MAP but not of cocaine, as assessed by conditioned place preference in mice. The present study examined using in vivo microdialysis whether THP would influence the extracellular dopamine (DA) levels in the nucleus accumbens and striatum of mice injected with MAP, as compared to scopolamine (SCP) and cocaine. In addition, locomotor activity was simultaneously measured during microdialysis. In vivo microdialysis experiments revealed that MAP (1 mg/kg) increased DA levels during 0-60 min after injection up to 600% in the NAc and 380% in the striatum as compared to the basal level. These increases were retarded by the treatment with 5 mg/kg THP to 280% in the NAc and 200% in the striatum. However, SCP (3 mg/kg) had no effect on increases in the extracellular DA levels in both the two regions after MAP injection. Cocaine (10 mg/kg) increased DA levels during the initial hour to 250% in the NAc and 200% in the striatum as compared to the basal level. These increases were unaffected by either treatment with THP or SCP. On the contrary, both THP and SCP enhanced the locomotor-stimulant action of MAP and cocaine. These results, together with our previous finding, suggest that THP may specifically antagonize rewarding properties of MAP through suppression of DA release in the mesolimbic area without retarding locomotor activity.

P01-E-36

Serotonergic signaling inhibits hyperalgesia induced by spinal cord damage

○Hideki Horiuchi, Tadanori Ogata, Tadao Morino, Haruyasu Yamamoto
Dept.Orthop.Surg.,Ehime Univ

Although dysesthesia is one of the serious problems in the patients with spinal cord injury, most of them are often refractory to conventional treatments. The intrathecal injection of metergoline, a non-selective 5-HT antagonist, induced thermal-hyperalgesia in the rat's hind paw indicating that serotonergic signaling regulates the pain sensation in physiological conditions. We established a rat thoracic spinal cord mild-compression model in this study that revealed thermal hyperalgesia in the hind paw. The thoracic spinal cord was compressed gently by a 20 g weight for 20 minutes (mild-compression model). The withdrawal latency of the heating stimulation to bilateral hind-limb paw was monitored using Hargreaves¹ Plantar test apparatus. In this model, heat-hyperalgesia was observed for one week after the injury. In histological examination, inflammatory responses including microglia proliferation were observed in the same period as the hyperalgesic period. Intraperitoneal application of fluvoxamine maleate, a selective serotonin reuptake inhibitor, reduced the hyperalgesia induced by spinal cord injury. The inhibitory effect of fluvoxamine maleate on thermal hyperalgesia was prevented by pretreatment with metergoline. These results suggest that the change of the serotonergic signal plays important roles in hyperalgesia after the spinal cord injury, and the application of selective serotonin reuptake inhibitors will be one of the candidates for new therapeutic methods against post-spinal cord injury dysesthesia.

P01-E-37

Nicotine inhibits ERE binding via MAPK cascade in the hippocampus.

○Shozo KITO, Akiko S. SHINGO

Hyogo Univ. Sch. of Health Sci.

The present study was undertaken to answer the question of why nicotine suppresses neuronal effects of estrogen, while signal administration of each sometimes shows actions toward the same direction.

In the rat hippocampus, intraperitoneal injection of 1 mg/kg nicotine induced a marked and immediate inhibition of ERE-DNA binding. The level of the inhibition remained almost unchanged up to 120 min. This nicotine-induced inhibition of ERE-DNA binding was dose-dependent. To study the mechanism of this inhibition, changes in expressions of B-Raf and heat shock protein 90 (hsp90) were examined by western blotting. Besides, western blotting experiments with use of an Erk1/2 phospho-antibody revealed activation of MAPK cascade following the nicotine injection.

P01-E-38

Functional interaction between GABAergic and DAergic system through the corticostriatal pathways

○Machiko Matsumoto¹, Hiroko Togashi¹, Manabu Kanno², Satoshi Ohashi¹, Ken-ichi Ueno¹, Mitsuhiro Yoshioka¹

¹Department of Pharmacology, Hokkaido University Graduate School of Medicine, ²Department of Rehabilitation Medicine, Hokkaido University Graduate School of Medicine

It is known that the differences in the sensitivity of DA neuronal activity to physical stress and pharmacological manipulations exist between the rat prefrontal cortex (PFC) and striatum (STR)⁽¹⁾⁽²⁾. Anatomically, the STR receives glutamatergic input from the cortex, named corticostriatal pathways. Recently, we reported that the PFC regulates DA release in the STR, which is mediated via GABAA receptors⁽³⁾. These findings lead us to speculate that the cortical GABAergic system may be implicated in the regional differences in DAergic neuronal activity. The present study was undertaken to elucidate the possible interaction between GABAergic and DAergic system by exposure to psychological stress, in focusing on the corticostriatal pathways, by use of *in vivo* microdialysis. DA levels in the PFC were enhanced by conditioned fear stress (CFS), which is proposed to induce the anxiety based on fear memories. Striatal DA levels did not exert any changes by exposure to the CFS. Extracellular levels of GABA in the PFC increased, but glutamate did not change by the CFS. An unconditioned anxiogenic stimuli using elevated plus maze task did not affect the GABA levels in the PFC. Striatal DA and glutamate levels increased by exposure to elevated plus maze. These findings suggest that the cortical GABAergic system functionally regulates the STR DA release through the corticostriatal pathways. In other word, GABAergic system may play a key role in the regional differences of DAergic neuronal activity.

(1)Broderick P.A.and Piercey M.F.(1998)*J. Neural Transm.*105:749-767 (2)Feenstra M.G.*et al.*(1998)*J. Neurochem.*70:1104-1113 (3)Matsumoto M.*et al.*(2002)*Japan J. Pharmacol.* 88,Suppl.I,190p

P01-E-39

Effect of Different Species of Aluminum on Glutamate-Induced Overexpression of PLC- δ 1 Protein Level in Primary Cultured Rat Cortical Neurons

○Sadaki Fujimoto¹, Kentaro Nishida¹, Shun Shimohama², Kazuki Nagasawa¹

¹Dept. Environ. Biochem., Kyoto Pharm. Univ., ²Dept. Neurol. Grad. Sch. Med., Kyoto Univ.

[Purpose] Aluminum (Al) is reported to induce oxidative stress in the brain and to have the potential to contribute to Alzheimer's disease (AD). On the other hand, we have previously showed the abnormal accumulation of PLC- δ 1, which is thought to be a stress responsive protein, in the AD brain. However, correlation of the accumulation of Al and PLC- δ 1 to neurodegenerative disorder is not clarified yet. Furthermore, it is unclear whether or not the difference between the Al species, that is, Al citrate which is the predominant species in the brain, and Al chloride which is the ionic species, has different effect on the glutamate (Glu)-induced alteration of PLC- δ 1 protein level. In this study, we evaluated the effect of Al citrate and Al chloride on Glu-induced alteration of PLC- δ 1 expression in primary cultured rat cortical neurons.

[Materials & Methods] Rat cortical neurons were cultured in B-27 supplement-containing EMEM medium. The cultured cells were confirmed to be pure neurons by immunohistochemistry and expression pattern of PLC isozymes. The neurons were pretreated with Al citrate or Al chloride for 4 days, and treated with Glu in Mg²⁺-free Locke's solution. After culturing for 24 hr, the protein levels of PLC- δ 1 and the other isozymes were determined by Western blot.

[Results & Discussion] The PLC- δ 1 protein level was increased by the treatment with Glu dose-dependently. This elevation of PLC- δ 1 protein level was suppressed to the control level by the pretreatment of Al citrate. These results suggest that Al citrate has suppressive effect on the Glu-induced overexpression of PLC- δ 1 protein. Now, we are investigating the effect of difference in the species of Al on the PLC- δ 1 protein level in Glu-stimulated response.

P01-E-40

5HT-1 and 5HT-3 serotonin receptor subtypes regulate pain sensation in the spinal cord level: Normal and hyperalgesic animal model

○Tadanori Ogata, Hideki Horiuchi, Tadao Morino, Haruyasu Yamamoto

Dept. Orthopaedic Surg. Ehime Univ. School of Med.

Serotonergic receptor function for pain-threshold against heat stimulation was tested in rat hind paw. We used two animal models; normal and hyperalgesic model after spinal cord injury. Twenty minutes¹ compression by 20g-weight in 11th thoracic vertebral level spinal cord induced heat-hyperalgesia. The withdrawal latency of the heating stimulation was measured using Hargreaves² Plantar test apparatus at 1 hr after the intrathecal injection of 5HT receptor agonists or antagonists. In the normal rat, selective 5HT-1 and 5HT-3 receptor antagonists reduced the withdrawal latency. On the other hand, 5HT-2 receptor antagonist showed no effect. In the spinal cord injury model, the withdrawal latency was significantly lower than that in the normal rat. Intrathecal administration of selective 5HT-1 and 5HT-3 receptor agonists completely diminished the spinal cord injury-induced hyperalgesia, while 5HT-2 receptor agonist showed no effect. When endogenous 5HT level was elevated by the addition of selective serotonin reuptake inhibitor (SSRI), the spinal cord injury-induced hyperalgesia was diminished. Selective 5HT-1 and 5HT-3 receptor antagonists counteract the effect of SSRI on the hyperalgesia. These results suggest that serotonin inhibits pain sensation in spinal cord level via 5HT-1 and 5HT-3, not 5HT-2, receptor subtypes.

P01-E-41 (MS5-4)

Stimulation of phospholipase A₂ activity by anandamide in PC12 cells

○Akiyoshi Someya, Syunji Horie, Toshihiko Murayama

Lab. Chem. Pharmacol., Grad. Sch. Pharm. Sci., Chiba Univ.

Anandamide (arachidonyl ethanolamine) is an endogenous ligand for cannabinoid receptors, and recent study revealed that it is also an endogenous ligand for vanilloid receptor subtype 1 (VR1). In the present study, we investigated the effects of anandamide and capsaicin on arachidonic acid (AA) release in PC12 cells, rat pheochromocytoma cell line. The addition of anandamide or capsaicin stimulated AA release in a concentration-dependent and in an extracellular CaCl₂-independent manner. The effects were not inhibited by capsazepine, an antagonist of VR1, and WIN55,212-2, a potent agonist of cannabinoid receptors, did not stimulate AA release. Anandamide shifted the concentration-response curve of mastoparan, which is a wasp venom toxin and stimulates cytosolic phospholipase A₂ (PLA₂) activity in PC12 cells, to the left. Bromophenacyl bromide, a nonselective inhibitor of PLA₂, inhibited AA release stimulated by anandamide. The effects of anandamide and capsaicin were not washed out, because AA release in PC12 cells treated with anandamide or capsaicin for 30 min and then washed, was much higher compared with that in the control cells. Anandamide also stimulated prostaglandin F_{2α} formation. These results suggest that anandamide and capsaicin stimulated PLA₂ activity in VR1- and cannabinoid receptor-independent pathway(s) in PC12 cells. Further studies concerning the subtypes of PLA₂ and the biochemical mechanism(s) involved in the activation of PLA₂ by anandamide in PC12 cells are currently in progress in our laboratory.

P01-E-42 (MS5-3)

Behavioral and neurochemical changes by repetitive transcranial magnetic stimulation: focused on anxiolytic properties during exposure to the elevated plus-maze test

○Manabu Kanno¹, Machiko Matsumoto², Hiroko Togashi², Mitsuhiro Yoshioka², Yukio Mano¹

¹Dept. Rehabilitation Med. Grad. Sch. Med., Hokkaido Univ., ²Dept. Pharmacol. Grad. Sch. Med., Hokkaido Univ.

Transcranial magnetic stimulation (TMS) is a procedure in which regional electrical activity in the brain is influenced by a pulsed magnetic field. The rapidly changed magnetic field produces electrical currents, thereby activating neurons. Repetitive TMS (rTMS) treatment is reported to transiently cause the functional changes of cortical region. Recently, attention was focused on the clinical availability of rTMS in view towards antidepressant and anxiolytic effects.

The present study was undertaken to elucidate the effects of rTMS treatment on behavioral and neurochemical changes during exposure to the elevated plus-maze (plus-maze) test in focusing on anxiety. Extracellular levels of dopamine (DA) and serotonin (5-HT) in the prefrontal cortex (PFC) were determined using *in vivo* microdialysis. Each rat received rTMS treatment consisting of 125 stimuli from 5 trains at a rate of 25 Hz for 1 s and the 2 min train interval per day. Three-days series of rTMS treatment (chronic rTMS treatment) caused significant increases in both number of entries into the open arms and time spent on the open arms, compared with non-treated and sham-treated rats. However, these behavioral changes were not observed in one-day series of rTMS treatment (acute rTMS treatment). Chronic rTMS treatment suppressed the enhancement of 5-HT levels produced by exposure to the plus-maze test, whereas elicited DA levels were not influenced by chronic rTMS treatment. These data suggest that chronic rTMS treatment to the frontal brain region has anxiolytic effects, which are related to the serotonergic neuronal system in the PFC. In other words, chronic rTMS treatment suppresses the serotonergic function and consequently may exert anxiolytic effects.

P01-E-43

The mutation of two amino acid residues of tyrosine hydroxylase dramatically enhances the ability of neuroendocrine AtT-20 cells to synthesize L-DOPA

○Akira Nakashima¹, Yoko S Kaneko¹, Keiji Mori¹, Kentaro Fujiwara², Toshimitsu Tsugu¹, Takahiro Suzuki³, Toshiharu Nagatsu³, Akira Ota¹

¹Dept. of Physiology, Sch. Med., Fujita Health Univ.,

²Dept. of Internal Medicine, Sch. Med., Fujita Health Univ., ³Div. of Molecular Genetics, Inst. for Compr. Med. Sci., Fujita Health Univ.

The sequence Arg³⁷-Arg³⁸ of tyrosine hydroxylase (TH) plays a significant role in the feedback inhibition of the catecholamine products including dopamine (DA). To clarify how deeply the sequence Arg³⁷-Arg³⁸ and the phosphorylated Ser⁴⁰ of human TH type 1 (hTH1) are involved in the regulation of this feedback inhibition in mammalian cells, we produced the following mutants: RR-GG, Arg³⁷-Arg³⁸ replaced by Gly³⁷-Gly³⁸; RR-EE, Arg³⁷-Arg³⁸ by Glu³⁷-Glu³⁸; S40D, Ser⁴⁰ by Asp⁴⁰; and S40A, Ser⁴⁰ by Ala⁴⁰. The inhibitory effect of DA for the RR-EE was smaller degree than that for the phosphorylation-mimicking S40D on the catalytic activity. AtT-20 neuroendocrine cells were earlier shown to be capable of fully converting L-DOPA into DA, whereby the catalytic activity of TH would be expected to be inhibited by the end product DA accumulating in the cells. The level of DA accumulation in the cells transfected with wild-type and mutated hTH1 genes was in the order: RR-EE > S40D > S40A = RR-GG > wild-type, which was in accordance with the observations on the catalytic activity. These results suggest that the sequence Arg³⁷-Arg³⁸ of hTH1 is a more potent determinant of the efficient production of DA in mammalian cells than is the phosphorylated Ser⁴⁰-hTH1. Several studies indicate that the direct virus vector-mediated delivery of transgenes of the enzymes involved in catecholamine synthesis in vivo may be available for gene therapy of Parkinson's disease. It is suggested that the transduction with RR-EE would be one of the useful methods for the gene therapy.

P01-E-44

The Expression of GTP Cyclohydrolase I mRNA in Murine Locus Coeruleus Is Enhanced by Peripheral Administration of Lipopolysaccharide

○Yoko S. Kaneko¹, Keiji Mori¹, Akira Nakashima¹, Ikuko Nagatsu², Akira Ota¹

¹Dept. of Physiology, Sch. Med., Fujita Health Univ.,

²Dept. of Anatomy, Sch. Med., Fujita Health Univ.

GTP cyclohydrolase I (GCH) is the first and rate-limiting enzyme for the *de novo* biosynthesis of tetrahydrobiopterin (BH4), which is the cofactor for tyrosine hydroxylase (TH). As we previously reported, peripherally-administered lipopolysaccharide (LPS) can modulate BH4 production by upregulating GCH protein expression in the locus coeruleus (LC) in the mouse brain. The increased supply of BH4 in the LC leads to increased TH activity without affecting the level of TH protein expression, resulting in an increase in norepinephrine (NE) turnover at the site. We next studied to address whether the increase in GCH protein is dependent on the *de novo* synthesis of GCH in the LC.

After a 5 or 500 μ g of LPS i.p. injection to C3H/HeN mice (δ , 8W), the mRNA expression of GCH in the LC was examined. The expression level increased within 2 hrs, and reached to maximum level at 4 hrs after the LPS injection. However, the mRNA expression level of 6-pyruvoyl-tetrahydropterin synthase (PTPS) or sepiapterin reductase (SR), both of which are involved successively after GCH in BH4 biosynthesis, were not affected by the LPS injection. These results suggest that GCH upregulation alone is enough to modulate BH4 production in the LC. In addition, the mRNA level of TH was also not affected by the LPS injection. Taken together, the data indicate that GCH plays a crucial role in regulating NE biosynthesis by a pathway which is triggered by LPS i.p. injection.

P01-E-45

The effect of ophiobolin A, a novel calmodulin antagonist, on neurotransmitter release from rat cerebellar granule cells and PC12 cells

○ Kosuke Ando¹, Yoshihisa Kudo¹, Masami Takahashi^{2,3}

¹Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo, Japan, ²Department of Biochemistry, Kitasato University School of Medicine, Kanagawa, Japan, ³CREST/JST

Neurotransmitter is released from synaptic nerve terminals by means of an exocytosis of synaptic vesicles. Neurotransmitter release is triggered by Ca²⁺ ion and the identification of Ca²⁺ sensor protein is quite important for the understanding of molecular mechanisms of presynaptic function. Synaptotagmin I, a synaptic vesicular protein having C2-domains in cytoplasmic region, has been suggested to be a good candidate for presynaptic Ca²⁺ sensor. However, agreement has not yet been achieved since recent accumulating evidences suggest that calmodulin also plays important roles in neurotransmitter release adrenal chromaffin cells. In order to reveal whether calmodulin has characteristic of the Ca²⁺ sensor for neurotransmitter release, we studied the effect of novel calmodulin antagonist, ophiobolin A, on neurotransmitter release from cultured rat cerebellar granule cells and clonal rat pheochromocytoma PC12 cells. Ophiobolin A suppressed releases of dopamine and acetylcholine from PC12 cells in concentration- and time- dependent manners. By the contrast, ophiobolin A did not inhibit glutamate release from cultured rat cerebellar neurons. These findings suggest that calmodulin may play an important role in Ca²⁺-dependent neurotransmitter release from PC12 cells but not from neurons.

P01-E-46

Effect of peripheral lipopolysaccharide administration on dopamine content in murine anterior olfactory nucleus

○Keiji Mori¹, Yoko S kaneko¹, Akira Nakashima¹, Kentaro Fujiwara², Ikuko Nagatsu³, Akira Ota¹

¹Department of Physiology, Fujita Health University School of Medicine, ²Department of Internal Medicine, Fujita Health University Hospital, ³Fujita Health University School of Medicine

Norepinephrine (NE) turnover in the murine locus coeruleus (LC) is known to be enhanced by the intraperitoneal (i.p.) injection of lipopolysaccharide (LPS). Approximately 40% of LC neurons project to the olfactory system. Therefore, we investigated whether the endotoxemia caused by an i.p. injection of LPS could modulate the catecholamine biosynthesis in the olfactory bulb (OB) and the anterior olfactory nucleus (AON). Following i.p. injection of LPS to C3H/HeN male mice, slices of OB and AON were removed at multiple time points (2, 4, and 6 h) and used as samples for analysis. In the AON, the change in the contents of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid was transient. The levels of both were highly increased at 4 h after the LPS injection. The contents of NE and its metabolites were not affected by the LPS injection. The mRNA expression level of the gene encoding tyrosine hydroxylase (TH) was not altered by LPS injection, whereas that of the gene encoding GTP cyclohydrolase I (GCH), the rate-limiting enzyme for the biosynthesis of the cofactor of TH, was enhanced. The mRNA expression levels of the genes encoding DA receptor subtypes were transiently enhanced by the LPS as well as the level of mRNA encoding the α 2C-adrenergic receptor subtype. On the contrary, in the OB, the contents of DA, NE and their metabolites were not affected by LPS. However, the mRNAs of GCH and DA transporter genes in the OB were enhanced by it. Although the AON has been considered not to belong to dopaminergic neuron system and the origin of the enhanced DA in the AON was not fully identified, our report is the first to show an elevated DA content occurred in the AON under a stressful condition such as LPS-induced endotoxemia.

P01-E-47

Changes in substance P and its processing enzyme activity in rat dorsal root ganglia following sciatic nerve ligation

○Ryoko Nakajima¹, Ken Tsuchida¹, Toshiyuki Chikuma², Takeshi Kato¹

¹Grad. Sch. Int. Sci., Yokohama City Univ., ²Dept. Hygien. Chem., Showa Pharmaceut. Univ.

Substance P (SP), a neuropeptides, which is present in afferent fibers projecting to dorsal horn. SP is known as a peptide transmitter closely associated with nociceptive stimulus. It has been reported that various peripheral nerve injuries alter SP-immunoreactivity level in rat dorsal root ganglia (DRG). The biosynthesis of SP is regulated by their processing enzymes, carboxypeptidase E (CPE) and peptidylglycine α -amidating monooxygenase (PAM). The aim of this study is to clarify the relation between SP and its processing enzymes following rat sciatic nerve ligation. After complete ligation of the nerves, we measured preprotachykinin (PPT) mRNA level, SP-immunoreactivity level, and the enzyme activity of CPE and PAM in rat DRG. There was no significant change in PPT mRNA level and the processing enzyme activity between injured and control animals. SP-immunoreactivity in the DRGs decreased in the injured animals compared with the sham-operated and intact control animals. We are planning to investigate the peptide level and its processing enzyme activity using a different model of nerve injury.

P01-E-48

Prenatal and lactational exposure of Bisphenol A to mice effects on monoaminergic neurons

○Toyoko Hiroi, Kazushi Okada, Takashi Yoneda, Yoshihiko Funae

Dept. Chem. Biol. Osaka City Univ. Grad. Med. Sch.

Bisphenol A (BPA) is commonly ingested by humans, being released by polycarbonate plastics, the lining of food cans, and dental sealants. BPA has an estrogenic activity *in vitro* and *in vivo* though the affinity of BPA for estrogen receptors is very low compared with that of 17 β -estradiol, and is suspected to be one of endocrine disrupting chemicals (EDCs). Some ECDs such as polychlorinated biphenyls (PCBs) are reported to influence not only on endocrine systems but also on central nervous systems. In this study, we focused on the effect of BPA on central nervous systems. We first investigated the effects of prenatal and lactational exposure of BPA to *ddy* mice on monoaminergic neurons. BPA was treated with foods to dams during pregnancy and lactational periods. After the weaning, whole brains were removed from offsprings, which were born and lactated from these dams. The levels of monoamine neurotransmitter such as dopamine, norepinephrine and serotonin were measured using HPLC-ECD. The mRNA levels of proteins such as dopamine receptors and tyrosine hydroxylase were measured using quantitative RT-PCR analysis. BPA-treatment decreased significantly the level of dopamine in all females offsprings. However, the mRNA level of tyrosine hydroxylase was not decreased, rather increased. Furthermore, we used rat pheochromocytoma PC12 cells, which respond to NGF and synthesize dopamine and norepinephrine, *in vitro* study. The level of dopamine in PC12 cells was decreased dose-dependently by BPA-treatment.

P01-E-49

Functional abnormalities of the brain serotonergic system in PACAP-deficient mice

○Norihito Shintani¹, Kazuhiro Tanaka¹, Chihiro Kawaguchi¹, Hitoshi Hashimoto¹, Toshio Matsuda², Akemichi Baba^{1,3}

¹Dept. Mol. Neuropharmacol. Grad. Sch. Pharm. Sci., Osaka Univ., ²Dept. Med. Pharmacol. Grad. Sch. Pharm. Sci., Osaka Univ., ³Dept. Mol. Pharmacol. Grad. Sch. Med., Osaka Univ.

Neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) has been conserved remarkably during evolution and is widely expressed in the mammalian brain. Recently we have generated PACAP-deficient mice (PACAP^{-/-} mice) exhibiting hyperactive and explosive jumping behaviors in an open field, increased exploration and reduced anxiety in the elevated plus maze, and decreased serotonin (5-HT) turnover in the brain.

To examine the changes in brain 5-HT function in PACAP^{-/-} mice, the hypothermic effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT, and the behavioral effects of a selective 5-HT reuptake inhibitor (SSRI) fluoxetine were examined. In wild-type mice, 8-OH-DPAT (0.5 mg/kg, s.c.) significantly lowered rectal temperature, while it had only a small effect in PACAP^{-/-} mice. In PACAP^{-/-} mice, 2.5-10 mg/kg fluoxetine effectively suppressed the hyperactivity and abnormal jumping behavior. Similarly, the administration of a 5-HT precursor, 5-hydroxytryptophan (5-HTP) significantly reduced the jumping behavior of mutants.

These results demonstrate that PACAP is required for maintaining normal 5-HTergic functions, and suggest the involvement of 5-HTergic dysfunctions in phenotypic changes of PACAP^{-/-} mice.

P01-E-50

Selective deficit of serotonin_{1A} receptor-mediated regulation of cortical dopamine release in isolated mice

○Toshio Matsuda¹, Yukio Ago¹, Masaki Sakaue², Yutaka Koyama¹, Akemichi Baba²

¹Lab. Med. Pharmacol., Grad. Sch. Pharm. Sci., Osaka Univ., ²Lab. Mol. Neuropharmacol., Grad. Sch. Pharm. Sci., Osaka Univ.

Social isolation induces aggressive behavior in several strains of rats and mice, and serotonin (5-HT)_{1A} receptor agonists reduce this aggressive behavior. Dopamine (DA), 5-HT and noradrenalin (NA) are considered to be involved in mediating the abnormal behavior, and the cortical release of these transmitters is regulated by 5-HT_{1A} receptors. Our previous microdialysis study using reuptake inhibitors shows that isolation-rearing reduces MKC-242-stimulated DA release in vivo in the frontal cortex, but does not affect MKC-242-induced modulation of NA and 5-HT release. However, the results in the presence of reuptake inhibitors do not always reflect in vivo situation. We using a system in the absence of reuptake inhibitors re-examines the effect of isolation-rearing on the 5-HT_{1A} receptor-mediated modulation of DA, 5-HT and NA release in the frontal cortex of mice. Male ddY mice were either housed in groups of 5-6/cages or isolated in the same size cage for more than 6 weeks before experiments. In microdialysis experiments, the probes were perfused with Ringer's solution in the absence of reuptake inhibitors. The locomotor activity of the mouse was measured by Supermex after a 2 h habituation period. The isolation did not affect basal extracellular levels of DA, NA and 5-HT. The effect of MKC-242 in increasing DA release was less pronounced in isolated than in grouped mice, while there was no difference in those of the drug on NA and 5-HT release between both groups. Amphetamine-induced increases in cortical DA release and locomotor activity were greater in isolated than in grouped mice. These findings suggest that isolation-rearing specifically alters dopaminergic neurons in the cortex.

P01-E-51

Estrogen Enhances Depolarization-induced Glutamate Release through Activation of Phosphatidylinositol 3-kinase in Cultured Hippocampal Neurons

○Daisaku Yokomaku^{1,2}, Tadahiro Numakawa^{1,2,3}, Yumiko Numakawa¹, Naoki Adachi¹, Tomoya Matsumoto^{1,2}, Satoru Yamagishi^{1,3}, Chika Nishio¹, Takahisa Taguchi², Hiroshi Hatanaka¹

¹Dept. Protein Biosynthesis, Inst. Protein Research, Osaka Univ., ²Neuronic RG, Special Div. Human Life Tech., AIST, ³Research Fellowships of the Japan Society for the Promotion of Science

The ovarian steroids, estrogen and progesterone, are known to influence an organization of structure and maturation of synaptic function in brain. The changes in synaptic efficacy are thought to be necessary for learning and memory. Recently, it has been suggested that estrogen controls synaptic function in central nervous system (CNS). However, it is still unclear how estrogen regulates synaptic function in CNS neurons. Here, we found that estrogen potentiated presynaptic function in cultured hippocampal neurons. The chronic treatment with estradiol (1 or 10 nM) for 24 h significantly increased the high potassium (HK⁺)-induced glutamate release. In contrast, estradiol had no effect on the GABA release evoked by HK⁺. Not only cultured hippocampal neurons but also cortical, amygdala and cerebellar neurons responded to estradiol. The HK⁺-evoked release with or without estradiol pretreatment was inhibited by Cd²⁺ and tetanus neurotoxin, suggesting that estrogen enhanced presynaptic function through the up-regulation of exocytotic machinery. In addition, it was revealed that the estrogen-potentiated glutamate release required the activation of phosphatidylinositol 3-kinase (PI 3-kinase). All these results suggested that estrogen reinforces excitatory synaptic transmission via potentiated-glutamate release from presynaptic sites through activation of PI 3-kinase in CNS neurons.

P01-E-52

Brainstem is a possible site of leptin's action in the brain

○Toru Kawagishi, Yasunobu Okuma, Toru Hosoi, Jun Tanaka, Yasuyuki Nomura
Dept. Pharmacol. Grad. Sch. Pharm. Sci., Hokkaido Univ.

Leptin is a circulating protein which is the product of *obese* gene, it regulates food intake and body weight, reported to mediate mainly in the hypothalamus via Ob-Rb receptor, which activates JAK-STAT pathway. Although leptin receptors exist in many regions of the brain, there have been few *in vivo* functional studies of leptin's target site other than the hypothalamus. Ob-Rb leptin receptor mRNA was expressed in the brainstem as well as in the hypothalamus. Intravenous injection of leptin increased STAT3 phosphorylation not only in the hypothalamus but also in the brainstem as assessed by Western blotting analysis. Moreover, administration of leptin induced expression of suppressor of cytokine signaling 3 (SOCS3) mRNA, a negative feedback regulator of JAK-STAT pathway, in the brainstem as well as in the hypothalamus. Using immunohistochemistry, we observed phosphorylated STAT3 immunoreactive cells in the arcuate nucleus, ventromedial hypothalamic nucleus and lateral hypothalamic area of the hypothalamus, and nucleus of the tractus solitarius, dorsal motor nucleus of the vagus nerve, lateral parabrachial subnucleus and central gray of the brainstem of leptin-injected mice. These findings represent physiologically functional leptin Ob-Rb receptor in the brainstem as well as in the hypothalamus. Consequently, it is suggested that circulating leptin may directly act in the brainstem to elicit autonomic and neuroendocrine control of food intake and energy expenditure.

P01-E-53

Augmentation of T-type Ca^{2+} -current by Neuropeptide Y in NG108-15 cells

○Masayoshi Okada, Ayumi Hasegawa, Hiroshi Higuchi
Div. Pharmacol. Grad. Sch. Med. Sci., Niigata Univ.

T-type Ca^{2+} -channel plays fundamental roles in both regulation of excitability of neurons and pacemaking of heart cells, because of its channel properties of low-threshold activation and fast inactivation. However, modulation of T-type Ca^{2+} -channel is poorly understood. Neuropeptide Y (NPY) is one of the most abundant neuropeptide in the central and peripheral nervous system. NPY, indeed, is involved in memory, regulation of food intake, sedation, and works as a co-transmitter of sympathetic nerves. NG108-15 cell is a hybridoma cell line of neuroblastoma and glioma cells, and endogenously expressed T-type Ca^{2+} -channel and NPY receptor. Here we report that NPY (3×10^{-8} M) significantly augmented the amplitude of T-type Ca^{2+} -current. Neither rise nor decay time course of the current was affected by the NPY treatment. The I-V relationship of the NPY treated cells was similar to that of control cells. These results suggested that NPY treatment increases the availability of the T-type Ca^{2+} -channel without affecting its channel property in NG108-15 cells.

P01-E-54

Acid-Sensing Ion Channels ASIC1a and ASIC3 Are Leading Acid Sensors Expressed in Polymodal Nociceptors

○Shinya Ugawa, Takashi Ueda, Shoichi Shimada
Dept. Anat. II, Nagoya City Univ. Med. Sch.

Many painful inflammatory and ischemic conditions are accompanied by local tissue acidosis, and extracellular protons provoke pain by opening cation channels in nociceptors. Sensory neurons from mice lacking VR1 (vanilloid [capsaicin] receptor subtype-1) are severely deficient in their responses to acidic stimuli, so it is generally accepted that the VR1 channel mediates the greater part of the acid-evoked pain. In our psychophysical experiments, however, direct infusion of acidic solutions (pH 6.0) into human skin caused localized pain, which was blocked by amiloride, an inhibitor of ASICs (acid-sensing ion channels), but not by capsazepine, an inhibitor of VR1. Amiloride itself neither blocked capsaicin-evoked localized pain in human skin nor inhibited acid-evoked currents in VR1-expressing *Xenopus* oocytes. *In situ* hybridization and coimmunoprecipitation studies revealed that ASIC1a, ASIC3, and ASIC1a/ASIC3 complexes were main ASIC entities located in small-to-medium-diameter sensory neurons, and that most of VR1-expressing neurons were ASIC-positive. ASICs are thus leading acid sensors expressed in human nociceptors and essential components of VR1-expressing polymodal neurons.

P01-E-55

Transport mechanism of aluminum citrate across the blood-brain barrier

○Kazuki Nagasawa¹, Shingo Ito¹, Ikumi Tamai², Akira Tsuji³, Sadaki Fujimoto¹

¹Dept. Environ. Biochem., Kyoto Pharm. Univ., ²Fac. Pharm. Sci., Tokyo Univ. Sci., ³Fac. Pharm. Sci., Kanazawa Univ.

[Purpose] Because aluminum (Al) accumulated in the brain of Alzheimer's diseases, it is thought to play a role in etiology of neurodegenerative disorders. It was suggested that Al exists as Al citrate in plasma up to 20%, and in the brain its predominant species is Al citrate. Distribution of Al citrate into the brain should be regulated by the blood-brain barrier (BBB). Nevertheless, the detail transport mechanism of Al citrate at BBB is not clarified yet, although it was speculated that a specific transport system, involving monocarboxylate transporter (MCT) 1, might mediate Al citrate transport at BBB. In this study, we investigated whether a transport system handled the permeation of Al citrate at BBB using rat BBB model cell line, RBEC1. [Materials & Methods] Expression of mRNA for MCT isoforms was evaluated by RT-PCR method. In uptake study, cells were incubated with Al citrate or AlCl₃ in HBSS for the indicated times at 0 or 37 °C, and the reaction was terminated by adding ice-cold PBS. The cellular Al concentration was determined using ICP-MS. [Results & Discussion] mRNA for MCT1, 2 and 4 was detected in RBEC1. AlCl₃, Al³⁺, uptake is concentration-, but not temperature-, dependent, indicating involvement of nonspecific diffusion manner. On the other hand, Al citrate uptake could not observed under physiological condition, but in ATP-depleted RBEC1, there was apparent uptake of Al citrate at 37 °C. There was no difference in Al citrate uptake between in the presence and absence of H⁺-gradient. These findings suggest that Al citrate was not taken up into RBEC1 under physiological condition because it was actively effluxed from the cells, and MCT isoforms did not involved in its transport.

P01-E-56

Autocrine/paracrine regulation of voltage-dependent calcium channels in adrenal chromaffin cells

○Toshio Ohta, Takahiko Kai, Shigeo Ito

Lab. Pharmacol., Grad. Sch. Vet. Med., Hokkaido Univ.

To assess autocrine/paracrine regulation on voltage-dependent Ca²⁺ channels (VDCC) in chromaffin cells, effects of vesicular contents on VDCC were studied using a whole-cell patch clamp technique. A depolarizing pulse to 0 mV (test pulse) from a holding potential of -80 mV evoked inward calcium (ICa) and barium currents (IBa) in the presence of Ca²⁺ and Ba²⁺, respectively. Stopping the flow of the external solution (flow-stop) decreased the amplitude of IBa but not ICa, which was recovered by re-superfusion. Application of a large depolarizing pulse to +100 mV (prepulse) prior to a test pulse increased amplitudes of IBa to the same extent regardless of superfusion or flow-stop. External Ba²⁺ produced a long-lasting increase of oxidative currents due to catecholamine, which were significantly increased by flow-stop, indicating accumulation of vesicular contents around the cell. Exogenous application of vesicular contents [noradrenaline (NA), adrenaline, ATP, methionine-enkephalin (Met-enk) and proton] decreased IBa in a dose-dependent manner. Application of a prepulse greatly relieved IBa inhibition by met-enk, but had a little effect on that by others. Phentolamine and suramin decreased IBa inhibition induced by flow-stop. Intracellular application of GDP β S and pretreatment with pertussis toxin (PTX) significantly decreased IBa inhibition induced by flow-stop, NA and ATP, but not proton. Flow-stop, NA, ATP and proton inhibited N-type and L-type VDCC to the same extent. These results suggest that the inhibition of VDCC by flow-stop is mediated by an accumulation of vesicular contents released by Ba²⁺ around the cell in the presence of external Ba²⁺. Catecholamines and ATP may be involved in the VDCC inhibition by flow-stop through activation of α -adrenoceptor and P₂-purinoceptor associated with PTX-sensitive G-proteins, respectively. VDCC in porcine adrenal chromaffin cells seem to be regulated by a negative feedback mechanism in an autocrine/paracrine fashion.

P01-E-57

Effect of Central or Peripheral IL-1 β on Hydroxyl Radical and Nitric Oxide Productions in the Medial Prefrontal Cortex in Conscious Rats

○Yuta Ishizuka¹, Yasushi Ishida¹, Hiroshi Kannan²

¹Department of Psychiatry, Miyazaki Medical College,

²Department of Physiology, Miyazaki Medical College

We examined whether peripheral or central administration of interleukin-1 β (IL-1 β) might change levels of nitric oxide (NO) and hydroxyl radical (OH) in the medial prefrontal cortex (mPFC) in conscious rats. Extracellular levels of NO metabolites (NOx) were measured by an NO detector and high-performance liquid chromatography (HPLC). Extracellular levels of 2,3-dihydroxybenzoic acid (2,3-DHBA), as a marker of OH production, were also determined using an inert microdialysis technique and an INERT-HPLC system. Peripheral administration of IL-1 β significantly increased NOx levels but not 2,3-DHBA levels in the mPFC. In contrast, central administration of IL-1 β into the mPFC resulted in dose-dependent increases in levels of both NOx and 2,3-DHBA. The present results show a different response in free radical productions in the mPFC between peripheral and central administration of IL-1 β . This finding should be useful for our understanding of the response of free radicals such as NO and OH in the mPFC after central and peripheral administration of IL-1 β .

P01-E-58

Disturbance of thyroid hormone-dependent synapse formation in cultured rat cortical neurons by environmental chemicals

○Ritsuko Hosoda^{1,3}, Midori Kato-Negishi¹, Kenji Nakayama², Kazuyo Muramoto¹, Masahiro Kawahara^{1,3}, Yoichiro Kuroda^{1,3}

¹Dept. Mol. Cell. Neurobiol., TMIN, ²Dept. Clin. Pathol., Hokkaido Inst. Public Health, ³CREST, JST

Thyroid hormones (THs) affect the development of CNS and the deprivation of THs leads to the severe mental retardation such as cretinism in human. Recently, many synthetic environmental chemicals are reported to affect the thyroid system and cause profound effects on structures and functions of the developing brain. We have previously established an assay system to investigate the synapse formation in rat cerebral cortical neurons using a multi-site calcium fluorometry. In this system, existence of 1 nM thyroxine (T4) increased the frequency of synchronous oscillatory changes in intracellular calcium levels, which corresponds to the number of synapses formed. We applied miscellaneous chemicals to this system along with 1 nM T4 and their effects on synapse formation were investigated by observing the frequency of synchronous oscillatory changes in intracellular calcium levels. Among various chemicals tested, we found that several pesticides and endocrine disrupting chemicals inhibited T4-induced synapse formation. This work was supported by CREST, Japan Science and Technology Corporation.

P01-E-59

Neuronal Toxicity of apoptotic injury induced by the long term treatment of typical and atypical antipsychotics

○Wataru Ukai, Hiroki Ozawa, Hiroshi Choei, Shingo Yamada, Rie Sakai, Masato Konno, Masaru Tateno, Megumi Yamamoto, Toshikazu Saito
Dept. of Neuropsychiatry, Sapporo Medical University

Neurotoxic effects of antipsychotic drugs have been demonstrated in relation to the production of extrapyramidal side effects (EPS) including tardive dyskinesia (TD) that may be irreversible in a large number of patients. The haloperidol induced apoptotic cell death mediated by reactive oxygen species (ROS), mitogen-activated protein kinases p38 and c-jun-NH2-terminal protein kinase (JNK) was reported in cultured neuron. In the present study, we examined the effects of long-term treatment of several kinds of antipsychotic drugs on the neuronal survival in rat cortical cell culture. Primary neurons dissociated from embryonic 17-19 rats and maintained in DMEM supplemented B27 medium. The neurons were exposed for 3-6 days to each drugs, then the levels of neuronal toxicity was analyzed using phase contrast photomicroscopy and evaluated by the MTT colorimetric assay, displayed the viable neurons. We also examined the apoptotic injury by the DNA fragmentation assay. Chronic exposure of haloperidol induced time and dose-dependent neuronal toxicity in cortical neurons. Haloperidol caused neuronal toxicity most potently in the tested typical and atypical antipsychotic drugs. There is clinical evidence that antipsychotics currently in development including serotonin-dopamine antagonist (SDA) cause few EPS. SDAs showed neurotoxic effect in higher doses than that of haloperidol. The lower potential of neuronal toxicity may relate to the low risk of EPS in psychotic patients. However, the neuronal cell injury profile of the drugs were not evaluated well, our findings indicate that the long-term treatment induced neuronal toxicity of antipsychotic drugs revealed at least part of the characteristic potentials and kinds of action contribute to its clinical appearances.

P01-E-60 (MS4-7)

Short-term memory impairment in juvenile stroke-prone spontaneously hypertensive rats, an animal model of attention-deficit/hyperactivity disorder, is improved by cholinergic intervention.

○Hiroko Togashi¹, Ken-ichi Ueno¹, Machiko Matsumoto¹, Hideya Saito², Mitsuhiro Yoshioka¹
¹Department of Pharmacology, Hokkaido University Graduate School of Medicine, ²Department Basic Science Japan Red Cross Hokkaido College Nursing

The present study was intended to elucidate a crucial role of cholinergic systems on short-term memory impairment based on attention-deficit in juvenile stroke-prone spontaneously hypertensive rats (SHRSP), an animal model of attention-deficit/hyperactivity disorder (ADHD)¹⁾. Short-term memory was assessed by spontaneous alternation behavior in a Y-maze task. Juvenile male SHRSP exhibited an impairment of spontaneous alternation performance and a significant increase in total arm entries in a Y-maze, as compared with the genetic control Wistar-Kyoto rats. (-)-Nicotine (0.1-1 mg/kg, SC) dose-dependently improved the lowered spontaneous alternation performance but not the increase total arm entries in SHRSP. Nicotine (1 mg/kg, s.c.)-induced improvement was significantly abolished by the centrally acting nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine (1 mg/kg, i.p.), but not by peripherally acting hexamethonium (5 mg/kg, i.p.). The $\alpha 4\beta 2$ nAChR antagonist dihydro- β -erythroidine (3-10 mg/kg, i.p.), dose-dependently counteracted nicotine-induced improvement of spontaneous alternation in SHRSP. Nefiracetam (3-30 mg/kg, i.p.), a pyrrolidone-type nootropic drug, which modulates cholinergic transmission, also ameliorated the impaired spontaneous alternation. These findings provide evidence that the cholinergic mechanism is responsible for short-term memory deficit of SHRSP. It is also suggested that nefiracetam treatment may be a new strategy as a non-psychostimulant therapy for improving attentional dysfunction in ADHD. 1) Ueno, K. et al.: Behavioral and pharmacological relevance of stroke-prone spontaneously hypertensive rats as an animal model of a developmental disorder. Behavioral Pharmacol. 2002, 13:1-13.

P01-E-61

Effect of valproate on agonist-induced intracellular calcium mobilization in human platelets

○Tatsuyuki Akimoto, Katsuzi Suzuki, Yuki Sasaki, Ichiro Kusumi, Tukasa Koyama
Dept. Psychiatry. Grad. Sch. Med., Hokkaido Univ

Serotonin (5-HT)- or thrombin (Thr)-induced calcium (Ca) mobilization in human platelets has been shown to be enhanced in patients with bipolar disorder. We have reported that in bipolar disorders, responder to mood stabilizers such as lithium and valproate (VPA) exhibited a significant higher Ca response to 5-HT than nonresponder, suggesting the possibility that the mechanism of enhanced Ca response may be associated with that of action of lithium or VPA.

We examined the effect of VPA on 5-HT- or Thr-induced intracellular Ca increase in the platelets with normal subjects, using calmodulin (CaM) antagonist W-7, myosin light chain kinase inhibitor ML-9, protein kinase C (PKC) inhibitor staurosporine and bisindolylmaleimide II (BIS), PKC activator phorbol 12-myristate 13-acetate (PMA). Intracellular Ca concentrations were determined by a Hitachi F-2000 fluorometer, after fluorescent Ca indicator fura-2 was loaded to adjusted platelets.

Pretreatment with VPA alone for 30 min at therapeutic relevant concentration (50-100 μ g/ml) inhibited both 10 μ M 5-HT-induced or 50 U/l Thr-induced Ca response in a concentration-dependent manner. This inhibitory action of VPA on the 5-HT-induced Ca response was not influenced by the pretreatment with CaM antagonists (30 μ M W-7, 30 μ M ML-9), but reduced by the pretreatment with PKC inhibitors (10 nM staurosporine, 10 nM BIS). Furthermore, the pretreatment with PKC activator (1nM PMA) reversed the inhibited Ca response by therapeutic relevant concentration (50-100 μ g/ml) of VPA. These findings suggest the possibility that the mechanism of action of VPA may be associated with PKC system rather than CaM system, and related to the "buffering" effect on PKC system.

P01-E-62

Dissociative role of GABA_B and GHB receptors in γ -butyrolactone induced absence epilepsy of developing rat brain

○Masayuki Tanaka¹, Noboru Takizawa¹, Kazuhiro Mawatari², Satoru Kato¹

¹Dept. Mol. Neurobiol, Grad. Sch. Med, Kanazawa Univ., ²Dept.Lab.Sci.,Fac.Med.,Kanazawa Univ.

Absence epilepsy is the age dependent childhood epilepsy that has a unique arrest and staring seizure in behavior and has spike and wave discharges (SWDs) in electroencephalography (EEG). γ -Aminobutyric acid B (GABA_B) receptors in reticular thalamic cells play an important role in generating SWDs in the thalamocortical circuit. A late long-lasting IPSP induced by GABA_B produces a low threshold calcium channel potential and activates a recurrent firing of thalamocortical cells. γ -Butyrolactone (GBL), a GABA_B receptor agonist, produces an abnormal seizure-like absence epilepsy and an increase of activator protein 1 (AP-1) DNA binding activity in the thalamus of adult mouse brain. There is a report that both GABA_B and γ -Hydroxybutyric acid (GHB) receptors are involved in absence epilepsy and that the GABA_B receptor expression is seen on postnatal day 3, while the GHB receptor expression is seen on postnatal day 20. In the present study, we investigated the role of both GABA_B and GHB receptors in absence epilepsy, an ontogeny of epileptic behavior, EEG, and AP-1 DNA binding activity in the GBL-induced absence epilepsy was studied in developing rat brain. Absence seizures first appeared on postnatal days 5-10, while AP-1 activation first appeared on postnatal days 30-40. A GABA_B receptor antagonist specifically inhibited both the absence epilepsy and AP-1 activation. On the other hand, a GHB receptor antagonist only inhibited AP-1 activation but not the absence epilepsy. The data strongly indicate that GABA_B receptors play a primary role in the generation of absence epilepsy induced by GBL, whereas the GHB receptors play a key role in the activation of AP-1 DNA binding activity, which is concerned with the maintaining of absence epilepsy.

P01-E-63

Measurement of metabolic rates for glutamate and glutamine in occipito-parietal lobes of human brain by non-invasive 2T ^{13}C -magnetic resonance spectroscopy (^{13}C -MRS)

○Tomoyuki Kanamatsu¹, Taisuke Otsuki², Kazuya Okamoto³, Hidehiro Watanabe³, Masaaki Umeda³, Yasuzo Tsukada¹

¹Institute of Life Science, Soka University, ²Depts. of Neurosurgery, Musashi Hospital, National Center of Neurology & Psychiatry, ³Toshiba Medical Systems Research & Development Center

We have developed the new technique to measure metabolic rates for glutamate and glutamine (Vglu, Vgln) in human brain and, as a final goal, to know the correlation between the changes of Vglu and Vgln and neurological disorders using 2T magnetic resonance spectroscopy. Young and aged volunteers and patients with brain disorders were examined by our newly developed ^{13}C -MRS system that has features of a high SNR, a clear separation of ^{13}C chemical compounds and a better localization (^1H - ^{13}C HSQC correlation spectroscopy). ^{13}C spectra were obtained from two voxels on bilateral occipito-parietal lobes of subjects after oral administration of 99% enriched [$1\text{-}^{13}\text{C}$] glucose (0.75g/kg body weight in 20% water solution). The time courses of ^{13}C incorporation into C4-glutamate and C4-glutamine were measured by ^{13}C -MRS. There was no difference in the values of Vglu and Vgln between the left and right occipito-parietal lobes of young healthy volunteers (n=7) and these values were 0.283 ± 0.069 and 0.020 ± 0.005 (mean $\pm$ S.D.; μ mole/g/min), respectively. In aged healthy volunteers (n=4), the Vglu was 0.250 ± 0.029 and the Vgln was 0.015 ± 0.002 . The Vglu and Vgln of mild dementia patients (n=4) were 0.254 ± 0.068 and 0.036 ± 0.009 , respectively. The Vgln of mild dementia was higher than that of aged and young volunteers ($p < 0.05$). In epilepsy patients with cerebral hemiatrophy (n=2), the Vglu and Vgln on the atrophied side were 0.150 and 0.043 (mean) and lower and higher than those on the normal side in the same patient and on both sides of young volunteers, respectively. ^{13}C -MRS revealed a decrease in the rate of ^{13}C incorporation from [$1\text{-}^{13}\text{C}$] glucose into glutamate and an increase in that into lactate in MELAS patient (n=1).

P01-E-64

Sleep-promoting system triggered by prostaglandin D_2 is involved in the homeostatic control of NREM sleep after sleep deprivation.

○Yoshihiro Urade^{1,2}, Naomi Eguchi^{1,2}, Yuko Kuwahata^{1,2}, Wei-Min Qu¹, Zhi-Li Huang¹, Osamu Hayaishi¹

¹Dept. Mol. Behav. Biol., Osaka Biosci. Inst., ²CREST, JST

Prostaglandin (PG) D_2 is a potent endogenous sleep-promoting substance in monkeys, rats, and mice. Lipocalin-type PGD synthase (PGDS), which is responsible for the biosynthesis of PGD₂ in the brain, is mainly produced in the leptomeninges of the entire brain, while PGD₂ receptor (DPR) is predominantly localized in the leptomeninges of the basal forebrain. PGD₂ infused into the subarachnoid space underlying the rostral basal forebrain increased in the extracellular adenosine level there and also induced sleep mediated by DPR in mice. The PGD₂-induced sleep was inhibited by an adenosine A_{2A}R receptor (A_{2A}R) antagonist. Furthermore, PGD₂ infusion significantly stimulated neural activities in the ventrolateral preoptic area (VLPO), one of the putative sleep centers, and simultaneously suppressed neural activities in the tuberomammillary nucleus (TMN), the histaminergic wake-center. Therefore, PGDS, DPR, and A_{2A}R are considered to be molecules involved in the sleep-promoting system triggered by PGD₂.

In this study, to evaluate the roles of PGDS, DPR, and A_{2A}R in sleep homeostasis *in vivo*, we performed sleep deprivation for 6 h and analyzed the recovery sleep by using gene-knockout (KO) mice for each gene. The circadian rhythm of rapid eye movement (REM) and non-REM (NREM) sleep was almost unchanged between each KO mice and their wild-type control (WT) mice as judged by recording electroencephalogram and electromyogram. However, we found that WT mice remarkably increased both NREM and REM sleep amounts during the recovery period for 12 h after sleep deprivation, whereas these KO mice for PGDS, DPR, or A_{2A}R did not show such a rebound in the amount of NREM sleep. The rebound of REM sleep was observed in these three KO mice after sleep deprivation. These results indicate that PGDS, DPR, and A_{2A}R are essential molecules that occur the NREM sleep rebound after sleep deprivation.

P01-E-65

Prostaglandin E₂ Activates Histaminergic System via EP₄ Receptor to Induce Wakefulness in Rats

○Zhi-Li Huang¹, Takatoshi Mochizuki¹, Tetsuya Okada^{1,2}, Wei-Min Qu¹, Atsushi Yamatodani³, Yoshihiro Urade^{1,4}, Osamu Hayaishi¹

¹Dept. Molec. Behav. Biol., Osaka Biosci. Inst., ²Dept. Med. Sci. III, Sch. Health Sport, Osaka Univ., ³Dept. Med. Physics, Sch. Allied Health Sci., Osaka Univ., ⁴CREST, Jpn. Sci. Tech. Corp.

Prostaglandin (PG) E₂ promotes the wakeful state when administered into the posterior hypothalamus where the histaminergic tuberomammillary nucleus (TMN) is located. To explore the neurotransmitter mechanisms responsible for the PGE₂-induced wakefulness in rats, we examined the effect of PGE₂ on the activities of the histaminergic system and the involvement of PGE₂ receptor subtypes in the response. PGE₂ perfusion in the TMN at doses of 40, 200, and 400 pmol/min for 2 hr significantly increased the histamine release from the medial preoptic area and frontal cortex in a dose-dependent manner, as measured by *in vivo* microdialysis. Among the agonists tested for 4 distinct subtypes of PGE₂ receptors (EP₁₋₄), only the EP₄ receptor-agonist (ONO-AE1-329) mimicked the excitatory effect of PGE₂ on histamine release from both the medial preoptic area and frontal cortex. RT-PCR analysis revealed that the EP₄ receptor mRNA was expressed in the hypothalamus but not in the cortex. Perfusion of either PGE₂ or the EP₄ agonist into the TMN at a dose of 200 pmol/min for 1 hr increased the histidine decarboxylase activity by 17 and 21 %, histidine decarboxylase mRNA level by 83% and 69%, and the histamine content by 27 and 67%, respectively, in the hypothalamus as compared with the values for the control group. Furthermore, EP₄ agonist perfusion into the TMN at doses of 200 and 400 pmol/min induced wakefulness in a dose-dependent manner. These findings indicate that PGE₂ induces wakefulness through activation of the histaminergic system via EP₄ receptors.

P01-E-66

Periodic nuclear/cytoplasmic shuttling of BMAL1 in the central and peripheral clocks

○Teruya Tamaru¹, Yasushi Isojima², Ken Takamatsu¹
¹Dept. of Physiology, Toho Univ. Sch. of Med., ²Div. of Protein Metabolism, Inst. for Protein Res.

BMAL1 is an essential component for mammalian circadian pacemaker that binds to CLOCK and drives clock-related transcription and self-sustaining oscillation.

We developed specific antibody against BMAL1 that detected heterogeneously phosphorylated BMAL1 proteins *in vivo*. Immunohistochemical analysis revealed periodic nuclear/cytoplasmic shuttling of BMAL1 in mouse suprachiasmatic nucleus (SCN) with dual nuclear accumulation patterns at dawn and dusk. However, BMAL1 accumulated to the nucleus with peaking once a day in mouse liver and NIH 3T3 cells. These data suggests the central clock-specific regulatory mechanism, which is related to photic entrainment and lack in the peripheral clock.

P01-F-1

Effect of MK-801, an NMDA Receptor Antagonist, on BACE and EP24.15 in Rat Brain

○Md. Mahiuddin Ahmed¹, Masaru Yamamoto¹, Toshiyuki Chikuma², Takeshi Kato¹

¹Grad. Sch. Int. Sci., Yokohama City Univ., ²Dept. Hygien. Chem., Showa Pharmaceut. Univ.

Recently a novel transmembrane aspartyl protease with all known characteristics of β -secretase was cloned and characterized, termed BACE (beta site APP cleaving enzyme), that cleaves the amyloid precursor protein (APP) at β -secretase site in Alzheimer's disease (AD). It has been also implicated that endopeptidase EP 3.4.24.15 (EP24.15) may be involved in the processing of APP in AD. In our present study, we have shown the effect of MK-801, as an early dementia model in AD, on BACE and EP24.15 in different rat brain regions. In order to find the physiological roles of both EP24.15 and BACE, we administered MK-801 (10mg/kg; i.p) in male rats and investigated the effect of EP24.15 enzyme activity and immunoreactivity as well as BACE immunoreactivity in different brain regions at 3h, 1 day and 3 days after the treatment. We found the enzyme activity of EP24.15 was significantly decreased in hippocampus and striatum, 1 day after the treatment and in posterior cortex after 3 days. However, the immunoreactivity of EP24.15 was unchanged in all brain regions after the treatment. BACE immunoreactivity was significantly decreased in posterior cortex and striatum, 1 day and 3 days after MK-801 treatment. From the present results, we suggest that the effect of MK-801 on BACE and EP24.15 is not unique in all brain regions and the effectiveness depends on duration of time for specific region. We also suggest that NMDA receptor antagonist performs both neurodegenerative and neuroprotective effect. We have also shown the effect of MK-801 on EP24.15 enzyme activity in female rat brains at different doses (0.2,1,0.3,0.10.0 mg/kg) and didn't get any changed of EP24.15 enzyme activity. We have a plan to investigate the effect of MK-801 on both enzymes by immunocytochemistry.

P01-F-2 (MS2-4)

Niemann-Pick C1 deficiency reduces the levels of cholesterol in detergent insoluble, low-density membrane domains leading to activation of MAP kinase pathway and increased phosphorylation of tau

○Naoya Sawamura^{1,2}, Jian-Sheng Gong¹, Ta-Yuan Chang³, Katsuhiko Yanagisawa¹, Makoto Michikawa¹

¹Dept. Dementia Res., NILS, ²JSPS, ³Dept. Biochemistry, Dartmouth Med. Sch.

Niemann-Pick type C (NPC) disease is characterized by an accumulation of cholesterol and other lipids in most tissues, and causes neurodegeneration in the central nervous system, with the formation of neurofibrillary tangles (NFTs); the major component of which is the hyperphosphorylated tau. Here, we examined the mechanism underlying hyperphosphorylation of tau using mutant Chinese hamster ovary (CHO) cell line (CT43) defective in NPC1. Immunoblot analysis using various anti-tau antibodies revealed that tau was hyperphosphorylated at multiple sites in CT43 cells, but not in their parental cells (25RA) or the wild-type CHO cells. In CT43 cells, MAP kinase (MAPK) was activated, while the amount of protein phosphatase 2A (PP2A) not bound to microtubules was decreased. The specific MAPK inhibitor, PD98059, attenuated the phosphorylation of tau in CT43 cells. Electron microscopy and filipin staining showed elevated intracellular cholesterol accumulation in CT43 cells. However, CT43 cells but not 25RA cells were amphotericin B-resistant, indicating that cholesterol concentration in the plasma membrane of CT43 is decreased. In addition, the level of cholesterol in the detergent-insoluble, low-density membrane (LDM) fraction of CT43 cells was markedly reduced compared with the other two types of CHO cells. Since LDM domain is known to play critical role in signaling pathways, these results suggest that the reduced level of cholesterol in LDM domain due to the lack of NPC1, may result in the activation of MAPK and PP2A, which in turn promotes tau phosphorylation in NPC1-deficient cells.

P01-F-3

p25 subunit, an activator of CDK5, induces intensive phosphorylation on Ser202/Thr205 residues of human tau40 *in vitro*.

○Mitsuko Hashiguchi¹, Taro Saito², Shin-ichi Hisanaga², Toshio Hashiguchi¹

¹Dept. Physiol. Sch. Med., Tokyo Med. Univ., ²Dept. Biol. Sci. Grad. Sch. Sci., Tokyo Met. Univ.

Hyperphosphorylated tau is a major component of neurofibrillary tangles, one of the hallmarks in Alzheimer's disease (AD) patient brain. Hyperphosphorylation of tau causes dissociation of tau from microtubules and irreversible tau aggregation resulting in microtubules dysfunction. CDK5, a member of proline-directed serine/threonine protein kinases, is one of tau kinases that phosphorylate tau protein. Its endogenous activator p35 regulates activity of CDK5. Recently calpain was found to digest p35 to its truncated product, p25. Several lines of evidence suggest that p25/CDK5 has much more powerful kinase activity and may cause abnormal hyperphosphorylation of tau. How and to what extent kinase activity of CDK5 is modulated by the activators, p35 or p25, has not been studied yet. We have examined kinetic characteristics of *in vitro* phosphorylation of human tau40 by CDK5 and its activators using recombinant proteins. The kinase activity of CDK5, as indicated by V_{max}, in phosphorylation of human tau40 was significantly higher in the presence of p25. Affinity of CDK5 to human tau40, as estimated from K_m value, did not show any difference. Analysis with 2D phosphopeptide mapping revealed enhanced phosphorylation of Ser202/Thr205 residues by p25/CDK5. These results suggest that the proteolytic cleavage of p35 to p25 not only greatly enhances the kinase activity of CDK5 but also increases the phosphorylation of specific sites, Ser202/Thr205, of human tau40. Considering the fact that phosphorylation on Ser202/Thr205 antagonizes the tau-mediated nucleation of tubulin, p25/CDK5 might play a pivotal role in the neuronal cell death in AD.

P01-F-4 (MS2-3)

Induction of differential responses in neurons and astrocytes by A β protein and non-A β component (NAC) of Alzheimer's disease amyloid through generation of reactive oxygen species and activation of NF- κ B

○Seigo Tanaka, Masanori Takehashi, Kunihiro Ueda
Lab. Mol. Clin. Chem., Instit. Chem. Res., Kyoto Univ.

Non-A β component of Alzheimer's disease (AD) amyloid (NAC), or a ≥ 35 amino-acid peptide derived from α -synuclein, is in association with A β protein in senile plaques. After exposure to A β ₁₋₄₀ and NAC fibrils preformed *in vitro*, cortical neurons of rat brain primary culture became apoptotic, while astrocytes were activated with extension of their processes. The neuron-specific injury by amyloid fibrils was also indicated by extracellular release of lactate dehydrogenase (LDH). A β ₁₋₄₀ and NAC fibrils decreased 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-reducing activity of cortical neurons more markedly than that of astrocytes. Induction of neuronal apoptosis was effectively suppressed by antioxidants, such as propyl gallate (PG) and N-*t*-butyl-phenylnitron (BPN), indicating a participation of reactive oxygen species (ROS) in the cytotoxic action of amyloid fibrils. Intracellular generation of ROS was demonstrated by oxidation of 2', 7' -dichlorofluorescein, and its location in mitochondria by staining with a fluorogenic probe, reduced chloromethyl-tetramethyl rosamine. Overexpression of human Bcl-2 efficiently inhibited A β ₁₋₄₀ or NAC-induced apoptosis of neurons. Exposure of cortical neurons and astrocytes to amyloid fibrils enhanced DNA-binding activity of NF- κ B, a transcription factor activated by oxidative or other stresses. These results suggest that a differential change in gene expression between neurons and astrocytes is induced by A β and NAC fibrils through mitochondrial ROS generation and NF- κ B activation in AD brain.

P01-F-5

CD36, a member of class B scavenger receptor family, serve as β -amyloid receptor and its expression on primary rat microglia stimulated with interleukine-4

○Kohichi Kawahara, Makoto Kajizono, Hitoshi Nakayama
Faculty of Pharmaceutical Sciences, Kumamoto University

Although imbalance between formation and degradation of β -amyloid peptide ($A\beta$) is thought to relate closely to the generation and progression of Alzheimer's disease, a little is known about the mechanism for its clearance in brain. Class A scavenger receptors (SRA) were involved in such a clearance in microglia (MG), but results from SRA-knock-out mice indicate that SRA is not the definitive molecule responsible for the mechanism. We investigated expression of CD36 in rat primary MG before and after activation and how they contribute to the clearance of $A\beta$. CD36 was not evident in untreated MG but was induced by IL-4. Neither LPS nor M-CSF were effective for the induction, while they enhanced SRA expression. Cellular association and degradation activities of [^{125} I] $A\beta$ 1-42 were significantly observed in CD36-overexpressed CHO cells. In the IL-4 stimulated MG, however, most of the cellular association activity of [^{125} I] $A\beta$ 1-42 was inhibited by addition of AcLDL, a favorable SRA ligand, whereas the considerable amount of the degradation activity retained by the addition of AcLDL. By contrast, the [^{125} I] $A\beta$ 1-42 degradation in MG was markedly inhibited by addition of a proteinase inhibitor thiorphan. These results indicate that scavenger receptors (SRA primarily and CD36 at rather late inflammatory stage) play roles for $A\beta$ clearance, but thiorphan-sensitive protease(s) also contributes to the degradation of $A\beta$ in activated microglia.

P01-F-6

A Novel Function of Monomeric Amyloid β -Protein Serving as an Antioxidant Molecule against Metal-Induced Oxidative Damage

○Kun Zou, Jian-Sheng Gong, Katsuhiko Yanagisawa, Makoto Michikawa
Dept. of Dementia Research, Natl. Inst. for Longevity sci.

Aggregated and oligomeric amyloid β -protein ($A\beta$) is known to exhibit neurotoxicity. However, the action of $A\beta$ monomers on neurons is not fully understood. We have studied aggregation-state-dependent actions of $A\beta$, and found an oligomer-specific effect of $A\beta$ on lipid metabolism in neurons (Michikawa et al., J. Neurosci., 2001; Gong et al., J. Neurosci. Res., 2002). Here, we show a novel function of monomeric $A\beta$ 1-40, which is the major species in physiological fluid and the conditioned medium of the cultured neurons, as a natural antioxidant molecule. Monomeric $A\beta$ 1-40 protects neurons cultured in a medium containing 1.5 μ M Fe(II) without antioxidant molecules. Metal ion chelators such as EDTA, CDTA and DTPA, an iron-binding protein, transferrin, and antioxidant scavengers such as catalase, glutathione and vitamin E also inhibit neuronal death under the same conditions. In addition, monomeric $A\beta$ 1-40 prevents neuronal death caused by Cu(II), Fe(II), and Fe(III), but does not protect neurons against H_2O_2 -induced damage, indicating that $A\beta$ protects neurons as a metal binding molecule but not as a radical scavenger. Monomeric $A\beta$ 1-40 inhibits the reduction of Fe(III) induced by vitamin C and the generation of superoxides, and prevents lipid peroxidation induced by Fe(II). $A\beta$ 1-42 remaining as a monomer also exhibits antioxidant and neuroprotective effects. In contrast, oligomeric/aggregated $A\beta$ 1-40 and $A\beta$ 1-42 lose their neuroprotective activity. These results indicate that $A\beta$ monomers protect neurons by quenching metal-inducible oxygen radical generation. Since aggregated $A\beta$ is known to be an oxygen radical generator, our results provide a novel concept that the aggregation-dependent biological effects of $A\beta$ are dualistic, being either an oxygen radical generator or its inhibitor.

P01-F-7

Isoform-Dependent Lipid Release from Astrocytes Prepared from Human Apolipoprotein E3- and E4-Knock-in Mice

○Jian-Sheng Gong¹, Mariko Kobayashi², Hideki Hayashi¹, Kun Zou¹, Naoya Sawamura¹, Shinobu C Fujita², Katsuhiko Yanagisawa¹, Makoto Michikawa¹

¹Dept. of Dementia Research, Natl. Inst. for Longevity sci., ²Mitsubishi Kagaku Institute for Life Sciences

We have previously reported that exogenous apolipoprotein E (apoE) promotes cholesterol release in an isoform-dependent manner (Michikawa et al., J. Neurochem, 2000). However, the molecular mechanism underlying this isoform-dependent promotion of cholesterol release still remains unknown. In this study, we demonstrate that the cholesterol release mediated by endogenously synthesized and secreted apoE is isoform-dependent and we also demonstrate the mechanism underlying the isoform-dependent cholesterol release using cultured astrocytes prepared from human apoE3 and E4 gene-knock-in mice. Cholesterol and phospholipids were spontaneously released into the culture media, resulting in the generation of two types of high-density lipoprotein (HDL)-like particles; one is associated with apoE and the other with apoJ. The amount of cholesterol released into the culture medium from the apoE3-expressing astrocytes was approximately 2.5-fold greater than that from apoE4-expressing astrocytes. In contrast, total amount of apoE3 released into the culture media was similar to that of apoE4. Most of apoE3 and apoE4 are associated with HDL-like particles. The molar ratios of cholesterol to apoE in the HDL fraction of the culture media of apoE3- and apoE4-expressing astrocytes were 250 ± 6.0 and 119 ± 5.1 , respectively. However, the sizes of HDL-like particles released from apoE3- and apoE4-expressing astrocytes were similar. These data indicate that apoE3 has an ability to generate lipid particles of similar size with less number of apoE3 molecules than apoE4, suggesting that apoE3-expressing astrocytes can supply more cholesterol to neurons than apoE4-expressing astrocytes. Since we have shown that a decreased cholesterol level induces tau phosphorylation in neurons (Fan et al., J. Neurochem., 2001), these findings may provide a basis for future studies that will delineate the relationship between cholesterol and the pathogenesis of Alzheimer's disease.

P01-F-8

small GTPase Rab1Aの過剰発現によるA β 産生の促進

○白石 博久¹, 川村 勇樹², 鈴木 亮¹, 蔡 曉蕊¹, 柳澤 勝彦¹, 駒野 宏人¹

¹長寿研・痴呆疾患, ²埼玉大・薬理

アルツハイマー病脳の老人斑を形成するアミロイド蛋白(A β)は、 β -セクレターゼおよび γ -セクレターゼと呼ばれる2種類のプロテアーゼによってアミロイド前駆体蛋白(APP)から切り出される。このうち γ -セクレターゼ活性は、家族性アルツハイマー病原因遺伝子翻訳産物プレセニリン(PS)を含む蛋白複合体が担っていることが示されているが、その実体および活性調節メカニズムについては未だ不明である。

我々は、 γ -セクレターゼ活性を調節しA β 産生を促進する因子を同定するために、独自のスクリーニング法を確立している。この方法により、小胞体からゴルジ体への蛋白輸送に関与するsmall GTPaseの一つ、Rab1Aを同定した。本研究において、Rab1AのA β 産生に与える影響について解析したので報告する。

マウス由来の線維芽細胞にRab1AとAPPを導入し強制発現させたところ、A β 40、A β 42の産生がそれぞれ3倍および2倍上昇した。このとき、細胞内APPの発現量には殆ど変化がなく、また、 α -セクレターゼおよび β -セクレターゼによって産生される分泌型APPの増加はいずれも2倍以下であった。これらの結果は、Rab1AによるA β 産生の上昇が、Rab1Aが単にAPPの細胞内輸送を促進したからではなく、 γ -セクレターゼによる切断を促進したために起きたことを示している。

γ -セクレターゼによる切断は、トランスゴルジ網以降の蛋白輸送経路で起きると考えられており、本研究から、小胞体・ゴルジ体蛋白輸送以外でのRab1Aの新たな機能が示唆される。おそらくRab1Aは、 γ -セクレターゼの活性調節を担っているか、もしくは、 γ -セクレターゼの基質であるAPPの β -セクレターゼ切断産物(C99)の細胞内輸送に機能しているのではないかと考えられる。

P01-F-9

Significance of glyceraldehyde-derived advanced glycation end-product (AGE-2) in Alzheimer's disease

○Masayoshi Takeuchi¹, Nobuyuki Sasaki², Takayuki Watai¹, Seiji Kikuchi³, Sho-ichi Yamagishi⁴, Zenji Makita⁴, Yukihiro Kameda¹

¹Dept. Biochemistry, Hokuriku Univ., ²Dept. Neuropsychiatry, Sapporo Med. Univ., ³Dept. Neurology, Hokkaido Univ. School Med., ⁴Dept. Medicine, Kurume Univ. School Med.

The recent identification of distinct AGEs that form in vivo together with the availability of antibodies directed against specific AGEs has helped to focus attention on the precise nature of glycosylated proteins in Alzheimer's disease (AD). Incubation of cortical neurons with six immunochemically distinct AGEs, designated AGEs-1 to -6, produced a dose-dependent increase in neuronal cell death. The structural epitope designated AGE-2 (glyceraldehyde-derived AGE structure) was found to have the greatest cytopathic effect and the neurotoxicity of AGE-2 was neutralized by the addition of an anti-AGE-2 specific antibody, but not by other types of anti-AGE antibodies. Distinct classes of AGE structures also have been established to circulate in the blood of individuals with diabetes mellitus and end-stage renal disease treated by hemodialysis (DM-HD). We fractionated serum from normal control and DM-HD patients by gel filtration and identified two fractions that contained AGE epitopes-1 to -6 and as well as the defined AGE structure carboxymethyllysine (CML). The addition of these two fractions led to the death of cultured neuronal cells and this cytotoxic effect was completely prevented by the addition of the anti-AGE-2 specific antibody. We also examined the immunohistochemical location of six distinct classes of AGEs in senile plaques, neurons and astrocytes from patients with AD. In AD brain, anti-AGE-2 specific antibody reacted with both senile plaques and granules in neurons and astrocytes. We propose that the structural epitope AGE-2 is an important toxic moiety for neuronal cells.

P01-F-10

Transfected mutant presenilin-1 affects intracellular trafficking in PC12D cell

○Fuyuki Kametani¹, Hideaki Kume¹, Hiroshi Mori²

¹Dept. Mol. Neurobiol., Tokyo Inst. Psychiatry, ²Dept. Neurosci., Osaka City Univ. Med. Sch.

Most familial early-onset Alzheimer's disease (FAD) is caused by mutations in the presenilin-1 (PS1) gene. Aβ₄₂ is derived from amyloid precursor protein (APP) and its increased concentration has been widely believed to be a pathological hallmark for abnormal PS function. Thus an interaction between PS1 and APP is a central theme to clarify the molecular mechanism of AD. To examine the effect of PS1 and PS1 mutations on APP metabolism, we made several PC12D cell lines to express human PS1 or mutant PS1 and analyzed concentrations of endogenous APP, transfected PS1, and their related proteins in membrane fractions. We found that cellular distributions of transfected human PS1 and APP C-terminal fragment (CTF) in membrane fractions changed to accumulate in heavy membrane fraction including traffic vesicle from trans-Golgi network to plasma membrane. Furthermore, we also found significant changes of some marker proteins related to the traffic vesicle from trans-Golgi network to plasma membrane and early endosome in these PC12D cells. These results suggest that transfected mutant PS1 affects the intracellular trafficking and disturbs APP CTF transport.

P01-F-11

Herp によるA β 産生増加機構の解析

○蔡曉蕊¹, 小亀浩市², 白石博久¹, 鈴木亮¹, 宮田敏行², 柳澤勝彦¹, 駒野宏人¹

¹国立長寿研・痴呆疾患研究部, ²国立循環器病セ・研

アミロイド蛋白 (A β) はアミロイド前駆体蛋白APP から β -セクレターゼと γ -セクレターゼと呼ばれるプロテアーゼによって産生される。 γ -セクレターゼ活性には、プレセニリン (PS) が必要であることが明らかとなっているが、その実体は依然不明である。昨年、本大会において、A β の産生を促進する蛋白として小胞体ストレスによって誘導されるHerpを同定したことを報告した (Sai X. et al. J.Biol.Chem. in press 2002)。また、Herpは、全長PSと結合することもすでに報告している。今回、HerpによるA β 産生増加の分子機構をさらに詳細に解析することを目的とした。APPおよびHerpを安定に過剰発現しているHEK 293細胞を樹立し、これを用いて、(1) Herp発現による γ -セクレターゼの基質となるC99の細胞内レベルの変化 (2) PS依存におこる γ -切断および ϵ -切断に及ぼすHerpの影響 (3) パルスチエイイス実験による、PSのendoproteolysisに対するHerpの影響を解析した。その結果、Herpの過剰発現により、細胞内C99のレベルが減少し、それに伴い γ -切断によって産生されるA β 40およびA β 42レベルの上昇、また、 ϵ -切断によって生じるAPP C末端断片 (ϵ -CTF) 量の上昇が認められた。特に、昨年、HerpはA β 40の産生を選択的に上昇することを報告したが、confluent細胞を用いるとA β 40およびA β 42ともにそのレベルがほぼ2倍上昇する結果が得られた。また、Herpの発現により、PSのendoproteolysisを促進する傾向が認められた。以上の結果から、Herpの過剰発現は、 γ -切断および ϵ -切断のどちらも上昇させることが明らかとなった。また、Herpにより、全長PSのendoproteolysisが促進されたことから、Herpは、活性型PSへの代謝を促進することにより、A β 産生を増加させている可能性が考えられた。

P01-F-12

Immunohistochemical study of ubiquitin and deposition of β -Amyloid protein in old dog brain

○Tatsuki Itoh, Takao Satou, Yoshiyuki Hayashi, Shigeo Hashimoto

Sec. Dep. Path., Kinki Univ. Sch.Med.

In recent years, there have been indications that the aging change induces deposition of β -Amyloid protein (AB) in the dog brain, and that a dementia symptom clinically. In last year study, we reported immunohistochemical study of AB deposited ratio, aging, memory and learning. In this study, we examined immunohistochemical study of ubiquitin, AB and, Memory and learning. Methods: Whole dog 13 brains were removed (11 years old-19 years old), and the resected brains were immersed in 4% paraformaldehyde. Cerebral frontal cortex were serially sliced at a thickness of 50 μ m with a micro slicer. Immunohistochemistry was performed using against ubiquitin and AB. Ubiquitin and AB positive area was measured by NIH 1.6 image software. Memory and learning was evaluated by Utino's score Results: Ubiquitin positive area and age, ubiquitin positive area and AB positive area, and ubiquitin positive area and score indicated significant negative correlation ($p < 0.05$). Discussion: Ubiquitin was related with changes of AB, memory and learning by aging.

P01-F-13

The preventive effect of propentofylline on the β -amyloid-induced apoptosis in rat hippocampal neurons

○Yoshiki Koriyama¹, Matsumi Yamazaki¹, Kenzo Chiba¹, Satoru Kato², Tetsuro Mohri¹

¹Dept. Biodynamics, Fac. Pharm. Sci., Hokuriku Univ,

²Dept. Mol. Neurobiol, Grad. Sch. Med., Kanazawa Univ

The amyloid protein (β 42) has been proposed to play an important role in the pathogenesis of Alzheimer's disease. We have previously reported the preventive effect of propentofylline (PPF) on the neurotoxicity of β 42 in primary cultured hippocampal neurons. As has been previously reported that necrosis and apoptosis was involved in the neurotoxicity by β 42, we were prompted to study the further mechanism of preventive effect of PPF on β 42-induced neurotoxicity in relation to apoptosis and Bcl-2 family proteins.

Primary hippocampal cells were obtained from 18 days fetal rat (Wistar) and grown with 10 % semi fetal calf serum in Dalbecco's modified Eagl medium (DMEM). Two days later, cell division was halted by addition of AraC for 24 hr. Neuronal cell layers were rinsed and cultured with serum-free DMEM to treat cells with or without β 42 and drugs. Cell viability were monitored by LDH release and MTT reduction assays. Nuclear damages were monitored by Hoechst33258 staining and ladder formation. Bcl-2 family proteins were detected by western blot analysis.

When the hippocampal cells were treated with 20 μ g/ml β 42 for 24~48 hr, typical nuclear condensation and DNA ladder were observed, and both nuclear damages were suppressed by PPF preincubated for 1 hr. β 42-induced neurotoxicity was prevented significantly in the presence of caspase inhibitor (10 μ M Ac-DEVD-CHO). Anti apoptotic protein Bcl-2 revealed a significant increase in the neuronal cells treated by 20 μ g/ml PPF for 6 hr, and revealed a significant decrease in the cells treated by 20 μ M β 42. These results strongly suggest that PPF induces upregulation of Bcl-2 protein and prevents apoptosis of hippocampal neurons induced by β 42.

P01-F-14

Therapeutic effects of Zokumei-to on spatial learning impairment and synaptic loss in a mouse model of Alzheimer's disease

○Takayuki Tamura, Chihiro Tohda, Katsuko Komatsu

Res. Center for Ethnomed., Inst. of Natural Med., Toyama Med. & Pharm. Univ.

Although Zokumei-to, a Kampo formula has been used for postapoplectic palsy, logopathy and dysesthesia, a basic research of this drug has hardly been done. We had an idea that Zokumei-to may affect the neuronal plasticity, and investigated whether this drug could improve learning impairment and synaptic loss observed in Alzheimer's disease. A β 25-35 (4.7 nmol) was i.c.v. injected into ddY mice (male, 6 weeks old). Fourteen days after the injection, mice were p.o. given Zokumei-to extract (500 mg/kg/day) for 15 days. In a memory acquisition test, the A β 25-35-injected mice required more time to master this task as compared with a saline- or A β 35-25 (reverse peptide)-treated groups. Zokumei-to-treated mice shortened escape latencies during trial days 3 to 5, but not significantly. Three days after the last drug treatment, the retention test was performed. Number of crossing over a platform position was significantly decreased in A β 25-35-injected mice compared with control groups. However, Zokumei-to-treated mice showed complete recover of that. Although A β 25-35-injection decreased the synaptophysin expression in the cerebral cortex and the hippocampus, Zokumei-to-treatment significantly increased the expression level and the distributed area of synaptophysin up to the control level. Donepezil hydrochloride (0.05 mg/kg/day, p.o.), clinically used for Alzheimer type dementia, shortened the escape latency, but didn't effect on the memory retention and the synaptophysin level. A β 25-35-induced neuronal loss was not observed in any region of the brain. These results suggest that the memory loss and axonal atrophy in Alzheimer's disease may be improved by treatment with Zokumei-to even after the impairment has already progressed.

P01-F-15

Exogenous HSP90-induced microglial activation and amyloid- β clearance

○Jun-ichi Kakimura¹, Yoshihisa Kitamura¹, Kazuyuki Takata¹, Shun Shimohama², Takashi Taniguchi¹

¹Dept. Neurobiol., Kyoto Pharm. Univ., ²Dept. Neurol. Grad. Sch. Med., Kyoto Univ.

Alzheimer's disease (AD) is characterized by accumulation of fibrillar amyloid- β peptides (A β) to form amyloid plaques. Understanding the balance of production and clearance of A β is the key of elucidating amyloid plaque homeostasis. Microglia in the brain, associated with senile plaques, are likely to play major role in maintaining this balance. Heat-shock proteins (HSPs) are induced by stressful conditions such as pathophysiological stimuli. One of them, HSP90 is well known as intracellular chaperones. But, its function in microglia is unknown. In the present study, we examined the effect of HSP90 on microglial function. In the microglial culture, the addition of HSP90 markedly induced the production of cytokines such as interleukin-6 or tumor necrosis factor- α in a concentration-dependent manner. In addition, HSP90 induced increase the phagocytosis and clearance of A β . The mechanism of microglial activation by exogenous HSP90 involves the NF- κ B and p38 MAP kinase pathways mediated by Toll-like receptor (TLR)-4 activation. In AD brains, levels of HSP90 were increased in both the cytosolic and membranous fractions, and HSP90 was co-localized with amyloid plaques. These results suggest that HSP90-induced microglial activation may serve a neuroprotective role by facilitating A β clearance and cytokine production.

P01-F-16 (MS4-2)

Role of oxidative protein in neuronal cell death of Parkinson's disease.

○Wakako Maruyama¹, Masayo Shamoto-Nagai¹, Masashi Tanaka², Makoto Naoi³

²Lab. Biochem. Metabol. Dept. Basic Gerontol. NILS¹, Gifu International Biotechnol., ³Dept. Brain Sci. Instit. Appl. Biochem.

In degenerating dopamine neurons of Parkinson's disease (PD), increased level of oxidized proteins was observed in the substantia nigra, and aggregation of modified proteins produce Lewy bodies, a pathological hallmark of PD. Recently alpha-synuclein, gene mutation of which causes autosomal dominant PD, was found to be a main component of Lewy bodies. Conformational change of mutated alpha-synuclein was proposed to result in aggregation of the protein, and finally cause neuronal cell death. In idiopathic PD without mutation of alpha-synuclein, the post-translational modification of alpha-synuclein, such as nitration of tyrosine and phosphorylation of serine residues, was detected in Lewy bodies, and increased 4-hydroxy-2-nonenal-modified proteins were detected in melanized dopamine neurons in PD. In this paper, we studied how oxidized proteins are aggregated and induce cell death in human dopaminergic neuroblastoma SH-SY5Y cells. The cells were cultured in the presence of a proteasome inhibitor, carbobenzoxy-L-isoleucyl-g-t-butyl-L-glutamyl-L-alanyl-L-leucinal (PSI) or a complex I inhibitor, rotenone, and the levels of HNE- and acrolein (ACR)-modified proteins were examined. In the cells treated with PSI, the level of HNE- or ACR-modified proteins increased markedly, and apoptotic cell death was induced in a dose-dependent manner. Most oxidized proteins were detected in soluble fraction, and protein aggregation was not observed. On the other hand, rotenone induced aggregation of oxidized proteins, and the most of them were detected in mitochondrial fraction. These results suggest that mitochondrial dysfunction may induce oxidized protein aggregation, and vice versa.

P01-F-17 (MS4-3)

Mechanism of neuroprotection by propargylamines

○Makoto Naoi¹, Wakako Maruyama², Yukihiro Akao³, Hong Yi³, Yoshihide Tsujimoto⁴

¹Dept. Brain Sci. Instit. Appl. Biochem., ²Lab. Biochem. Metabol. Dept. Basic Gerontol. NILS, ³Gifu International Biotechnol., ⁴Lab. Molecular Genetics and CREST of JST, Osaka Univ. Grad. Sch. Med.

Propargylamines, (-)-deprenyl, N-(2-heptyl)-N-methylpropargylamine, N-propargyl-1(R)-aminoinidan (rasagiline), have been proved to protect neurons by in vivo and in vitro experiments. However, the molecular mechanism of the neuroprotection remains to be elucidated. Propargylamines suppressed apoptotic cell death induced by peroxynitrite and an endogenous neurotoxin, N-methyl(R)salsolinol [NM(R)Sal], in human dopaminergic neuroblastoma, SH-SY5Y cells. Among propargylamines examined, rasagiline was found to be the most effective. Rasagiline prevented decline of mitochondrial membrane potential by NM(R)Sal and following apoptotic signal, release of cytochrome c, activation of caspase 3 and nuclear DNA fragmentation. Using mitochondria isolated from rat liver, NM(R)Sal was proved to induce permeability transition (PT) directly, which was antagonized by rasagiline. It was suggested that rasagiline interacted with the molecule regulating PT at mitochondria. In addition, rasagiline was found to increase the level of neuroprotective proteins such as BCL-2, glial cell line-derived neurotrophic factor (GDNF) and superoxide dismutase (SOD), and their mRNA. The increase was inhibited by sulfosaladine, an inhibitor of NFκB inhibitor. The activation of NFκB was confirmed by nuclear translocation of p65 and increase of binding of p65 to NFκB binding site. Rasagiline and other propargylamines may be neuroprotective through inhibition of PT and induction of neuroprotective proteins.

P01-F-18 (MS4-6)

ALS2 gene accounting for amyotrophic lateral sclerosis 2 encodes multi guanine exchanging factor (GEF) domains acting for small GTPases

○Joh-E Ikeda, Asako Otomo, Ryota Kunita, Eri Yoshida, Shinji Hadano, Hitoshi Osuga
SORST/JST, Department of Molecular Neuroscience, The Institute of Medical Sciences, Tokai University School of Medicine

Amyotrophic lateral sclerosis (ALS) is a heterogeneous group of inexorable neurodegenerative disorders characterized by a selective loss of upper and lower motor neurons in the brain and spinal cord. Although most cases reported are sporadic, 5-10% are familial. The autosomal dominant form, ALS1, has been identified with the association of missense mutations in SOD1 gene. Since then to this today, ALS genes other than ALS1 have not been identified. We recently identified a causative ALS2 gene accounts for a type 3 autosomal recessive amyotrophic lateral sclerosis (ALS2) encoding a protein (ALS2 protein, 184-kD) comprises multi guanine exchanging factor (GEF) domains, RCC1(Ran GEF), DH/PH(Rho GEF), and VPS9(Rabex5GEF), as well as the MORN motifs (Nature Genetics 29: 166-173, 2001). Two independent ALS2 families with the deletion mutations in the coding exons of the ALS2 gene were identified. These deletion mutations result in frameshifts generating premature stop codons, and the truncated ALS2 protein can be generated from mutated gene and does not contain any of the putative functional domains in the normal ALS2 protein causing a loss of function. We also isolated the mouse ortholog of ALS2, Als2, and the entire Als2 ORF is well conserved between human and mouse. The ALS2 and Als2 transcripts are expressed to a variable degree in neuronal cells throughout the brain and spinal cord. We will present the expression of Als2 protein during the neuronal development in the mouse and subcellular localization of the ALS2 protein. The characterization of the ALS2 GEF (GTPase regulator) will take new turn in quest of the molecular pathogenesis of ALS2 as well as sporadic ALS and also the molecular mechanism of neuronal cell survive and integrity.

P01-F-19 (MS4-5)**Functional analysis of the putative transcription factors for the human Huntington's disease gene**

○Kazunori Tanaka^{1,2}, Junko Shouguchi-Miyata², Natsuki Miyamoto¹, Joh-E Ikeda^{1,2}

¹SORST, Japan Science and Technology Corporation (JST), ²Department of Molecular Neuroscience, The Institute of Medical Sciences, Tokai University School of Medicine

Huntington's disease (HD) is a neurodegenerative disorder caused by an expansion of a CAG triplet repeat in exon 1 of the HD gene. Though HD gene is ubiquitously expressed, disease is characterized by a specific neuronal loss and dysfunction in the striatum and cortex. The relationship between HD gene expression and selective cell death remains elusive. On the other hand, the study of the conditional mouse model showed that abolishing expression of mutated huntingtin led the disappearance of aggregate formation, suggesting that HD is reversible. To aid in the delineation of the transcriptional regulation of the HD gene for the elucidation of selective cell death, we explored protein factors binding to the HD promoter region. Using a yeast one hybrid screening, we isolated two full-length clones (clone 2L and clone 8) which showed the binding activity to the HD promoter region containing 20bp direct repeats. They were found to be ubiquitously expressed in human tissues. Analysis of the deduced amino acid sequences of clones 2L and 8 revealed that putative proteins (HTF2L, HTF8) encoded similar domain structures: a single Zn finger domain, the C-terminal conserved region and potential nuclear export signal (NES). By gel-shift assay using GST-fusion protein, the C-terminal conserved regions of both proteins were necessary to mediate the DNA-protein interaction. Subcellular localization of GFP-HTF2L and -HTF 8 in HeLa cell was studied. Those fusion protein localized in nuclear of a few cells, mainly in cytoplasm. As a GFP fusion protein deleted N-terminal region of HTF 8 has localized in nuclei, it evidences that there is the functional NES in HTF8. The DNA binding sequence and the activity of HTF2L and HTF8 as transcription factors shall be discussed.

P01-F-20 (MS4-1)**尾懸垂試験を用いたMPTP誘発パーキンソン病モデルマウスの行動評価**

○森 厚詞^{1,2}, 中井 正三¹, 光本 泰秀¹, 森泉 哲次²

¹大塚製薬(株) 探索第二研究所, ²信州大・医・第二解剖

神経毒1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)は脳内でB型モノアミン酸化酵素(MAO-B)によってMPP⁺(1-methyl-4-phenylpyridinium)に変換され、ドパミントランスポーターを介してドパミン(DA)神経細胞内に特異的に取り込まれる。マウスにおいて、MPTPの全身投与により、中脳黒質-線条体系のDA神経細胞の変性脱落に伴った線条体DA含量の低下が引き起こされる。しかしながら、霊長類へのMPTP投与で見られるような振戦、固縮、無動、姿勢反射障害といったパーキンソン病(PD)様神経症状、痲呆症状やうつ症状等の露呈に関しては、一様な見解が未だ得られていない。今回我々はMPTP処置したPDモデルマウス(MPTPマウス)において行動異常が観察される試験系を見出し、さらにその機序について神経化学的及び薬理的に検討した。

C57BL/6雄性マウスにMPTP (20 mg/kg, i.p.)を2時間間隔で4回投与することによりPDモデルマウスを作製した。選択的MAO-B阻害剤であるDeprenyl (2.5 mg/kg, i.p.)の投与はMPTP初回投与18時間前に行った。自発運動量はtilting-cage法により測定した。尾懸垂試験は試験開始から6分間の無動時間を測定した。モノアミン含量はHPLC-ECD法で測定した。

MPTP処置3日後において、コントロール群に比べ、MPTPマウスの自発運動量に変化は見られなかったが、尾懸垂試験では有意な無動時間の延長が認められた。また、線条体におけるDA及びセロトニン含量の有意な低下も観察された。これらの行動学的、神経化学的变化は、Deprenylを前処置することにより完全に抑制された。

以上の結果から、尾懸垂試験においてMPTPマウスで観察された無動時間延長は、モノアミン含量低下によるものであり、同試験系がMPTPマウスの新規な行動評価系になり得る可能性が示唆された。ここに見出された行動異常がPD様神経症状を反映したものであるのか、もしくは情動の変化を示すものであるのかを現在解析中である。

P01-F-21

Effects of isatin, an endogenous MAO inhibitor, on striatal dopamine level in rats of Japanese encephalitis virus-induced Parkinson model.

○Masaru Minami¹, Naoya Hamaue¹, Mutsuko Terado¹, Toru Endo¹, Masahiko Hirafuji¹, Akihiko Ogata², Kunio Tashiro²

¹Dept. Pharmacol. Health Sci. Univ. Hokkaido, ²Dept. Neurol., Hokkaido Univ. Sch. Med.

We previously reported that striatal dopamine (DA) and acetylcholine levels significantly increased after the administration of isatin, an endogenous monoamine oxidase (MAO)-A and B inhibitor. Furthermore, urinary isatin concentrations of patients with Parkinson's disease tended to increase according to the severity of disease. We used a rat model for Parkinson's disease that was developed by infection with the Japanese encephalitis virus (JEV) in order to elucidate the efficacy of isatin for Parkinson's disease. In Fisher rats infected with JEV at 13 days after birth and sacrificed at 90 days, the major pathological changes resembled those found in Parkinson's disease. Tyrosine hydroxylase (TH)-positive neurons were significantly decreased in the substantia nigra of JEV-rats as compared with those of non-treated control rats. The motor activity measured by pole-test in JEV-rats for 90 days was decreased. The striatal DA levels determined by HPLC-ECD were significantly decreased in JEV-rats. The motor activity of JEV-rats receiving selegiline, a selective MAO-B inhibitor, was improved as compared with that of untreated JEV-rats. Selegiline prevented the decrease in striatal DA levels in JEV-rats. Isatin also demonstrated the similar effects on motor activity and striatal DA levels in JEV-rats. Isatin, but not selegiline, significantly decreased DOPAC/DA ratio of JEV-rats. Selegiline and isatin did not exert a significant influence to the TH-positive neurons in JEV-rats. These results suggest that exogenously administered isatin can improve motor activity of parkinsonism by increasing DA levels and decreasing DOPAC/DA ratio.

P01-F-22

A role of MxA protein, anti-viral GTPase, in Parkinson's disease

○Daisuke Ishibashi, Kenichi Mishima, Naoya Aoo, Hisako Furukawa, Mitsuo Takahashi, Michihiro Fujiwara

Dept. Clin. Pharmacol. Facult. Pharm. Sci., Fukuoka Univ

Epidemiological findings of Parkinson's disease (PD) have shown that pandemics of influenza A virus infection has an influence on the incidence of subsequent PD. MxA protein is one of the superfamily members of large GTPases. It is known to have anti-viral properties against influenza A virus. Auto-aggregated MxA protein is deposited in the cytoplasm of host cells during virus infection and Lewy bodies (LBs) of PD. It is possible that protein seed comprised of virus-induced MxA protein involved in the formation of cytoplasmic LBs inclusions, resulting in consequent dopaminergic neuronal loss in PD. In the present study, we detected that the effect of MxA protein on behavioral, histochemical and molecular biological changes using transgenic mice constitutively expressing MxA protein (MxA-Tg). MxA-Tg group showed significant impairments in the performance of a Pole-test and a Rota-rod test, indicating the disturbances of coordinated movements. Decreases in a Traction meter test showed abnormal muscle tone compared to those of Wild-type (WT) group, without any alteration in spontaneous motor activities between these two groups. These abnormal motor activities were suggestive of parkinsonism. By TUNEL method to detect makers of neuronal cell death, TUNEL positive cells were observed only in nigral cells of MxA-Tg mice. These results suggest that hyperexpression of MxA protein in the central nervous system produced coordinated motor abnormalities.

P01-F-23

Identification of genes that function in the ER stress using a randomized ribozyme library

○Reiko Onuki^{1,2}, Yoshio Bando^{3,4,5}, Eigo Suyama^{1,6}, Hiroaki Kawasaki^{1,6}, Taiichi Katayama^{3,5}, Masaya Tohyama^{3,5}, Kazunari Taira^{1,6}

¹AIST, ²Grad. Sch. Life and Env. sci., Tsukuba Univ., ³Dept. Anat. and Neurosci., Grad. Sch. Med., Osaka Univ., ⁴Dept. Anat., Asahikawa Med. college, ⁵CREST, JST, ⁶Dept. Chem. Biotech., Tokyo Univ.

We have shown that the ER stress-induced cell death is concerned with neurodegenerative disorder, such as ischemia and Alzheimer's disease. In this study, we tried to identify pro-apoptotic factors in ER stress-induced apoptosis by the gene discovery system using a hammerhead ribozyme. The ribozyme is a small RNA molecule that can catalyze cleavage of RNAs in a sequence-specific manner. Thus, they have been used successfully to knock down the intracellular expression of a variety of specific viral and cellular targets. In the gene discovery system, we constructed a ribozymes library that has randomized substrate-binding arms to cleave any mRNA. When the phenotype of cells changes upon introduction of a ribozyme library, genes that are responsible for the changes in phenotype can be identified by sequencing the active ribozyme that is expressed in the cell. The ribozyme library was transiently introduced into the SKNSH cell, and these cells were supplemented with tunicamycin as an inducer of ER stress. When the active ribozyme is able to cleave a pro-apoptotic gene in the cells, we expect that the cells must be avoided from cell death. Thus we isolated the ribozymes from these cells, which remained after selection, and based on the sequence of binding arms, we performed homology analysis. Several important genes were identified in this gene discovery selection. Taken together, our gene discovery system is the effective method for identification of key molecules in various neurodegenerative disasters.

P01-F-24

Immunocytochemical analyses for subcellular localization of EAOH gene *aprataxin*

Hidetoshi Date, Shuichi Igarashi, Yasuteru Sano, Tetsuya Takahashi, Osamu Onodera, ○Shoji Tsuji
Dept.Neurol. BRI Niigata University

Early-onset ataxia with ocular motor apraxia and hypoalbuminemia (EAOH) is an autosomal recessive neurodegenerative with clinical presentations partly similar to those of Friedreich's ataxia. By a positional cloning approach, we have recently identified the causative gene, *aprataxin*. *Aprataxin* has two major mRNA species coding for short and long forms as a result of alternative splicing involving exon 3. Both forms of *aprataxin* mRNAs are expressed at comparable levels in various human tissues. Homology search revealed that both forms of *aprataxin* have a highly conserved Histidine triad motif (HIT). The C-terminal domain contains a zinc-finger motif. The N-terminus portion of the long form of *aprataxin* mRNA shows a homology to the polynucleotide kinase-3'phosphatase (PNKP), and contains three nuclear localizing signals. To elucidate the physiological functions of *aprataxin*, we first investigated localization of the two forms of *aprataxin* by expressing myc-tagged *aprataxin* in COS-7 cells. Short-form *aprataxin* protein is expressed both in the nucleus and cytoplasm, while the expression of long-form proteins is localized in nucleus. Taken together with the homology of long form *aprataxin* with polynucleotide kinase-3'phosphatase (PNKP) these data suggest that *aprataxin* is involved in repair of single-stranded DNA break. Interestingly, ataxia telangiectasia, an autosomal recessive diseases caused by a defect in DNA double-strand break sensor, exhibits clinical presentations similar to EAOH, raising a possibility that cerebellar neurons are exquisitely sensitive to DNA damage.

P01-F-25

The analysis of aprataxin, the product of causative gene of early-onset ataxia with ocular motor apraxia and hypoalbuminemia

○Yasuteru Sano¹, Shuichi Igarashi¹, Osamu Onodera¹, Hidetoshi Date¹, Mutsuo Oyake¹, Nobuyoshi Hukuhara³, Takao Makifuchi³, Mitsunori Morimatsu⁴, Hitoshi Takahashi², Shoji Tsuji¹

¹Dept. Neuro. Brain, Niigata Univ., ²Dept. Patho. Brain, Niigata Univ., ³Dept. Neuro. Saigata Hp., ⁴Dept. Neuro, Yamaguchi Univ.

Early-onset ataxia with ocular motor apraxia and hypoalbuminemia (EAOH) is an autosomal recessive neurodegenerative disorder characterized by early age of onset, progressive ataxia, absence of tendon reflexes, distal loss of sense of position and vibration, pyramidal weakness of the legs and hypoalbuminemia. Recently, the causative gene of EAOH, APTX, has been identified. There are two mRNA species of APTX. We called the shorter splicing variant "short form" and the longer one "long form". In Northern blot analysis, the long form of APTX was more strongly expressed in various tissues including CNS than the short form. The long form of aprataxin is a protein consisting of 342 amino acids harboring 3 NLS with a calculated molecular weight of about 39100. To characterize the physiological functions of aprataxin, we developed polyclonal antibody raised against C-terminal fragment of aprataxin. On Western blotting of control human tissues this Ab strongly detects strongly a 39kDa protein that is reduced or absent in the case of EAOH patients. A 20kDa protein is also detected in control human tissues, but this protein band is much fainter than that of a 39kDa and also absent in those of EAOH patients. We estimate that the 39kDa protein represents the translational product derived from the long form APTX and the 20kDa protein corresponds to that of short form. By subcellular fractionation using differential centrifugation we found that aprataxin is mainly located in the nucleus. These data suggest that the 39kDa protein is the major product of APTX and localized in the nucleus, and furthermore, that defective physiological functions of this larger protein lead to the EAOH phenotype.

P01-F-26

The role of PKR in neurodegenerative diseases induced by ER-stresses.

○Yoshio Bando^{1,3}, Reiko Ohnuki^{4,6}, Taiichi Katayama^{2,3}, Shigetaka Yoshida¹, Kazanari Taira^{4,5}, Masaya Tohyama^{2,3}

¹Dept. Anatomy, Asahikawa Medical college, ²Dept Anat. and Neurosci., Osaka Univ. Grad. Sch. of Med., ³Core Research for Evolutional Science and Technology, ⁴Gene Discovery Research Center, AIST, ⁵Dept. of Chem. and Biotech., Tokyo Univ. Grad. Sch. of Engineering, ⁶Institute of Applied Biochemistry, Univ. of Tsukuba

We have reported that ER-stress triggered neuronal cell death in neurodegenerative diseases, such as CVD, AD, and PD. We attempt to gene discovery using ribozyme method based on gene knockdown and expression cloning systems for the isolation of effective genes (Ohnuki R. reports in this meeting). We determined the target genes from Rz sequences and analyzed the role of isolated genes in apoptosis. Further we have examined about one of key molecules, pro-apoptotic factor PKR. It is well known that over-express or activation of this gene leads to apoptosis. We found this gene was induced and activated by ER-stress, and Rz for this gene actually suppress its expression, and protected by ER-stress mediated apoptosis. Moreover, PKR was expressed in autopsy tissues of various neurodegenerative diseases, such as AD, PD, and HD. We conclude that ER-stress will be common pathway in various neurodegenerative diseases and PKR will be one of key molecule in these diseases.

P01-F-27

Molecular mechanism in neuroprotective properties of a dopamine agonist, cabergoline.

Michiyo Yoshioka, Ken-ichi Tanaka, Naoko Fujita, Ikuko Miyazaki, Masato Asanuma, Norio Ogawa
Dept. Brain Science, Okayama Univ. Grad. Sch. Med. & Dent.

Free radicals are involved in the pathogenesis and/or progression of Parkinson's disease (PD). Several ergot derivative dopamine (DA) agonists have been reported to scavenge free radicals in vitro and to show a neuroprotective effect in vivo. We investigated the in vitro free radical scavenging and antioxidant activities of cabergoline, a long-acting ergot DA agonist, as well as its ability to activate glutathione (GSH), catalase (Cat) and superoxide dismutase (SOD) activating effects and its in vivo neuroprotective properties against 6-hydroxydopamine (6-OHDA) intracerebroventricularly (i.c.v.) in mice. The striatal DA turnover induced by i.c.v. injection of 6-OHDA was completely normalized by pretreatment with cabergoline. Moreover, cabergoline scavenged free radicals in vitro and significantly reduced lipid peroxidation in vitro and in vivo. Furthermore, daily administration of cabergoline to mice significantly increased striatal GSH levels by activation of RNA expressions of GSH-related enzymes, although striatal catalase and SOD activities did not change. In addition, our present results suggest that repeated administration of cabergoline attenuates both 6-OHDA-induced striatal DAergic dysfunction and nigrostriatal DA neuronal cell death, since cabergoline also had a neuroprotective effect in the immunohistochemical experiment.

In conclusion, our findings indicate that the multiple antioxidant mechanisms of cabergoline, such as activation of the GSH system and the direct free radical scavenging activity, may explain the neuroprotective effect of this ergot DA agonist.

P01-F-28 (MS4-4)

Activation of CREB-dependent transcription system suppresses expanded polyglutamine induced cytotoxicity.

Mitsuteru Shimohata, Takayoshi Shimohata, Shuichi Igarashi, Satoshi Naruse, Shoji Tsuji
Dept. Neurol, Brain Res. Ins, Niigata Univ,

At least nine neurodegenerative diseases are known to be caused by expanded CAG repeats encoding polyglutamine (polyQ) stretches. The molecular mechanisms of neurodegeneration, however, remain unknown. We recently found that expanded polyQ stretches preferentially bind to TAF II 130 and that the interaction strongly suppresses CREB-dependent transcriptional activation. To further elucidate the involvement of CREB-mediated transcription in polyglutamine diseases, we investigated how expression of cyclic AMP-responsive gene products is affected in Neuro2a cells expressing the nuclear localization signal (NLS)-tagged truncated DRPLA protein (Q0, 19, 57) fused with GFP. On Western blot analysis, we found that expression of phosphorylated CREB and c-fos was substantially suppressed in a polyglutamine length-dependent manner after the treatment with 0.1mM of CPT-cAMP, and this length-dependent downregulation of CREB-dependent transcription was restored by the treatment with 2mM of CPT-cAMP. Furthermore nuclear fragmentation in Neuro2a cells and COS7 cells expressing the truncated DRPLA protein (Q57) was substantially suppressed in the presence of CPT-cAMP (2mM) or 100 μ M of Forskolin in the culture medium, and co-expression of dominant-negative CREB abrogated the effects of these compounds, indicating that phosphorylation of CREB is involved in the cytotoxicity induced by expanded polyQ stretches. These findings suggest that the interference of CREB-dependent transcription by expanded polyQ stretches is involved in the pathogenesis, and that phosphorylation of CREB or supplementation of agents which enhance CREB-dependent transcriptional activation are targets of treatment for these disorders.

P01-F-29

ATF3 protects neurons from JNK induced apoptosis by upregulating Hsp27.

○Saya Nakagomi¹, Yasuhiro Suzuki^{1,2}, Hiroshi Kiyama¹

¹Dept. Anat. Grad. Sch. Med. Sci., Osaka City Univ.,

²Dept Internal Med1. Med., Asahikawa Medical Coll.

Activating transcription factor 3 (ATF3) is an immediate-early gene, which is rapidly induced during stress responses such as UV irradiation and nerve injury. However, neither the physiological function nor the mechanism of activation has been unknown. We demonstrate here that ATF3 protected PC12 cells from JNK induced apoptosis. Moreover, ATF3 also protected SCG neurons against cell death after NGF deprivation. By using microarray technology, we found that Heat shock protein 27 (Hsp27), known as a molecular chaperone and antiapoptotic proteins, was increased in ATF3 overexpressing cells. This upregulation was seen only when c-Jun NH-terminal Kinase (JNK) was activated. BIM and Fas ligand, the major proapoptotic genes upregulated by c-Jun/JNK activation, was not changed during inhibition of JNK induced apoptosis by ATF3. ATF3 may modulate apoptosis by regulating stress-induced factors, such as heat shock proteins and affect the fate of neurons.

P01-F-30 (MS2-1)

Transcription factors induced in cultured cortical neurons by beta amyloid peptide

○Yoko Uchida, Fujiya Gomi

Dept. Neuropathol. TMIG

Beta amyloid (A beta) may play a important role in pathogenesis of AD, because A beta has toxic effect to cultured neuronal cells. However, about half of non-demented aged individuals have A beta plaques in neocortex but do not show neuronal loss in neocortex. These controversial findings in vitro and in vivo suggests that A beta induces molecules not only activating cell death pathway but also protecting neurons from A beta toxicity in neocortex. To understand the molecular mechanisms of A beta actions, we analyzed gene expression in rat cerebral cortical neurons treated with A beta 1-42 peptide by using cDNA macroarray and RTQ-PCR analysis. Out of 1176 cDNAs, 2 genes, PAX and MASH1 showed a reproducible induction in their expression by the addition of A beta. MASH1 is a basic helix-loop-helix transcription factor and known to function in determination and differentiation of neurons. Then we examined the effects of MASH1 over-expression in cultured cortical neurons, on neurite outgrowth and on neuronal death by serum withdrawal. Neurons expressing MASH1-hrGFP had longer neurites than those expressing hrGFP. Moreover, neurons expressing MASH1-hrGFP were less sensitive to serum withdrawal than those expressing hrGFP. These results indicate that induction of embryonic gene MASH1 in neurons by A beta may be protective response in brain. The updated information of the expressions of other transcription factors in A beta treated neurons will also be discussed.

P01-F-31

Temporary damage by trimethyltin in dentate granule neurons of mouse

○Hiroaki Okuda¹, Norito Nishiyama¹, Yasuhiro Yamamoto¹, Yukio Yoneda², Kiyokazu Ogita¹

¹Dept. Pharmacol. Setsunan Univ. Fac. Pharm. Sci.,

²Dept. Mol. Pharmacol. Kanazawa Univ. Fac. Pharm. Sci.

We examined the effect of trimethyltin (TMT), a well-known neurotoxicant, on hippocampal neurons of mouse. An intraperitoneal injection of TMT into adult ddY mouse led to neuronal loss in dentate granule neurons, in a dose-dependent manner. However, CA1-CA3 pyramidal neurons of the hippocampus were not damaged by the injection of TMT. Cresyl violet staining revealed that neuronal loss was seen in the dentate granule neurons 1 day after injection of TMT at a dose of 3 mg/kg. At 2 days later, surviving granule neurons in the dentate were less than 30% of those in the control animals. At 7 days after TMT injection, interestingly, surviving dentate granule neurons were increased to about 60% of those in the control animals, with returning to the control levels 14 days afterward. Immunohistochemical analysis using an anti-single-stranded DNA (ssDNA) antibody revealed that DNA fragmentation was seen in the dentate granule neurons, but not in the pyramidal neurons, 1 to 7 days after TMT injection, with the highest level at 2 days later. However, there was no positive cell for anti-ssDNA antibody 14 days afterward. These results suggest that a systemic administration of TMT into mouse selectively damages granule neurons in the hippocampal dentate gyrus, where has the ability of repairing TMT-induced neuronal damage.

P01-F-32 (MS2-2)

Activity-dependent expression of *alivin 1* and its involvement in the survival of cerebellar granule neurons

○Tomio Ono^{1,2}, Naoko Suzuki¹, Yoshiaki Kikkawa², Hiromichi Yonekawa², Seiichi Kawashima¹

¹Dept. Mol. Biol., Tokyo Metro. Inst. Med. Sci., ²Dept. Lab. Animal Sci., Tokyo Metro. Inst. Med. Sci.

Neurons survive in an activity-dependent manner. Their survival requires Ca²⁺-dependent gene transcriptions. To identify genes that are required for the survival of neurons, we have screened genes that were expressed in an activity-dependent manner in rat cerebellar granule neurons, and identified and cloned rat *alivin 1* (*ali 1*). This gene encodes a novel transmembrane protein that has seven leucine-rich repeats, one immunoglobulin-loop, and a single transmembrane domain. This protein shows similarity to the proteins encoded by *Drosophila kek* family and also mammalian *trk* family. In cultured rat cerebellar granule neurons, expression of *ali 1* mRNA was confined to the cells that were cultured in the medium containing either 25 mM KCl or 150 μ M N-methyl-D-aspartic acid, both of which promote the survival of the granule neuron. On the other hand the expression of *ali 1* mRNA was hardly detected when the cells were cultured in the medium containing 5 mM KCl. The expression of *ali 1* mRNA was regulated at the transcriptional step by the Ca²⁺ influx through voltage-dependent L-type Ca²⁺ channels when the cells were stimulated by 25 mM KCl. Addition of the specific antiserum against rat Ali 1 to the culture medium resulted in the decreased survival rate of cerebellar granule neurons, suggesting that Ali 1 is involved in the survival of the granule neuron. We have also cloned *ali 1* cDNA from mouse and human brains. Mouse *ali 1* was mapped to chromosome 15 by the radiation hybrid method. This region is syntenic with a region on human chromosome 12, which is a positional candidate locus for familial Alzheimer's disease type 5.

P01-F-33

Essential role of Rho/Rho-kinase signaling pathway in target-independent survival of developing motor neurons

○Kenta Kobayashi¹, Masanori Takahashi², Kazuaki Takahashi¹, Hiroyuki Yaginuma³, Noriko Osumi², Kozo Kaibuchi⁴, Kazuto Kobayashi¹
Dept. Mol Genet. Inst. Biomed. Sci., Fukushima
¹Medical Univ., ²Dept. Dev Neuro. Grad. Sch. Med.,
Tohoku Univ., ³Dept. Anat., Fukushima Medical Univ.,
⁴Dept. Cell Pharmacol. Grad. Sch. Med., Nagoya Univ

About 50% of spinal cord motor neurons (MNs) undergo programmed cell death during embryonic development. A general mechanism that controls MN death has been considered to depend on the target-derived trophic factors derived from target tissues. The Rho GTPase participates in signaling pathways that regulate actin cytoskeletons including stress fibers and focal adhesions. Rho is also involved in the regulation of neurite retraction presumed to be driven by a contraction of the actomyosin system. Rho-kinase is one of the major effector molecules in the Rho signaling pathway. Here we report an important role of Rho/Rho-kinase signaling pathway in MN development. Transgenic (Tg) mice expressing dominant negative form of RhoA in specific neuronal types showed an evident reduction in MN number in the spinal cord at early embryonic stages because of enhanced apoptosis. Tg mice expressing dominant negative form of Rho-kinase exhibited similar developmental defects. The enhanced apoptosis occurred in the developing MNs prior to the target innervation. These results suggest that Rho/Rho-kinase signaling pathway plays a pivotal role in target-independent survival of MNs during development.

P01-F-34

Dlxin-1, a necdin/MAGE homologous protein, enhances E2F1-induced neuronal apoptosis

○Takaaki Kuwajima, Hideo Taniura, Kazuaki Yoshikawa
Div. Regu.Macro.Mol.Func.Ins.Protein Res.,Osaka Univ.

Necdin is a growth suppressor expressed predominantly in postmitotic cells. Necdin has been implicated in the pathogenesis of Prader-Willi syndrome, a genomic imprinting-related neurobehavioral syndrome. Necdin binds to the transcription factor E2F1, and ectopic expression of this protein induces cell growth arrest. In search of necdin homologous proteins, we screened mouse brain cDNA library using cDNA encoding a necdin/MAGE homologous region. One of the positive clones encodes Dlxin-1, a protein that interacts with the Dlx family homeodomain protein Dlx5. Recently, NRAGE, a rat Dlxin-1 homolog, has been identified as a protein that mediates p75 neurotrophin receptor-dependent apoptotic signaling. We raised a rabbit polyclonal antibody against Dlxin-1 and examined the expression of Dlxin-1 in the brain. Western blot analysis revealed that the expression level of Dlxin-1 was high in the brain of mouse embryo but was reduced in mature brain. Immunohistochemistry showed that Dlxin-1 was enriched in the hypothalamus, brain stem and spinal cord and also observed in peripheral neurons, including the trigeminal and dorsal root sensory ganglia. Dlxin-1 was distributed predominantly in neuronal cytoplasm. Forced expression of Dlxin-1 induced growth suppression as assessed by bromodeoxyuridine uptake assay and colony formation analysis. A carboxyl terminal region of Dlxin-1, which encompasses the necdin/MAGE homology domain, bound to E2F1. In differentiated N1E115 neuroblastoma cells, ectopic expression of E2F1 induced apoptosis. Coexpression of Dlxin-1 accelerated E2F1-induced apoptosis, whereas coexpression of necdin suppressed apoptosis. These results suggest that Dlxin-1 suppresses cell growth and enhances apoptosis through interaction with E2F1 in differentiated neurons.

P01-F-35

Nitric oxide(NO)-induced apoptosis is mediated by tyrosine kinase activation in PC12 cells

○Toshifumi Yamamoto¹, Kohei Yuyama¹, Hideko Yamamoto²

¹Lab. Mol. Recognition. Grad. Sch. Integrated Sci., Yokohama City Univ., ²Dept. Mol. Psychiatry, Tokyo Inst. Psychiatry

We previously reported that low concentrations of nitric oxide (NO) induce an apoptosis through caspase-independent mechanisms. We also demonstrated that the antioxidant ascorbate blocked NO-induced apoptosis suggesting that an increase in the production of reactive oxygen species (ROS) contributes the cell death. In the present study, we investigated the role of production of ROS and activation of protein kinases in this apoptotic process. An NO-donor, NOR3 increased the formation of ROS from 1 h after treatment in PC12 cells using the fluorescent dye 2',7'-dichlorofluorescein diacetate (DCFH-DA). Furthermore, NOR3-induced cell death was completely attenuated by pretreatment with genistein, an inhibitor of protein tyrosine kinase, in dose-dependent manner, whereas inhibitors of protein kinase A, protein kinase G and CaM kinase unaffected NOR3-induced cell death in PC12 cells. Calphostin C, an inhibitor of protein kinase C, also partially inhibited cell death induced by NOR3. These findings suggest that the cell death induced at low concentrations of NO is mediated by the production of ROS and resulting most likely in the activation of protein tyrosine kinase and/or protein kinase C activities as an initiator of caspase-independent apoptosis

P01-F-36

The involvement of conversion of the p35 Cdk5 activator to p25 in neuronal cell death

○Taro Saito, Yuichi Fujita, Akiko Asada, Shin-ichi Hisanaga

Dept. Biol. Sci. Grad. Sch. Sci., Tokyo Metropolitan Univ.

Cyclin-dependent kinase 5 (Cdk5), a cdc2-related kinase expressed in post-mitotic neurons, is activated by association with a brain specific activator p35. Cdk5 is also activated by p25, an N-terminal truncated form of p35. Cleavage of p35 to p25 is suggested to change the subcellular distribution of active Cdk5 from the particulate to the cytosolic fraction, thereby allowing aberrant phosphorylation of neuronal proteins. Conversion of p35 to p25 was shown to be catalyzed by calpain, a protease which is involved in various types of cell death. Accumulation of p25 has been observed in cultured neurons undergoing cell death and in the brains of Alzheimer's patient and brains of ALS model mice. However, it is still unclear whether the conversion to p25 is essential for neuronal cell death. Here we examined the involvement of the p35 cleavage to p25 in neuronal cell death using a mutant p35, which was not cleaved to p25. A mutant p35 whose amino acids including the cleavage site were deleted, exhibited the resistance to calpain. We generated an adenovirus expression vector containing mutant p35 cDNA. We expressed the uncleavable p35 in primary cultured cortical neurons by infecting the adenovirus vector, and analyzed whether the p35 cleavage is necessary for neuronal cell death.

P01-F-37

Mechanism of the inhibition of oligodendrocyte apoptosis by NF- κ B p50 subunit.

○Makoto Hamanoue, Ken Takamatsu
Dept. Physiol. Sch. Med., Toho Univ.

p50 subunit is one of the NF- κ B family known to be expressed in ubiquitous cells and to inhibit apoptosis of sensory neurons, hippocampal neurons and striatal neurons in the central nervous system. Although activation of p50 in the oligodendrocyte are detected by several stimuli, the p50 function remains unknown. In last meeting, we have demonstrated that p50 prevent, does not induce, oligodendrocytes apoptosis effectively. Although p65 subunit of NF- κ B are supposed to prevent apoptosis by modifying caspase activity in neurons, the precise mechanism of p50 remains unknown.

In the present study, to elucidate the mechanism of p50, we established oligodendrocyte progenitors cell lines, CG4 cells, which carry activated exogenous human p50 subunit in the nucleus. CG4 cells carrying p50 were lysed and analysed by using specific antibody against c-IAP-2, which is able to inhibit caspase activity. Expression level of c-IAP-2 was not increased by p50-overexpression compared to that of empty vector, suggesting that c-IAP-2 and it's target, caspase, could not be related to apoptosis of oligodendrocytes induced by TNF- α . Indeed, we ascertained that pan-caspase inhibitor, v-VAD, failed to block CG4 cells apoptosis, indicating that p50 subunit of NF- κ B could function in preventing apoptosis of oligodendrocytes through the mechanism besides c-IAP-2 and caspase pathway.

P01-F-38

Effect of caspase-3 on the primary cultured mice cortical neurons with HSV-1 infection

○Jiawei WANG, Dexin WANG, Zijing FENG et al.
Department of Neurology, Beijing Friendship Hospital, Beijing 100050

[Abstract] Object To study the effect of caspase-3 on the primary cultured mice cortical neuron with HSV-1 infection. **Methods** By Using spectrophotometer to detect the absorbance units at 405 nm of the substrate of caspase-3, the chromophore p-nitroanilide (pNA). Comparing with the normal group, virus group, sorbital group, and sorbital group with virus in the absorbance units of chromophore substrate. Analyze the changes in inner mitochondrial membrane potential of the different group cells with flow cytometry. **Results** The absorbance units in the pNA of normal samples were higher than negative control, the sorbital group were higher than normal group, the virus group were much lower than normal group, and the sorbital with virus were lower than of the sorbital group. The mock-infected cells exposed to sorbitol or ceramide-2 showed a decreased mitochondrial membrane potential compared to untreated or HSV-1-infected cells. **Conclusion** We can find the apoptosis at the normal fetal mice cortical neurons primary cultured in vitro, HSV-1 infection prevents the depolarization of the inner mitochondrial membrane induced by sorbital, the pathways of programmed cell induced by sorbitol is caspase-3-dependent, HSV-1 may have the functions that block caspase-3.

P01-F-39 (MS4-8)

Activation of Forkhead transcription factor following transient forebrain ischemia in gerbil hippocampus

○Kohji Fukunaga¹, Takayuki Kawano^{1,2}, Yukitaka Ushio², Eishichi Miyamoto¹

¹Dept. Pharmacol. Kumamoto Univ. Sch. Med, ²Dept. Neurosurgery Kumamoto Univ. Sch. Med

In transient brain ischemia, an inactivation of protein kinase B (Akt) is involved in delayed neuronal death in gerbil hippocampus. We recently reported that a protein tyrosine phosphatase inhibitor, sodium orthovanadate rescues cells from delayed neuronal death following transient forebrain ischemia through phosphatidylinositol 3-kinase-protein kinase B (Akt) pathway (Kawano et al., JCBFM, 21: 1268, 2001). Here we demonstrated that activation of FKHR, which is a member of Forkhead transcription factors and a substrate for Akt, preceded delayed neuronal death in CA1 regions after transient forebrain ischemia. Adult Mongolian gerbils were subjected to 5-min forebrain ischemia. Immunoblotting analysis with anti-phospho-FKHR antibody showed that phosphorylation of FKHR at serine-256 in the CA1 region decreased immediately, 0.5 and 1 h after reperfusion. The dephosphorylation of FKHR was correlated with the decreased Akt activity. Intracerebroventricular injection of orthovanadate inhibited dephosphorylation of both Akt and FKHR after reperfusion, and blocked delayed neuronal death in the CA1 region. Gel mobility shift analysis using nuclear extracts from the CA1 region prepared immediately after reperfusion demonstrated increases in DNA binding activity for FKHR related element on Fas ligand promoter. The orthovanadate injection before ischemia inhibited its binding activity. Two days after reperfusion, expression of Fas ligand increased in the CA1 region and the orthovanadate injection inhibited its increased expression. These results suggest that the inactivation of Akt results in activation of FKHR and thereby induces expression of Fas ligand related to cell death in the CA1 region after transient forebrain ischemia.

P01-F-40

Leukotriene D₄ activates microglia through the CysLT₁ receptor

○Tetsuya Hirabayashi^{1,2}, Takao Shimizu¹

¹Dept. Biochem. Mol. Biol., Grad. Sch. Med., Univ. Tokyo, ²Lab. Chem. Pharmacol., Grad. Sch. Pharm. Sci., Chiba Univ

The cysteinyl leukotrienes (cysLTs) LTC₄, LTD₄, and LTE₄ are a class of peptide-conjugated lipids formed from arachidonic acid. They are produced in brain during ischemia, seizures and HIV-associated dementia. While they have been proven to be potent inflammatory lipid mediators in bronchial asthma, their pathological role in the central nervous system is still unclear. Northern analysis and *in situ* hybridization demonstrated that expression of CysLT₁-receptor mRNA in mouse brain was localized primarily to microglia. In culture, LTD₄ stimulated migration and elevated intracellular calcium levels of primary microglia as well as microglial cell lines. These responses were blocked by the competitive CysLT₁ receptor antagonists, MK-571 and ONO-1078. The effects of LTD₄ on cytokine secretion, nitric oxide synthesis, superoxide production, glutamate release, and matrix metalloproteinase production were also examined in microglia. Our findings suggest that LTD₄ released from damaged tissue attracts and activates microglia through the CysLT₁ receptor and contributes to the inflammatory glial response in pathological states.

P01-F-41

Inhibitory Effect of 17 β -estradiol Administration on Some Factors Implicating in Progression of Neuronal Apoptosis in Gerbil Hippocampus Induce by Transient Ischemia/Recirculation Insult

○Kimikazu Fujita¹, Toshiaki Kato¹, Masamitsu Yamauchi², Nobuko Yoshimoto³, Kenzo Shibayama¹, Yutaka Nagata¹, Eiichi Miyachi¹

¹Dept.Physiol.Sch.Med.,Fujita Hlth,Univ., ²Aichi Gakusen College, ³Nagoya Bunri College

We have already reported that enzymic activity and induced protein of tissue transglutaminase(tTGase), a marker of tissue degeneration process,had been continuously increasing in the hippocampus area after application of transient ischemia/recirculation insult to mongolian gerbil. Daily injection(i.p.) of 17 β -estradiol(17 β -ED; 10 μ g/ml) to the treated animal reduced the enhanced tTGase activity. Then we investigate throughly the mechanism of neuronal degeneration process, and examined alterations of some factors implicating in the process such as Bcl-2, an apoptosis suppressor gene, caspase-3, an enzyme to proceed tissue degeneration, and glial glutamate transporter(GLT-1,GLAST) in the hippocampus. Then we measured the effect of 17 β -ED administration to the animal after the transient ischemia/recirculation insult. After the insult, as the apoptotic changes proceed, immunochemically identified Bcl-2 gene expression tended to decrease, caspase-3 activity to increase and GLT-1 to become dysfunction. Through daily i.p injection of 17 β -ED to the operated animal showed some protecting effects for the progression of apoptotic changes by increasing Bcl-2 expression, suppressing caspase-3 activity, and recover the GLT-1 function in the damaged gerbil hippocampus tissues, produced by transient ischemia/recirculation insult.

P01-F-42

Subcellular localization of Sir2 NAD-dependent histone deacetylase homologues from brain

○Yoshiyuki Horio, Jun Sakamoto

Dept. Pharmacol., Sapporo Med. Sch.

The yeast silent information regulator 2 (Sir2) is an NAD-dependent histone deacetylase. It silences transcription at the mating type loci, telomeres and the ribosomal DNA. Over-expression of Sir2 extends the life span of yeast and *C. elegans*, while Sir2 knockout in yeast shortened life span to about 50%. Mammalian cells also express several Sir2 homologues. Recently, mammalian Sir2 α (SIRT1) has been shown to deacetylate not only histone H3 and H4 but also P53 in an NAD-dependent manner, suggesting that Sir2a has some functional roles in P53-mediated apoptosis. However, most of physiological functions of Sir2 homologues seem to be unknown. Neurons have an extremely long life span. To examine the functions of Sir2 homologues in the brain, we isolated Sir2 α (SIRT1) and Sir3(SIRT3/Sir2L3) cDNAs from mouse brain library. The deduced amino acid sequences of these clones showed that SIR2 α have two motifs resembling a nuclear localization signal (NLS) in N-terminal region (aa31-38, NLS1) and in the middle (aa223-230, NLS2) of the protein, while SIR3 has one NLS-like motif in C-terminal region (aa284-290). To assess whether these putative NLSs act as true nuclear import signals, we constructed various GFP-fused deletion mutants. We found both of NLSs in SIR2 α were functional, while that of SIR3 was not. Thus, target proteins of SIR2 α and SIR3 may be different.

P01-F-43

A novel gene containing ankyrin-repeat expressed in senescence accelerated mouse (SAMP8) hippocampus and cerebrum

○Naoshi Yamamura^{1,2}, Nobuya Koike¹, Yasuyuki Nomura³, Yasunobu Okuma³, Takashi Uehara³, Yoshiyuki Sakaki¹, Tomoko Tashiro², Chie Furihata^{1,2}
¹Human Genome Cent., Insit. Med. Sci., Univ. Tokyo, ²Sch. Sci. Eng., Aoyama Gakuin Univ., ³Dept. Pharmacol. Grad. Sch. Pharm. Sci., Hokkaido Univ.

Senescence-accelerated mouse prone 8 (SAMP8) is a disease mouse model of learning impairment. These mice grow normally to adults but then suffer accelerated aging. SAMP8 is a suitable animal model to examine the decline in learning ability with senescence. Gene expression changes in SAMP8 brain were studied by fluorescent differential display analysis. Total RNAs were extracted from hippocampus and cerebrum of groups of 5 male SAMP8 and SAMR1 (normal control), 8 months of age. The first cDNA strands were prepared with reverse transcriptase with GT15(A, C G). The second cDNA strands were prepared with Taq DNA polymerase with GT15(A, C G) and arbitrary 10mer 5' primers. PCR products were separated by 5%PAGE. A cDNA fragment of 165 bp encoding a part of EST was cloned. A 5-fold increase in expression was observed in hippocampus and cerebrum in SAMP8 determined by PCR and Northern blot. EST was highly expressed in hippocampus and cerebrum but not in other organs in SAMP8. An mRNA sequence of 2.5 kb that contains ORF (684 bp) could be linked to chromosome 15 by using EST clusters in NCBI database. Expression of 2.5 kb cDNA was confirmed in hippocampus and cerebrum by PCR, and sequenced. This is a novel gene. The ORF started from Met and contained ankyrin-repeat (294 bp). An mRNA sequence of 2.6 kb of a human homologue (also a novel gene) could be identified in chromosome 8 in NCBI database. The gene was sequenced and the expression confirmed by PCR. It contains ORF with 684 bp (87% homology) and 228 amino acids (98% homology). In conclusion we found a novel gene containing ankyrin-repeat expressed in SAMP8 hippocampus and cerebrum, and identified a human homologue. The gene may be related to brain aging.

P01-F-44

Effect of Japanese herbal medicine (Toki-Shakuyaku-San) on DNA damage in aged rodent brain and C6 glia cell death

○Midori Hiramatsu

Tohoku University of Community Service and Science

Japanese herbal medicine, Toki-Shakuyaku-San (Tsumura & Co., Tokyo) has free radical scavenging activities against superoxide, hydroxyl and 1,1-diphenyl-2-picryl-hydrazyl radicals. It has been shown to decrease elevated lipid peroxide level in aged rat brain. It has an enhancing activity on cholinergic neuronal functions in the striatum of aged rat. DNA damage has been evaluated by the assay of 8-hydroxy-2'-deoxyguanosine formation, and the damage in rodent brain was found to increase with age. Cell death is related with reactive oxygen free radicals. In the present study Toki-Shakuyaku-San was examined to affect DNA damage in brain with age and C6 glia cell death. 8-Hydroxy-2'-deoxyguanosine was assayed using high performance liquid chromatography and cell death was evaluated by induction with glutamate using lactate dehydrogenase assay. Senile accelerated mice (SAMP8) of 3-4 and 7-12 months old were used for experiment. The following results were obtained; (1) DNA damages in brain and liver of SAMP8 at 3-4 months old were accelerated compared to those of control SAMR1 mice, and Toki-Shakuyaku-San decreased the DNA damage in brain of SAMP8. (2) DNA damage was elevated in aged rat brain of 24 months old, and Toki-Shakuyaku-San lowered it. (3) Toki-Shakuyaku-San inhibited glutamate-induced C6 glia cell death. These results suggest that inhibitory effects by Toki-Shakuyaku-San may be due to its free radical scavenging activities. In conclusion, Toki-Shakuyaku-San may protect or may delay to have a lapse of memory and dementia.

P01-F-45

Reduction of histamine-induced phosphoinositide hydrolysis in mouse frontal cortical slices after repeated methamphetamine treatment

○Junichi Kitanaka, Nobue Kitanaka, Motohiko Takemura
Dept. Pharmacol., Hyogo Col. Med.

Chronic as well as acute administration of psychostimulants, such as cocaine and amphetamines, results in enhanced behavioral responses termed sensitization. This phenomenon is thought to be relevant to the development of drug addiction. Therefore, it is important to elucidate the exact molecular and cellular mechanisms underlying sensitization induced by psychostimulants for a better understanding drug addiction. The purpose of this study was to investigate whether repeated treatment of methamphetamine alters neurotransmitter-induced signal transduction of frontal cortex in mice. In this presentation, the effect of repeated administration of methamphetamine on locomotion (determined by Animex and Supermex systems, Muromachi Kikai Co., Ltd.) and change of brain signal transduction (phosphoinositide hydrolysis assay) was investigated. Male ICR mice (30-35 g on day 1) received the methamphetamine injection (1.0 mg/kg, i.p.) once a day for 5 consecutive days showed behavioral sensitization when they challenged methamphetamine on day 10. On day 1, the locomotor activity increased with regard to time up to 30 min, and on day 3 the maximal locomotor activity was attained immediately after the methamphetamine injection. The activity of phosphoinositide hydrolysis induced by histamine but not glutamate was reduced on day 10 in frontal cortical slices of mice exposed to repeated methamphetamine compared with the results from control group (repeated saline injection). The reduction recovered 25 days after the last injection of methamphetamine. We will discuss the possible developmental mechanism of methamphetamine-induced behavioral sensitization in mice.

P01-F-46

BDNF inhibits apoptosis induced by methamphetamine in primary cortical neurons through Activation of Akt kinase

○Hideo Matsuzaki¹, Kazuhiko Namikawa², Hiroshi Kiyama², Norio Mori³, Kohji Sato¹
¹Dept. Anat. and Neurosci., Hamamatsu Univ. Sch. Med., ²Dept. Anat., Grad. Sch. Med., Osaka City Univ., ³Dept. Psychiatr. and Neurol., Hamamatsu Univ. Sch. Med.

Recent study showed methamphetamine (MAP) induced neuronal apoptosis through the increase of cellular Ca^{2+} influx at acute phase. On the other hand, Ca^{2+} influx upregulates BDNF transcription activity in cortical neurons, and growth factors can promote neuronal cell survival by acting on phosphatidylinositol 3-kinase (PI3kinase) and its downstream target, serine-threonine kinase Akt. Here, we examined whether BDNF prevents neuronal cell death induced by MAP. Cultures of rat primary cortical neurons were prepared from embryonic day 18 Harlan Sprague Dawley rat embryos. At 24 h after the replacement of medium, cells were treated for 6 h with BDNF or vehicle (saline) and exposed to MAP. Neuronal cell viability was assessed by the release of LDH into the culture medium. Apoptosis of the neurons was detected by TUNEL. In PI3-kinase inhibition studies, the PI3-kinase inhibitor (wortmannin and LY294002) was added to the culture 15 min before treatment with BDNF. Both LDH assay and TUNEL assay suggested BDNF was capable of preventing neuronal cell death induced by MAP. BDNF-stimulated Akt kinase activity in cortical neurons was blocked by PI3kinase inhibitor. Incubation of neurons with either of the PI3-kinase inhibitors blocked the effects of BDNF on MAP-induced neurotoxicity. Adenovirus-mediated overexpression of the activated form of Akt significantly inhibited the MAP-induced neuronal cell death. Moreover, expression of the kinase-defective form of Akt almost completely blocked the effect of BDNF. These findings suggest that activation of Akt is necessary and sufficient for the neuroprotective effect of BDNF, and is capable of preventing MAP-induced apoptosis in primary cortical neuron.

P01-F-47

Paraquat induces long-lasting dopaminergic neurotoxicity through the excitotoxic pathway

○Kazuo Matsubara¹, Keiko Shimizu², Ko-ichi Ohtaki¹, Osamu Saito², Hiroshi Shiono²

¹Departments of Hospital Pharmacy & Pharmacology and Legal Medicine, Asahikawa Medical College,

²Hospital Pharmacy & Pharmacology and Legal Medicine, Asahikawa Medical College

The cause of Parkinson's disease (PD) has been unclear yet, but epidemiological studies suggest an association with pesticides and other environmental chemicals. The herbicide paraquat might be a possible environmental factor the etiology of Parkinson's disease due to the structural similarity to the active metabolites of MPTP, N-methyl-4-phenyl pyridinium (MPP⁺), induce specific dopaminergic toxicity in the brain. We have reported the manner of BBB penetration and neural cell uptake of paraquat. But the toxic mechanism of paraquat has not been well documented. The GBR12909, a selective dopamine transporter inhibitor, reduced the paraquat uptake into the striatal cell, indicating that the paraquat is uptaken into the striatal cells partially by the dopamine transporter. The systematic and subchronic treatment of paraquat decreased brain tissue monoamine content only in the striatum and its acidic metabolites content in striatum, midbrain and cortex comparing with the control rats, indicating that the herbicide exactly damaged to dopaminergic neuron and would be act on the dopamine nerve terminals as a toxin. Paraquat increased transitory the extracellular glutamate levels then elevated the extracellular NOx levels and caused dopamine overflow which is small but long-lasting, more than 24 h after the paraquat treatment. This long-lasting dopamine overflow was inhibited by L-NAME, MK-801, DNQX and Deprenyl. These results suggested that paraquat would stimulate glutamate efflux to initiate the excitotoxicity on dopaminergic neurons, resulting in evoked depolarization of NMDA receptor channel associating Ca²⁺ penetration into cells by activation of non-NMDA receptor channel, and the mitochondria dysfunction induced by the activation of NOS causing continuous and long-lasting dopamine overflow. The chronic mild exposure to paraquat may lead to the vulnerability of dopamine neuron easy to make the neurodegeneration if adding to other various stress.

P02-E-1

Compensatory neuroprotective events in kainic acid-induced neuronal cell death

○Kazuyuki Takata, Yoshihisa Kitamura, Mitsuhiro Okazaki, Jun-ichi Kakimura, Takashi Taniguchi
Dept. Neurobiol., Kyoto Pharm. Univ.

Kainic acid (KA)-induced neuronal cell death within the hippocampus might involve both the activation of glutamate receptors and the production of oxidative stress, which underlie neurodegenerative disorders. However, the processes of KA-induced neurodegeneration have been studied, the details and the compensatory neuroprotective mechanisms are still unclear. In this study, we have found that c-Jun, c-Fos and cyclooxygenase 2 (COX-2) were induced in the neurons throughout the entire hippocampus after KA-injection. The phosphorylation of c-Jun by c-Jun N-terminal kinase (JNK) was observed in the CA3 pyramidal neurons, which undergo apoptosis. On the other hand, the expression of HO-1, which catalyzes the production of antioxidants such as biliverdin and bilirubin, and aryl hydrocarbon receptor nuclear translocator (ARNT) were observed in microglia around CA3 region. Furthermore, we investigated the protective effect of adenosine using *N*⁶-cyclopentyladenosine (CHA) and 8-cyclopentyltheophylline (CPT) which are A1 adenosine receptor agonist and antagonist respectively. The co-administration of CHA with KA attenuated the neuronal loss, while the combination of CPT and KA induced neuronal loss both in CA3 and CA1. These results indicate the activation of JNK may participate in KA-induced neurodegeneration. In contrast, COX-2, ARNT, HO-1 and endogenous adenosine may involve in the compensatory neuroprotection.

P02-E-2

Association of protein kinase C alpha and of p38 mitogen-activated protein kinase with the induction of tumor necrosis factor alpha in lipopolysaccharide-stimulated microglia

○Kazuyuki Nakajima^{1,2}, Yoko Tohyama¹, Shinichi Kohsaka², Tadashi Kurihara¹

¹Neurochem Div. Inst. Life Sci., Soka Univ., ²Dept. Neurochem. Natl Inst. Neurosci.

Although activated microglia have been suggested to produce in brain tumor necrosis factor alpha (TNF α), a cytotoxic and inflammatory cytokine, under many pathological conditions, little has been known about the molecular mechanism of this induction. Using lipopolysaccharide (LPS) as an experimental inducer of TNF α , we investigated the regulation mechanism by which TNF α is induced or suppressed in microglia in vitro. LPS-induced TNF α was suppressed by pretreatment with the p38 mitogen-activated protein kinase (p38MAPK) inhibitor SB203580. Comparable suppression was achieved by pretreatment with conventional protein kinase C (PKC) inhibitor, Go6976. These results suggest the association of PKC α activity and of p38MAPK activity with TNF α induction in LPS-stimulated microglia. Further, the analysis of activation of these protein kinases revealed that p38MAPK and PKC α activation pathways are linked intimately to the TNF α induction signaling cascade. It is concluded that the induction of TNF α in LPS-stimulated microglia was mediated by the PKC α -linked p38MAPK signaling pathway.

P02-E-3

Reciprocal localizations and functional aspects of CD9 and CD81 in the nervous system.

○Tomoko Ishibashi¹, Kazuhiro Ikenaka², Eisuke Mekada³, Hiroko Baba¹

¹Dept. Molecular Neurology, Tokyo Univ of Pharmacy and Life Science, ²National Inst for Physiological Science, ³Reserch Institute for Microbial Disease, Osaka Univ.

CD9 is a member of transmembrane-4 superfamily (TM4SF), which has some roles in cell signaling, adhesion, cell growth and motility by forming complexes with a variety of membrane proteins including other TM4SF members. In the nervous system, CD9 is enriched in myelin, especially along the outermost membrane of myelin as well as in the paranodal region. CD9 knockout mouse (KO) showed the disruption of paranodal junction in addition to abnormally thick myelin predominantly observed in the PNS. To identify a functional role of TM4SF in the nervous system, we investigated the detailed localization of CD9 and CD81, which is the most closest member of CD9, in the same family. As a result, CD9 distributed predominantly in the PNS myelin. In contrast, CD81 was present more in the CNS myelin than in the PNS. Furthermore, such reciprocal localization of these two proteins was also found within the CNS myelin itself. The analysis of CD81 KO showed the abnormal distribution of Caspr, suggesting that CD81 have some roles in paranodal axo-glial junction predominantly in the CNS. Thus, myelin membrane mutually utilizes two members of TM4SF in the CNS and PNS.

P02-E-4

ATP selectively suppresses the synthesis of the inflammatory protein, MRF-1, through Ca^{2+} -influx via P2X₇ receptors in cultured microglia

○Shuuitsu Tanaka, Naoki Kaya, Tatsuro Koike
Div. Biol. Sci., Grad. Sch. Sci., Hokkaido Univ.

Microglia are known to express purinergic receptors for extracellular ATP of both P2Y and P2X subtypes. In CNS, ATP is released from neurons and acts as a signal between neuron and glia. The *mrf1* gene encodes 17-kDa protein with a single calcium-binding (EF-hand) motif and is expressed specifically in microglia. The gene was isolated from activated microglia in response to apoptosis of cerebellar granule neurons in culture and is upregulated in response to neuronal death and degeneration *in vivo*. We have found that ATP suppresses synthesis of the inflammation-related protein, MRF-1, in cultured rat microglia. When microglia were treated with ATP (1 mM) for 6 h, *mrf1* mRNA levels decreased to approximately 50% compared to those in the control. This effect was dependent on both the treatment period and the dose of ATP. After ATP (1 mM) treatment for 16 h, *mrf1* mRNA or MRF-1 levels decreased to 37.3 or 55.0%, respectively, compared to those in the control. A decrease in MRF-1 or its mRNA was also induced by benzoylbenzoyl-ATP (0.1 mM), a P2X₇ receptor agonist, and by the Ca^{2+} -ionophore (2 μ M), dependent on extracellular Ca^{2+} . Moreover, ATP modified neither the MRF-1 degradation rate nor total protein synthesis. These results indicate that ATP selectively suppresses MRF-1 synthesis at a transcription level via Ca^{2+} influx through P2X₇ receptors.

P02-E-5

Down-regulation of cholesterol metabolism by serotonin (5HT) in rat astrocytes

○Sachiko Ueno^{1,2}, Jinichi Ito², Yuko Nagayasu², Schinji Yokoyama²

¹Department of Psychiatry and Cognitive-Behavioral Science, Nagoya City University Graduate School of Medical Sciences, ²Department of Biochemistry, Cell Biology and Metabolism, Nagoya City University Graduate School of Medical Sciences

Astrocytes play a central role in cholesterol homeostasis in the brain, by synthesizing apolipoprotein E and secreting it as HDL-like particles with cellular cholesterol and phospholipid. This is one of the key reactions to develop and maintain structure and function of brain by regulating inter- and intracellular cholesterol transport in the central nervous system. Previous studies demonstrated functional expression of various serotonin [5HT] receptors in astrocytes and implicated association of their function with cellular cholesterol metabolism. We here report that 5HT down-regulates cholesterol biosynthesis in rat astrocytes. The treatment of the cell with 5HT for 5 minutes resulted in decrease of biosynthesis and release of cholesterol. 5HT decreased biosynthesis of mevalonic acid but not cholesterol biosynthesis from mevalonic acid, and decrease of HMG-CoA reductase activity was directly demonstrated. The effects were reproduced by the agonists for the 5HT_{2A/2C} receptors and inhibited by the antagonists for the same receptors. U-73122, a phospholipase C inhibitor, and W7, a calcium/calmodulin antagonist, canceled the effects of 5HT on cellular cholesterol metabolism. These findings suggested that 5HT causes down-regulation of HMG-CoA reductase activity by stimulating the 5HT_{2A/2C} receptors and subsequently phospholipase C β and calcium/calmodulin presumably through the activation of G_q protein. Since the down-regulation takes place within a short-time treatment, it is unlikely to be mediated through gene expression.

P02-E-6

Formation of floating cell aggregates from bipotential glial cells in the presence of primary microglia.

○Ryuuj Hisamura^{1,2}, Kenji F Tanaka^{1,3}, Makiko Kawakami¹, Haruo Kashima³, Makoto Sawada^{1,4}

¹JRDTAID, ICMS, Fujita Health Univ., ²Grad. Sch. Agri., Nagoya Univ., ³Dept. Neuropsychia. Grad. Sch. Med., Keio Univ., ⁴PRESTO, JST

From late embryonic period, neural stem cells differentiate into glioblasts rather than neuroblasts. Subsequently the number of macroglial cells, astrocytes and oligodendrocytes, dramatically increases in the brain during perinatal period. Microglial cells, which could be derived from hematopoietic lineage, also proliferate in same period. We hypothesized that microglia were involved in both generation and destination of glioblasts. To clarify this, we studied the possible interaction between glioblasts and microglia in culture. We used a glial cell line, OS3, as a glioblast. OS3 cells were previously established from a neonatal mouse primary mixed glial cell culture by transfection with an origin-defective simian virus 40 DNA. OS3 cells expressed both GFAP and nestin in the presence of 10% FBS, whereas, they differentiated into O4 positive immature oligodendrocytes in the presence of 1-2% FBS. When co-cultured with primary microglia in a serum free condition, OS3 survived, proliferated, and formed floating cell aggregates like neurospheres. OS3 cells in the floating spheroid were still nestin-GFAP double positive. Since Notch signaling may participate in the maintenance of immature state and the formation of spheroids, we investigated the expression of Notch signaling related molecules in OS3 and microglia. OS3 expressed mRNA of known all Notch receptors (Notch1~4), ligands (Dll1, 3, 4 and Jagged1, 2), and L-, M-, R-fringes. Primary microglia extensively expressed mRNA of Jagged1. Semi-quantitative PCR revealed that HES1 and HES5 mRNAs in OS3 spheroids did not change by microglial stimulation in co-culture. This suggested that the spheroid formation of OS3 cells might be controlled by a distinct mechanism from Notch signaling pathway.

P02-E-7

オリゴデンドロサイトの発生の起源

○中平 英子¹, 鹿川 哲史¹, Martyn M Guldin², 池中 一祐¹

¹生理研・神経情報, ²The salk institute

大脳皮質オリゴデンドロサイト (OL) は、腹側の予定線条体領域の限局した部分から発生し、移動によって前脳全体に広がるといわれている。しかし分裂するOL前駆細胞を恒久的に標識する方法がなかったためにin vivoで実際に移動しているのか、また予定線条体領域以外にOLの発生する部位があるのかはまだ証明されていない。我々は前回の学会で子宮内マウス胎仔にプラスミドDNAを導入するin utero electroporation法を独自に確立した事を報告した。また我々はすべての体細胞に発現しているROSA26遺伝子座に[loxP-転写終結シグナル-loxP-GAP43-EGFP]を挿入したROSA reporterマウスを作製し、cre遺伝子を含むプラスミドをin utero electroporation法で導入し、DNA組み換えを起こした細胞を恒久的にGFPでマーキングする事に成功した。今回はこの方法を発展させ、マウス胎生12日目の前脳、腹側、背側上皮細胞を8割以上の確率で選択的に標識し、それぞれの細胞が生後どのような細胞へと分化したかを検討した。標識したマウスを生後30日目に固定し、凍結切片を作成しミエリン蛋白質であるPLPとGFPを二重抗体染色したところ、大脳皮質内でミエリンを形成するOLにGFP陽性細胞が存在した。この結果より、in vivoで予定線条体領域で発生したOLが移動して大脳皮質内でミエリンを形成するOLとなる事が証明された。

P02-E-8

Expression of P2Y₁₂ receptors in the brain

○Yo Sasaki², Shizuyo Honda¹, Chihiro Akazawa¹, Kuninobu Johkohji¹, Yasuko Nakamura¹, Kazuhide Inoue³, Shinichi Kohsaka¹

¹Department of Neurochemistry, National institute of Neuroscience, Kodaira, Tokyo, Japan, ²Japan Science and Technology Corporation Domestic Research Fellow, ³Division of Pharmacology, National Institute of Health Sciences, Tokyo, Japan

We have previously shown that ATP and ADP induced membrane ruffling and chemotaxis of microglia. These effects were inhibited by pertussis toxin and AR-C69931MX, a selective antagonist against P2Y₁₂, suggesting that Gi/o-coupled receptor P2Y₁₂ is involved in these ATP or ADP effects. Recently platelet derived P2Y₁₂ receptor has been cloned. However, P2Y₁₂-expressing cells are not fully characterized in the brain. Then, we further examined expression of P2Y₁₂ in the brain. Northern blot analyses demonstrated that P2Y₁₂ is predominately expressed in the brain, and to a smaller extent in the spleen. We also found P2Y₁₂ was expressed in various brain regions, relatively high in the hippocampus. To further investigate the cellular distribution of P2Y₁₂, we performed in-situ hybridization in adult rat brain. P2Y₁₂ is strongly expressed in small cells, which is distributed throughout the brain. In contrast, lower expression could be detected in motor neurons in facial nucleus and granule-like cells in dentate gyrus. Moreover, we also found that P2Y₁₂-expressing small cells were increased in the facial nucleus following axotomy. To characterize the P2Y₁₂-expressing small cells, we performed double labeling with in-situ hybridization and immunocytochemistry of P2Y₁₂ and microglia specific protein Iba1. P2Y₁₂-expressing small cells were stained with Iba1. Taken together these results, P2Y₁₂ is strongly expressed in microglia, and is slightly expressed in certain population of neuronal cells.

P02-E-9

The basic helix-loop-helix factor Olig2 is essential for development of motoneuron and oligodendrocyte lineages

○Hirohide Takebayashi¹, Yoko Nabeshima², Shosei Yoshida², Osamu Chisaka³, Yo-ichi Nabeshima², Kazuhiro Ikenaka¹

¹National Institute for physiological Sciences, ²Dept. Pathol., Grad. Sch. Med., Kyoto Univ., ³Grad. Sch. Biostudies., Kyoto Univ.

Sonic hedgehog (Shh), an organizing signal from ventral midline structures, is essential for the induction and maintenance of many ventral cell types in embryonic neural tube. Olig1 and Olig2 are related basic helix-loop-helix factors induced by Shh in the ventral neural tube. Although gain-of-function experiments suggested that these factors were involved in motoneuron and oligodendrocyte development, they do not clearly define the functional differences between Olig1 and Olig2. We generated mice with a homozygous inactivation of Olig2. These mice did not feed and died on the day of birth. There were no motoneurons and no oligodendrocytes in the spinal cord of the mutant mice. Olig2^{-/-} neuroepithelial cells in the ventral spinal cord failed to differentiate into motoneurons or oligodendrocytes, and expressed an astrocyte marker, S100beta, at the time of oligodendrogenesis. Olig1 or Olig3, another family member, were expressed in the descendent cells that should have expressed Olig2. We concluded that Olig2 is an essential transcriptional regulator in motoneuron and oligodendrocyte development. Our data provide the first evidence that a single gene mutation leads to loss of two lineages, motoneuron and oligodendrocyte.

P02-E-10

Influence of astrocyte-inducers on glial cell development in primary cultures of embryonic rat cerebral hemisphere

○Tatsumi Adachi^{1,2}, Hiromi Takanaga^{2,3}, Manabu Kunitomo^{2,3}, Hiroaki Asou⁴

¹Dept. Basic Med. Sci., Natl. Inst. Minamata Dis., ²Natl. Inst. Environmental Studies, ³School Pharm. Sci., Kitasato Univ., ⁴Dept. Neurobiology, Tokyo Metropolitan Inst. Gerontology

The influence of leukemia inhibitory factor (LIF) and bone morphogenetic protein (BMP) 2 on differentiation and development of astrocytes and oligodendrocytes was investigated in primary cultures prepared from rat cerebral hemisphere on embryonic day 18. The cells were maintained in serum-free defined medium with or without LIF and/or BMP2 for 2 days, and western blot analysis of glial fibrillary acidic protein (GFAP), a specific marker of astrocytes, and immunocytochemical staining against galactocerebroside (GalC), a specific marker of oligodendrocytes, were performed. The level of GFAP expression markedly increased by addition of LIF or BMP2, and was further enhanced by the addition of both proteins. Number of GalC-positive oligodendrocytes increased by the addition of LIF, whereas decreased by the addition of BMP2. The number did not change by the addition of both proteins. When platelet-derived growth factor (PDGF) was neutralized, the number of oligodendrocytes increased under all conditions examined. As the results of neutralization, the effect of BMP2 on oligodendrocyte differentiation was disappeared, although LIF was still effective. These results suggest that the differentiation of oligodendrocytes is partially delayed by PDGF, which is probably produced and released by astrocytes. It is also suggested that LIF and BMP2, each of them accelerates the differentiation and development of astrocytes, have different effects on those of oligodendrocytes: LIF alone can directly affect the differentiation and development of oligodendrocytes.

P02-E-11

Analysis of Astrocyte Lineage Developmental

○Yasuhiro Ogawa¹, Matthew Rasband², Hirohide Takebayashi¹, Yasuno Iwasaki¹, James Trimer², Kazuhiro Ikenaka¹

¹National Institute for Physiological Sciences, ²SUNY Stony Brook

Central nervous system (CNS) consists of many types of neurons and glial cells. The diversities of many cell types become more and more complicated in the systems with higher function. Especially the diversities of neurons are studied well, and thought to depend on graded inductive and repressive signals derived from local organizing centers. Analysis of neuronal lineage only is insufficient to understand the regulated of CNS functions, because astrocytes not only occupy the major portion of the CNS but are thought to play important roles in CNS functions. However, astrocyte lineage have not been well understood developmentally due to lack of specific markers that label its lineage from early to mature stage. In order to approach the systems, it is necessary to establish many markers expressed with the astrocyte lineage, therefore we screened several monoclonal antibodies stained astrocyte lineages by immunohistochemistry. We have found some antibodies for radial glia-astrocyte lineage which did not be recognized at least known marker proteins, for example GLAST and Vimentin, by Western analysis and staining patterns.

P02-E-12

小脳顆粒ニューロン前駆細胞の増殖・分化調節

○岡野 (内田) 孝幸, 小宮 義璋, 石崎 泰樹
群馬大・医・分子病態

小脳顆粒ニューロンはその前駆細胞 (GNPs) が外顆粒層において増殖した後、細胞周期を脱して分化を開始し、やがて成熟した神経細胞となる。しかし、この増殖から分化へと向かう機構については未だ不明な点が多い。本研究では、昨年本大会で発表したimmunopanning法により幼若マウス小脳から未分化なGNPsだけを選択的に単離し、この機構について検討した。培養開始時からGNPsの増殖促進因子であるShhを添加することにより、この細胞は長期間増殖能を保持した未分化な状態に維持される。しかし、Shh存在下においてもGNPsは次第に増殖を停止して、ニューロンに特異的なマーカーである β -チューブリンIIIを発現し、成熟した顆粒細胞へと分化する。このとき同時にBMP2を添加すると、GFAP (グリア線維酸性蛋白) を発現する細胞の著しい増加がみられた。しかしBMP2を最初から単独で加えた場合には、GFAP陽性細胞の増加率は低かった。さらに、Shh存在下で培養した細胞では、ニューロンへの分化を開始した後でも、BMP2の添加によりGFAP陽性細胞の増加が認められた。これらの細胞の中には β -チューブリンIIIとGFAPを同時に発現しているものが存在していた。すなわち、Shh存在下で培養したGNPsは、いったんニューロンへと分化を開始した後でもアストロサイトへと分化転換しうることが明らかになった。

P02-E-13

Microglial apoptosis induced by a low concentration of polyamine

Katsura Takano¹, [○]Yoichi Nakamura^{1,2}, Yukio Yoneda¹

¹Dept. Mol. Pharmacol., Kanazawa Univ. Facul. Pharm. Sci., ²Dept. Physiol. Osaka Pref. Univ. Grad. Sch. Veterinary. Sci.

Pathological activation of microglia, which quiescently reside in physiological CNS, causes various neurodegenerative diseases. Endogenous polyamines, spermidine (Spd), and spermine (Spm), are known to be activators of cell proliferation and differentiation. We examined the effect of polyamines on microglial activation in culture.

Cultured microglia prepared from the whole brains of newborn rats produced nitric oxide (NO) by the stimulation with lipopolysaccharide (LPS). LPS-induced NO production was remarkably inhibited by Spd and Spm; a half effective concentration (EC₅₀) for Spd and Spm were about 3 and 1 μ M, respectively. Cell viability assessed by total mitochondrial activity decreased by the incubation with Spd and Spm for 24 hrs. In astrocyte culture, on the other hand, polyamines showed no significant effect on both LPS-induced NO production and cell viability. After the treatment with Spm for 24 hrs, the cells changed into small round morphology, and were markedly stained with propidium iodide. By the staining with Hoechst333424, concentration and fragmentation of nucleus were often observed. Semiquantitative analysis of fragmented DNA with ELISA technique revealed that a large amount of fragmented DNA appeared in cytosol prior to disruption of the cell membrane, suggesting that polyamines induced apoptosis. Fragmented DNA in cytosol increased dose dependently with spermidine; EC₅₀ was less than 10 μ M.

The elucidation of the mechanisms of transport and/or metabolism of polyamines should be important that might be a base for the protection against microglia-evoked neurotoxicity.

P02-E-14

Neuroprotection against Oxidative Stress by Microglia and Astrocyte

[○]Satoru Oshiro¹, Chun Zhang¹, Shigetaka Kitajima¹, Chihiro Miyazawa³, Ken-ichi Kawamura³, Kazuyuki Nakajima²

¹Dept. Biochem. Genet., Med. Res. Inst., Tokyo Med. & Dent. Univ., ²Dept. Neurochem., Life Sci. Inst., Soka Univ., ³Product Develop., TK Craft, Co. Ltd

In patients with Alzheimer's disease, there are significant evidence for disruption of iron homeostasis: selective accumulation of iron in neurofibrillary tangles (NFT) (1,2), high levels of serum melano-transferrin p97(3), abnormal localization of iron regulatory protein (4,5) in NFT and senile plaques(6). Recently, we reported that glial cells contribute more to iron accumulation but are more resistant to oxidative stress than neuronal cells(7). In the present study, we examined microglia(MG) and astrocytes(AS) to clarify which contribute to neuroprotection against iron or oxidative stresses. When rat primary cortical neurons(N), MG and AS (8,9) were solely incubated with an excess iron or hydrogenperoxide (H₂O₂), neuronal and MG cell death occurred but MG were less sensitive to the stress than neurons. Contrarily, no cell death of AS were substantially seen under the condition. However, when N were cocultured with rat primary MG cells or AS, iron- and H₂O₂-induced neuronal cell death markedly decreased. These results suggest that MG and AS protect N from both iron- and H₂O₂-induced neuronal cell death, and that a mechanism of neuroprotection by MG is different from that of AS.

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P02-E-15

Kainic acid induces leukemia inhibitory factor mRNA expression in the rat brain with different time courses between the dentate gyrus and hippocampal CA1/CA3 subfields

○Masabumi Minami, Keiko Maekawa, Hisashi Yamakuni, Takahiro Katayama, Junji Nakamura, Masamichi Satoh
Dept. Mol. Pharmacol., Fac. Pharm. Sci., Kyoto Univ.

Leukemia inhibitory factor (LIF) is a pluripotent cytokine which affects survival and differentiation of various types of cells in the hematopoietic and nervous systems. LIF mRNA expression in the rat brain following kainic acid-induced seizures was examined by northern blot analyses and in situ hybridization. Northern blot analyses examining the LIF mRNA expression at 8 h after the intraperitoneal injection of kainic acid (10mg/kg) demonstrated that the expression was intense in the hippocampus and moderate to weak in the cerebral cortex, thalamus and hypothalamus, but slight or negligible in the striatum, midbrain, pons-medulla and cerebellum. Further northern blot analyses revealed that the expression peaked at 8-24 h in the hippocampus and cerebral cortex and at 8 h in the thalamus and hypothalamus. In situ hybridization examining the LIF mRNA expression at 0, 6, 8, 10, 12 and 24 h after the injection revealed different time courses of the mRNA expression depending on the area of the hippocampus. The expression peaked at 8-10 h in the granule cell layer of the dentate gyrus, at 12 h in the polymorph and molecular layers of the dentate gyrus, and at 24 h in the strata oriens and radiatum of the CA1/CA3 subfields. Reportedly, seizures induce increment of neurogenesis and ectopic migration of newly generated granule cells in the dentate gyrus, the region where the remarkable upregulation of LIF mRNA expression was observed in the present study. This upregulation may be involved in the increment of neurogenesis and ectopic migration of granule cells in the dentate gyrus.

P02-E-16

Functional Expression of the Monoamine Transporter in Cultured Rat Astrocytes.

○ Hiroshi Takeda, Masato Inazu, Teruhiko Matsumiya
Dept. Pharmacol., and Intractable Diseases Research Center, Tokyo Med. Univ.

Astrocytes contain transport systems that are capable of removing various neurotransmitters from the synaptic cleft by transporters present in the plasma membrane. Glial monoamine transporter plays an important role in the reuptake of monoamines. Brain astrocytes are located around synapses and at the periphery of capillaries, so their uptake systems are likely to be important for regulating the neuronal environment. We assessed the functional expression of the monoamine transporter in cultured rat astrocytes. RT-PCR demonstrates the presence of serotonin transporter (SERT), DA transporter (DAT) and NE transporter (NET) mRNA in rat astrocytes. Astrocytes take up [³H]5-HT, [³H]DA and [³H]NE in a time-dependent manner that were sensitive to a reduction in temperature. Tricyclic antidepressants as well as selective serotonin re-uptake inhibitors were very potent inhibitors of [³H]5-HT uptake in astrocytes. In contrast, the inhibitory effects of NE or DA uptake inhibitors and cocaine were weaker than those of 5-HT uptake inhibitors. Astroglial [³H]DA uptake was inhibited by nisoxetine (selective NE uptake inhibitor) in a concentration-dependent manner, and nisoxetine was more potent than DA or 5-HT uptake inhibitors. Moreover, nisoxetine, desipramine and imipramine very potently inhibited [³H]NE uptake in astrocytes. DA or 5-HT uptake inhibitors and cocaine had weaker inhibitory activities. These data indicate that the SERT and NET are functionally expressed in cultured rat astrocytes. Glial cells may exert significant control of monoaminergic activity by inactivating monoamines that escapes neuronal re-uptake in sites distant from terminals, and are thus cellular targets for antidepressant drugs.

P02-E-17

Generation of BRINP1 knock-out mice

○Toshiyuki Nakatani¹, Hiromi Kawano¹, Miwako Kobayashi¹, Ken-ichi Matsumoto², Hiroyoshi Ariga², Ichiro Matsuoka¹

¹Dept. Neurosci. Grad. Sch. Pharm. Sci., Hokkaido Univ., ²Dept. Mol. Biol. Grad. Sch. Pharm. Sci., Hokkaido Univ.

We have previously reported that BMP and retinoic acid (RA) act on developing sympathetic neurons to promote their differentiation and induce a novel protein, BRINP1 (BMP/RA-Inducible Neural-specific Protein 1). Characterization of BRINP1 has indicated that 1) BRINP1 forms a novel protein family with subsequently identified BRINP2 and BRINP3. 2) BRINP1 translated as 88 kDa protein (pre-BRINP1) is processed to produce 40 kDa protein (mature BRINP1) that is located at cell membranes. 3) The expression of BRINP1 starts from late embryonic stage and continues into adulthood in the both CNS and PNS. 4) Elevation of neural activity induces BRINP1-mRNA in brain regions such as hippocampus and olfactory bulb. These observations suggest that BRINP1 has important roles in higher order neural functions such as learning and memory. To further clarify the physiological functions of BRINP1, we employed a gene targeting strategy.

At first we established ES cell lines with the 1st Met in exon-2 replaced by neomycin resistance gene. Then, we obtained *BRINP1* 1st Met-disrupted mice. RT-PCR analysis indicated that these mice expressed shorter BRINP1-mRNA lacking exon-2. Interestingly, western blot analysis revealed that mature BRINP1 with the same MW as that of wild mice was produced in the exon-2 knockout mice, probably processed from a truncated form of pre-BRINP1 translated from 2nd Met in exon-3. Then, we designed to generate another type of BRINP1 knock-out mice with disrupted exon-8 which covers entire part of mature BRINP1. At present, we have obtained the chimeric mice and we will discuss on the physiological functions of BRINP1 from phenotypic alterations of the exon-8 these mice.

P02-E-18

Characterization of intercellular Ca²⁺ wave propagation by endogenous ATP in cultured rat hippocampus

○Schuichi Koizumi¹, Kayoko Fujishita^{1,2}, Kazuhide Inoue^{1,2}

¹Div. Pharmacol., Natl. Inst. Hlth. Sci., ²Grad. Sch. Pharm. Sci., Kyushu Univ.

Much attention has focused on a role of ATP as an intercellular mediator in the brain. Especially, astrocytes release ATP to form interastrocytic Ca²⁺ wave propagation. In the present study, we investigated Ca²⁺ wave propagation in response to mechanical stimulation in cultured rat hippocampal astrocytes using fura-2 and fluo-3 based Ca²⁺ imaging techniques. When an astrocyte was mechanically stimulated with a glass pipette, the increase in intracellular Ca²⁺ concentration ([Ca²⁺]_i) was observed in the cell, which was subsequently propagated into adjacent astrocytes with a propagation velocity of 20 μm/s. This Ca²⁺ wave propagation was due to efflux and diffusion of endogenous ATP from astrocytes because the Ca²⁺ wave was inhibited by apyrase or antagonists to P2 receptors. Neither glutamate antagonists nor gap junction inhibitors affected on the Ca²⁺ wave propagation. Although the hippocampal astrocytes possess functional P2Y1 and P2Y2 receptors, the responsible P2 receptors for the Ca²⁺ wave was P2Y1 receptors. The Ca²⁺ wave was dependent upon stored Ca²⁺ but independent of extracellular Ca²⁺. The release of ATP is also dependent upon stored Ca²⁺. The existence of back propagation of Ca²⁺ wave and the slow Ca²⁺ wave velocity would predict the regenerative ATP release in a manner dependent upon intracellular Ca²⁺. We will also discuss communication by ATP between neurons and astrocytes in the hippocampus.

P02-E-19

Perineuronal oligodendrocytes protect against neuronal apoptosis in a genetic demyelinating model by upregulation of lipocalin-type prostaglandin D synthase

○Naomi Eguchi^{1,2}, Masako Taniike³, Ikuko Mohri³, Kinuko Suzuki⁴, Yoshihiro Urade^{1,2}

¹Dept. Mol. Behav. Biol., Osaka Biosc. Inst., ²CREST, JST, ³Dept. Developmental Medicine (Pediatrics), Osaka Univ. Grad. Sch. Med., ⁴Dept. Pathology and Labo. Med., and Neurosci. Center, Sch. Med., Univ. of North Carolina at Chapel Hill

The genetic demyelinating mouse, twitcher, is a model of the human globoid cell leukodystrophy caused by galactosylceramidase (GALC) deficiency. Demyelination in the twitcher brain is secondary to apoptotic death of oligodendrocytes (OLs). Lipocalin-type prostaglandin D synthase (PGDS) serves as PGD₂-producing enzyme and also as a transporter protein for small lipophilic ligands. PGDS is expressed in OLs after commencement of myelination in the CNS of rats, mice, and humans, but not in Schwann cells of the peripheral nerve system. Therefore, PGDS is a specific marker for mature OLs.

In this study, we found that mRNA for PGDS was progressively upregulated in twitcher, whereas expression of various other OL-associated proteins, such as carbonic anhydrase II, myelin basic protein, and myelin-associated glycoprotein, was downregulated during demyelination in twitcher brains. The upregulation of PGDS was more remarkable in perineuronal OLs than in interfascicular OLs. A larger number of PGDS⁺ OLs was found in selected fiber tracts of twitcher brains where fewer apoptotic cells were detected. The distribution of PGDS⁺ OLs was inversely related to the severity of demyelination as assessed by accumulation of scavenger macrophages. Mice doubly deficient for PGDS and GALC disclosed a large number of apoptotic neurons, which were never seen in twitcher brains, in addition to an increased number of apoptotic OLs. A linear positive correlation was observed between the population of PGDS⁺ OLs in the twitcher brain and the ratio of apoptotic nuclei in the double mutant versus those in the twitcher, suggesting a dose-dependent effect of PGDS against apoptosis. These results suggest that PGDS is an anti-apoptotic molecule protecting neurons and OLs from apoptosis in the twitcher mouse. This is a novel example of OL-neuronal interaction.

P02-E-20

Transcriptional regulation of the mouse 3-phosphoglycerate dehydrogenase (3-PGDH) gene in astrocytes

○Motohiro Shimizu^{1,2}, Shigeki Furuya¹, Yoko Shinoda¹, Kazuyuki Yoshida^{1,3}, Junya Mitoma⁴, Yasuo Suzuki², Yoshio Hirabashi¹

¹Neuronal Circuit Mechanism Research Group, BSI, RIKEN, ²Dept. Biochem. Sch. Pharm. Sci., University of Shizuoka, ³Unit. Grad. Sch. Agri. Sci., Tokyo University of Agriculture and Technology, ⁴The Burnham Institute

Astrocytes are considered to regulate the survival, development, and function of immature central neurons. We have demonstrated that L-Ser serves as a major astrocyte-derived neurotrophic factor, which strongly promotes the survival and maturation of developing neurons such as hippocampal neurons and cerebellar Purkinje neuron (PN) (Mitoma et al., J. Biol. Chem., 273, 19363, 1998; Furuya et al., PNAS, 97, 11528, 2000). The *de novo* synthesis of L-Ser from a glycolytic intermediate 3-phosphoglycerate involves three sequential reactions initiated by 3-phosphoglycerate dehydrogenase (3PGDH). Our Northern blot analysis showed a cell-type specific regulation of 3PGDH gene transcription with high expression of transcripts in astrocytes, and weak expression in neurons. To establish the molecular basis for the transcriptional regulation of the 3PGDH gene in neural cells, we cloned 3.5 kb of the mouse 3PGDH gene 5' -flanking region and characterized the promoter structure. Using luciferase reporter gene transfection analysis of 5' -deletion constructs, we demonstrated positive regulatory elements within 1.8 kb upstream sequence. Deletion of the -1792 and -1508 region caused a reduction of the functional promoter activity in astrocytes, but not in neurons. Electrophoretic mobility shift assay (EMSA) showed that nuclear proteins extracted from astrocytes bind to this region, which contains sequences with no similarities to known DNA-protein binding elements. Detailed characterization of DNA-protein binding in the region will be presented.

P02-E-21

Establishment and characterization of immortalized Schwann cells from murine disease models

○Kazuhiko Watabe¹, Tsuyoshi Sakamoto¹, Yoko Kawazoe¹, Jin-Song Shen^{1,2}, Toya Ohashi², Yoshikatsu Eto², Makoto Michikawa³, Katsuhiko Yanagisawa³, Katsuichi Miyamoto⁴, Takashi Yamamura⁴, Norie Araki⁵

¹Dept. Molecular Neuropathol., Tokyo Metropol. Inst. Neuroscience, ²Dept. Gene Therapy, Inst. DNA Medicine, Jikei Univ. Sch. Med., ³Dept. Dementia Research, Natl Inst. Longevity Sciences, ⁴Dept. Demyelinating Disease and Aging, Natl Inst. Neuroscience, ⁵Dept. Tumor Genetics and Biol., Kumamoto Univ. Sch. Med.

Previously we have established spontaneously immortalized cell lines from long-term cultures of normal adult mouse Schwann cells (J Neurosci Res 41:279-290,1995). Establishment of such Schwann cell lines derived from mutant mice may greatly facilitate the studies of the cellular mechanisms of their peripheral nervous system involvement in the relevant diseases. In this study we maintained long-term cultures of Schwann cells derived from dorsal root ganglia and consecutive peripheral nerves of 9 week-old Niemann-Pick disease type C mice (NPC; spm/spm, spm/+, npc^{nih}/ npc^{nih}, npc^{nih}/+), 3 week-old globoid cell leukodystrophy mice (twitcher), 21-35 week-old P0-deficient mice (P0-/-, P0+/-), and 11 week-old neurofibromatosis-1-deficient mice (Nf1^{Fcr} /+) for 8-10 months, and established spontaneously immortalized cell lines from all these animals. These cell lines showed spindle-shaped Schwann cell morphology and distinct Schwann cell phenotypes such as expression of S100, laminin and p75^{NTR} as shown by immunofluorescence microscopy and RNA transcripts of L1, PMP22, GAP43 and GDNF as demonstrated by RT-PCR. These cells retained genomic and biochemical abnormalities, sufficiently representing the in vivo pathological features of the mutant mice, such as accumulations of free cholesterol and GM2 ganglioside in spm/spm and npc^{nih}/ npc^{nih} cell lines (J Periph Nerv Syst 6:85-94,2001), and elevated psychosine levels in twitcher cell lines (J Neurosci Res, in press). These immortalized Schwann cell lines can be useful in studies of nervous system lesions in these mutant mice and relevant human disorders.

P02-E-22

Involvement of microglia activation and p38 phosphorylation in rat neuropathic pain

○ Kazuhide Inoue^{1,3}, Makoto Tsuda¹, Akito Mizokoshi^{1,3}, Yukari Shigemoto-Mogami¹, Shuichi Koizumi¹, Shinichi Kohsaka²

¹Division of Pharmacology, National Institute of Health Science, ²Department of Neurochemistry, National Institute of Neuroscience, ³Graduate School of Pharmaceutical Science, Kyusyu University

We already reported that ATP activates microglia to cause morphological change with membrane ruffling followed by chemotaxis to ATP, and to release plasminogen, or TNF- α and IL-6 through phosphorylation of mitogen-activated protein kinases (MAPK). It is also known that microglia are dramatically activated in the spinal cord of neuropathic pain models. In the present study, we investigated whether MAPK is activated in the spinal cord of the neuropathic pain model in rats, using antibody for the phosphorylated active form of MAPK (phospho-MAPK). Left L5 spinal nerve was tightly ligated and cut at the distal side of ligation. Two weeks after nerve injury, rats displayed a marked mechanical allodynia, confirming the induction of neuropathic pain. In these rats, the level of phospho-p38MAPK but not phospho-ERK1/2 MAPK was increased and many phospho-p38MAPK-positive cells were observed in the injury side of the spinal dorsal horn. Almost all phospho-p38MAPK-positive cells were immunostained by OX42 (microglial marker), but not by GFAP (astrocytes) or NeuN (neuron), indicating that activation of p38MAPK is restricted to microglia. The injection of SB203580, an inhibitor of p38-activation, decreased the strength of allodynia. These findings suggest that activation of p38MAPK in spinal microglia would play an important role in neuropathic pain.

P02-E-23

Binding of transcription factor AP-1 to the non-coding region in mitochondrial genome

○Yoshiaki Fujinami¹, Masahiro Kitano¹, Yukio Yoneda², Kiyokazu Ogita¹

¹Dept. Pharmacol. Setsunan Univ. Fac. Pharm. Sci.,

²Dept. Mol. Pharmacol. Kanazawa Univ. Fac. Pharm. Sci.

We have shown that transcription factor activator protein-1 (AP-1) complex is translocated into mitochondria, as well as into nucleus, in murine hippocampus after a systemic injection of kainate. To elucidate functional significance of mitochondrial AP-1 DNA binding enhanced by kainate treatment, we examined binding of AP-1-like sites in the mitochondrial DNA non-coding region in hippocampal mitochondrial extracts. There are 10 sites with sequences similar to the AP-1 site in the non-coding region of mitochondrial DNA. Among 10 pieces (MT-1 to MT-10) of synthesized double-stranded oligonucleotides containing each sequence similar to AP-1 site, MT-3, MT-4, and MT-9 were most effective in competing for mitochondrial AP-1 DNA binding enhanced by kainate. Electrophoresis mobility shift analysis using radiolabeled MT-3 and MT-9 as probes demonstrated that marked enhancement was seen with binding of the probes in hippocampal mitochondrial extracts prepared 1 to 6 h after kainate treatment. Enhanced MT-9 binding competed with unlabeled AP-1 probe and MT-3. In addition, supershift analysis revealed participation of at least c-Fos and Fos-B proteins in potentiation by kainate of mitochondrial MT-9 binding. These results suggest that AP-1 complex expressed by in vivo treatment with kainate would bind to AP-1-like sites in the non-coding region of mitochondrial DNA following translocation into the mitochondria in murine hippocampus.

P02-E-24

Effect of exposure to static magnetic field on AP1 DNA binding in cultured rat hippocampal neurons

○Takao Hirai, Yukio Yoneda

Dept. Mol. Pharmacol. Fac. Pharm. Sci. Kanazawa Univ., Ishikawa, Japan

Static magnetic field at 100 mT was generated by permanent magnets placed at both sides of culture dishes. Exposure to static magnetic field for 15 min led to marked but transient potentiation of DNA binding activity of the nuclear transcription factor activator protein-1 (AP1) in hippocampal neurons cultured for 3 days in vitro (DIV). Both immunoblotting and supershift analyses revealed that brief exposure to magnetic field increased AP1 DNA binding through expression of Fra-2, c-Jun and Jun-D proteins in immature cultured hippocampal neurons. These immature cultured neurons were exposed to static magnetic field for 15 min, followed by culturing for additional 24 h and subsequent loading of fluo-3 for determination of fluorescence intensity following the cumulative addition of NMDA at 1 to 100 mM in the absence of MgCl₂ using laser-scanning microscope. Significantly less potent increases were seen in intracellular free Ca²⁺ concentration following the addition of NMDA in these immature neurons exposed to magnetism 24 h before. No significant alteration was seen with the activity of lactate dehydrogenase released into culture medium when determined 24 h after brief exposure to magnetic field. These results suggest that brief exposure to static magnetic field may lead to desensitization of NMDA receptor channels through modulation of de novo synthesis of particular inducible target proteins at the level of gene transcription by the AP1 complex expressed in immature cultured rat hippocampal neurons.

P02-E-25

Augmentation of Ethanol-Induced Cell Damage and Activation of NF κ B by Over-Expression of Annexin IV in C6 Cells

○Hiroko Ohkawa^{1,2}, Hitoshi Sohma¹, Rie Sakai^{1,3}, Eri Hashimoto³, Wataru Ukai³, Toshikazu Saito³, Yoshio Kuroki¹

¹Dept. Biochem. Sapporo Med. Univ. Sch. Med., ²Dept. Occup. Ther. Sapporo Med. Univ. Sch. Health Sci., ³Dept. Neuropsychiatry Sapporo Med. Univ. Sch. Med.

We have previously shown that the expressive amount of annexin IV, a Ca²⁺ and phospholipid binding protein, is increased in cultured cells by exposure to ethanol (50-400 mM). In the present study, we investigated the effect of annexin IV on ethanol-induced cytotoxicity in C6 cells transfected with annexin IV-cDNA. The mitochondrial dehydrogenase activity, which is widely used as an index for monitoring cell viability, was decreased to a greater extent by exposure to ethanol in the annexin IV over-expressed C6 cells than in the cells transfected with vector alone. It has been demonstrated that ethanol augments the transcription factor NF κ B activation by cytokines such as tumor necrosis factor- α and interferon α . Electrophoretic mobility shift assay showed that the amount of the shifted band with the ³²P-labeled κ B oligonucleotide probe was augmented significantly in the nuclear extract from the annexin IV over-expressed C6 cells by exposure to ethanol without a cytokine, compared with the cells transfected with vector alone. In addition, Western blot analysis with anti-NF κ B (65 kDa) antibody revealed that the intensity of the NF κ B band was also increased significantly in the nuclear extract from the annexin IV over-expressed C6 cells by exposure to ethanol, compared with the cells transfected with vector alone. From these results, we conclude that over-expression of annexin IV augments both ethanol-induced cell damage and NF κ B activation by ethanol in C6 cells. Thus, the increase in the amount of annexin IV by ethanol in the cells might facilitate ethanol-induced cell damage via NF κ B activation.

P02-E-26 (MS6-4)

A novel human HRD1 involved in ER-associated degradation is induced by unfolded protein response and protects from ER stress-induced cell death via its ubiquitin ligase activity

○Masayuki Kaneko, Masataro Ishiguro, Yoshifumi Niinuma, Yasuyuki Nomura

Dept. Pharmacol. Grad. Sch. Pharm. Sci., Hokkaido Univ.

The unfolded protein response (UPR) is involved in a variety of gene expressions in response to stress that perturbs the function of the endoplasmic reticulum (ER). It has been reported that a number of proteins implicated in ER-associated protein degradation (ERAD) are induced by the UPR in *Saccharomyces cerevisiae*. ERAD requires retrograde transport of the unfolded proteins from the ER back to the cytosol via the Sec61 translocon for degradation by the ubiquitin-proteasome system. Hrd1p is a UPR-induced ER membrane protein that has a RING finger motif and acts as a ubiquitin ligase (E3) in the ERAD system. We report here the isolation and identification of a human homolog to yeast Hrd1p. The predicted structures in its transmembrane domain and RING finger motif are highly conserved from yeast to human. In fact, overexpressed human HRD1 was localized to the ER and ubiquitinates its substrates in HEK293 cells. Furthermore, it was found that human HRD1 was up-regulated in response to ER stress and overexpression of human IRE1 and ATF6, which are ER stress-sensor molecules in the ER. Therefore, we concluded that the function and induction mechanism of human HRD1 are conserved from yeast to human. Finally, 293 cells overexpressing wt-HRD1, but not the C329S mutant, exhibited resistance to cell death induced by the ER stress. These results suggest that human HRD1 up-regulated by the UPR contributes to protection from the ER stress-induced cell death by degrading unfolded proteins accumulated in the ER.

P02-E-27

Induction of SEL1L, a human homolog of Hrd3p, by ER stress and identification of the region for transcriptional induction by ATF6

○Yoshifumi Niinuma, Masataro Ishiguro, Saori Yasui, Masayuki Kaneko, Yasuyuki Nomura
Dept. Pharmacol. Grad. Sch. Pharm. Sci., Hokkaido Univ.

The endoplasmic reticulum (ER)-associated degradation (ERAD) system requires retrograde transport of malformed proteins from the ER to the cytosol and elimination by the proteasomal degradation. Hrd3p and Hrd1p are known to be components involved in the ERAD of *Saccharomyces cerevisiae*. These components are colocalized in the ER and interact with each other. Hrd1p possesses a ubiquitin ligase (E3) activity, and it is suggested that Hrd3p regulates E3 activity and the stability of Hrd1p. SEL1L, which has highly homologous sequences to *HRD3*, is a novel human gene, whose function remains unknown. In addition, we found high similarities in predicted localizations and transmembrane domain between Hrd3p and SEL1L. We confirmed that: 1) SEL1L is actually colocalized with human HRD1 in the ER and 2) SEL1L interacts with HRD1 as well as Hrd3p, suggesting that SEL1L is a human homolog of yeast Hrd3p. It is known that Hrd3p is induced by ER stress. SEL1L mRNA expression was also induced by ER stress-inducing agents: thapsigargin, tunicamycin or brefeldin A, and by transient overexpression of IRE1, a proximal ER stress sensor in the ER membranes. These results indicate that the SEL1L induction mechanism is conserved from yeast to humans. Moreover, ATF6, another sensor acting as a transcription factor in response to ER stress in mammals, induced SEL1L mRNA expression. It has been reported that ATF6 binds to a CCACG motif for transcription of the ER stress-induced genes. To identify the acting elements responsible for induction of SEL1L, we analyzed the SEL1L promoter region using the firefly luciferase assay, and found that the region from -297 to -283, including ⁻²⁸³CGTGG⁻²⁸⁴ complementary to CCACG, may be critical for induction of SEL1L.

P02-E-28

EXPRESSION OF XRE BINDING PROTEINS IN RAT CEREBELLUM

○Noriko Sanada, Katsuhiko Baba, Nobuyuki Kuramoto, Yukio Yoneda
Dept. Mol. pharmacol., Kanazawa Univ. Pharm. Sci.

Toxic effects of environmental pollutants are believed to involve aryl hydrocarbon receptor that activates the gene expression through binding to the core nucleotide sequence xenobiotic responsive element (XRE) on inducible target genes in the nucleus. In this study, we have attempted to detect XRE binding using gel retardation electrophoresis technique to investigate mechanisms underlying neurotoxicity of these pollutants in rat brain. Among discrete central structures examined, XRE binding was most potent in cerebellum, followed by cerebral cortex, hippocampus and striatum in a rank order of decreasing potency. Subcellular fractions were prepared from liver and cerebellum for determination of XRE binding in each fraction. XRE binding was higher in both S2 and P1 fractions compared with other fractions in liver, while both S3 and P3 fractions exhibited the highest activity among different subcellular fractions in cerebellum. In primary cultures of rat cerebellar neurons, both tryptamine and indigo potentiated XRE binding in a concentration-dependent manner when determined immediately after sustained exposure for 2 h. These results suggest that XRE binding proteins may be highly expressed in rat cerebellum for amplification and/or consolidation of transient extracellular signals carried by exogenous pollutants with subcellular distribution profiles different from those in liver. Particular compounds with an indole skeleton could be endogenous ligands for AhR in cultured rat cerebellar granule cells.

P02-E-29

Estrogen elevates PKA activity via the putative membrane receptor in hippocampal neurons.

○Akiko S. SHINGO, Shozo KITO
Hyogo Univ. Sch. of Health Sci.

For the past several years, estrogen actions rapid in onset, short in duration have attracted considerable attention. In relation with such obviously nongenomic estrogen effects, membrane estrogen receptors have been reported on pituitary, uterine, ovarian granulosa cell and liver cell membranes. In these putative membrane receptors, it has been advocated that the membrane receptor is linked to G-protein-conjugated adenylyl cyclase activation and thereby induces phosphorylation of proteins by cAMP-dependent protein kinase (PKA).

This study was undertaken to confirm estrogen-evoked PKA activation in the living cell with use of immortalized hippocampal neurons. 17β -estradiol-stimulated PKA activation in living differentiated H19-7 cells was visualized using DR2, a cell-permeable fluorescent PKA substrate. Estradiol induced marked changes of fluorescence in cytosolic areas and these were inhibited by a PKA inhibitor.

P02-E-30

A Novel FERM Domain Including Guanine Nucleotide Exchange Factor Is Involved In Rac Signaling and Regulates Neurite Remodeling

○Tateki Kubo, Toshihide Yamashita, Masaya Tohyama
Dept. Anat. Neurosci. Grad. Sch. Med., Osaka Univ., CREST

The Rho family of small GTPases, key regulators of the actin cytoskeleton in eukaryotic cells from yeast to humans, is implicated in the control of neuronal morphology. Guanine nucleotide exchange factors (GEFs) are upstream positive regulators of Rho GTPases, and integrate extracellular signaling for appropriate activation of Rho GTPases at specific subcellular regions. Here, we describe the identification of a novel Dbl family GEF for Rho GTPases in *Homo sapiens* and *Mus musculus*. It contains a tandem DH/PH domain and FERM domain, characteristic of the plasma membrane proteins linker. This gene, termed FERM domain including RhoGEF (FIR), was abundantly expressed in brain, lung and testis as well as embryonic hippocampal and cortical neurons. FIR was found to activate the biochemical pathway specific for Rac1, but not for RhoA or Cdc42. Ectopic expression of FIR in the cortical neurons resulted in significantly shortened neurites and excessive growth cones, presumably mediated by Rac. These results suggest that FIR may regulate neurite remodeling by mediating the signaling pathways from membrane proteins to Rac.

P02-E-31

Biological activity of neurotrophins is dependent on proper recruitment of Rac1 to lipid rafts

○Akiko Honda^{1,2,3}, Toshihide Yamashita^{1,2}, Tateki Kubo^{1,2}, Hiroyuki Tanaka^{1,2}, Taiichi Katayama^{1,2}, Masaya Tohyama^{1,2}

¹Anatomy and Neuroscience, Grad. Sch. Med, Osaka Univ, ²CREST,JST, ³Discovery Research Laboratory, Tanabe Seiyaku Co.Ltd

The Rho family of small GTPases, key regulators of the actin cytoskeleton in eukaryotic cells, is implicated in the control of neuronal morphology. Here we report that neurotrophins dependent cytoskeletal changes, characteristic of the phenotype of Rac1, in the hippocampal neurons or PC12 cells are perturbed by the disruption of lipid rafts integrity. Activation of Rac1 induced by NGF is impaired in cholesterol-depleted PC12 cells. Pretreatment with gamma-GTP shifted significant amount of Rac1, presumably in a GTP-bound form, from non-raft to raft fractions. Proper recruitment of activated Rac1 to lipid rafts, structures that represent specialized signaling organelles, is of fundamental importance in determining neurotrophins bioactivity.

P02-E-32

Morphological changes in mouse neuroblastoma caused by disruption of glycosylation

○KOUSUKE BABA, TAIICHI KATAYAMA, MASAYA TOHYAMA

Department of Anatomy and Neuroscience, Graduate School of Medicine, Osaka University, CREST,JST

Recently, implication of post-translation and function on cell is one of the major current topics. Several studies have indicated that oligosaccharide play important roles in normal cells, and alternations in their structures and binding may cause serious problems in cell function. To address this issue, we examined the possible morphological and physiological changes of the cells treated with various inhibitors of glycosylation. As used cell lines and reagent, we employed CAD cells a mouse neuroblastoma and PC12 cells, and 2-deoxy-D-galactose(2DG), which hinders glycoprotein fucosylation. More than 50% of the CAD cells treated with 1-20mM 2DG for 6-12hours showed vacuole-like morphological changes. In contrast, similar treatment of PC12 cells did not cause any morphological changes. To clarify the morphological basis of the vacuoles and its molecular mechanisms in CAD cells, we perform molecular biological and immunohistochemical analysis of the present results in both cells.

P02-E-33

Identification of GRP78 as a type-1 sigma receptor (sigmaR1) -associated protein.

○Hideko Yamamoto¹, Fuyuki Kametani¹, Yoshiko Namiki¹, Toshifumi Yamamoto^{1,2}, Junnichi Karasawa^{1,3}, Ichiro Sora^{1,4}, Toshihide Nukada¹

¹Tokyo Institute of Psychiatry, ²Yokohama City University, ³Nihon Shering Co., ⁴Tohoku University

Several psychoactive agents including antipsychotics, phencyclidine, neurosteroids, benzomorphans and cocaine have high affinities for sigma receptors. Sigma receptors were pharmacologically classified into type-1 (sigmaR1) and type-2 subtypes. SigmaR1, which is recently cloned, is expressed at high levels in the brain, liver and immune cells, and thought to play critical roles in the action of these psychoactive agents. It remains to be elucidated, however, how sigmaR1 transduces signals into the cells and what kinds of molecule are involved in this signaling pathway. Moreover, there is a large discrepancy between the molecular weights of sigmaR1 as estimated by immunoblot analysis (30-kDa) and those by gel filtration (440- to 450-kDa), indicating the presence of sigmaR1-associated proteins in native tissues. To address these issues, proteins associated with sigmaR1 were purified from rat liver by affinity chromatography on glutathione S-transferase (GST) proteins fused with a cytoplasmic region (amino acid residues 118-223) of sigmaR1. Affinity-purified proteins with an apparent molecular weight of 80-kDa on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were subjected to partial amino acid sequencing. One of these proteins was identified as 78-kDa glucose-regulated protein (GRP78/BiP), an endoplasmic reticulum stress-response protein. The association of sigmaR1 with GRP78 in the cells was confirmed by co-immunoprecipitation, in which an anti-GRP antibody immunoprecipitated sigmaR1 in the extracts of rat brain and liver and in PC12 cells as well. These results indicate that sigmaR1 may regulate the stress responses through interaction with GRP78.

P02-E-34

Regulation of exocytotic events and granular movements by protein kinase C in single chromaffin cells

○Nobuyuki Sasakawa, Sanae Hosaka, Mitsunori Hayashi, Konosuke Kumakura

Life Sci. Inst. Sophia Univ.

To clarify the implication of protein kinase C (PKC) in the regulation of exocytosis, we studied the effects of TPA on the movement of vesicles and on the kinetics of exocytosis of single vesicles in intact chromaffin cells. The track motion of LysoTracker Green DND26-labeled secretory granules were detected using fluorescence microscopy-cooled CCD system. Granule-positions were recorded every 2 s for 2min in normal buffer after stimulation with high K⁺ for 2 min. If we classify the vesicle movements into three groups on the basis of the velocity, about 60% of the vesicles showed a rapid movement, about 30% of the vesicles showed a moderate movement and about 10% of the vesicles showed a slow movement in control cells. The population of granules with a moderate movement was remarkably increased in TPA-treated cells. These results suggest that this type of granule movement may be regulated through PKC activation. Carbon fiber electrode-amperometric detection showed that the event frequency of initial 1 min and that after 2 min during 5min of 30 mM KCl stimulation was remarkably increased by TPA, suggesting that the size of readily releasable pool and the vesicle recruitment are regulated by PKC. We characterized the kinetics of single events by analysis of rise time, middle width and spike area. The rise time and middle width increased by treatment with TPA, while the spike area was not affected. These results suggested an important role of PKC for the regulation of fusion processes in adrenal chromaffin cells.

P02-E-35

Identification of a trans-acting protein interacting with cis-element for dendritic targeting of alpha-CAMKII transcript.

○Yasutake Mori, Shingo Miyata, Masaya Tohyama
Dept. Anat. and Neurosci. Grad. Sch. Med., Osaka Univ., CREST

An increasing number of mRNAs are dendritically targeted in the central nervous system (CNS) neurons. Of these transcripts, alpha-CAMKII mRNA is actively transported along the dendrites of pyramidal neurons in the hippocampus and translated *in situ* in response to external stimuli. We previously determined a cis-acting element for dendritic localization of alpha-CAMKII transcript in its own 3'-untranslated region (3'-UTR). The element entails at most 94 nucleotide-long region in the 5'-most side of the 3'-UTR and mediates active transport of alpha-CAMKII mRNA along dendrites of hippocampal culture neuron. Using *in vitro* transcribed ³²P-labeled oligonucleotide corresponding to this cis-element, we performed RNase protection assay to seek for interacting protein(s). S3 fraction from rat brain homogenate was incubated with the radiolabeled riboprobes in the presence or absence of the cis-acting element, followed by UV-crosslinking and RNase A treatment. Each reaction mixture was isolated by denatured gel. A 55kDa band could be reproducibly detected by incubating with riboprobes containing the cis-acting element, while deletion of the element from radiolabeled riboprobes completely abolish the interaction. Excess amount of non-labeled nucleotide could decrease the interaction in a dose-dependent manner. We demonstrated the possibility that the protein we detected is a trans-acting factor for dendritic targeting of alpha-CAMKII transcript.

P02-E-36

Influx of extracellular Ca⁺⁺ induced the expression of arc mRNA in PC12

○Hisashi Ichikawa, Eiichi Taira, Naomasa Miki
Dept. Pharmacol., Osaka Univ. Med. Schl

Arc, an activity-regulated cytoskeleton-associated protein, was first isolated from a rat brain. Arc expression can be rapidly induced in the specific brain regions by physiological synaptic activity, presenting an immediate-early gene (IEG) response such as c-fos, junB, zif-268. But much less is known about the mechanism of arc mRNA expression. To investigate the mechanism of expression of arc mRNA in detail, we initially analyzed the expression level of arc in neuronal cell lines. We detected significant expression of arc mRNA in four cell lines, NG108-15, N18TG2, Neuro2A and PC12, but not in C6. PC12 cells expressed the most abundant in above four cell lines. Next we examined whether the expression of Arc mRNA could be changed by various stimuli which change the second messenger in PC12. We found that NGF, forskolin, depolarization by KCl and A23187 markedly induced the expression of arc mRNA. We speculated that the induction of arc requires the influx of extracellular Ca⁺⁺ from the results of induction by A23187. To confirm this, we treated PC12 with A23187 under the Ca-free condition. The absence of Ca⁺⁺ resulted in the block of the induction. Moreover, the addition of Ca⁺⁺ to this condition resulted in recovery of the induction by A23187. We concluded that the influx of extracellular Ca⁺⁺ is one of the triggers in induction of arc mRNA.

P02-E-37

Activation and phosphorylation of Ca²⁺/calmodulin-dependent protein kinases in cultured rat hippocampal neurons

○Akiyoshi Uezu, Kohji Fukunaga, Jiro Kasahara, Eishichi Miyamoto
Dept. Pharmacology, Kumamoto Univ. School of Medicine

We have focused on activation mechanisms of CaM kinase I and CaM kinase IV in the cultured hippocampal neurons. In the immunohistochemical studies, CaM kinase I was predominantly localized in the cytosol of the neurons. This is contrast to the predominant localization of CaM kinase IV in the nuclei. We prepared a specific assay method for CaM kinase I. Similar to CaM kinases II and IV, CaM kinase I in neurons was essentially activated by stimulation with the NMDA glutamate receptor. The activation of CaM kinase I was persistent during stimulation with glutamate contrast to a transient activation of CaM kinase IV. In addition, CaM kinase I was activated by a lower concentration of glutamate than that of CaM kinase IV. Depolarization-induced activation of CaM kinase I was also evident in cultured neurons and was largely blocked by L-type calcium channel antagonists. In the experiment with ³²P-labeled cells, phosphorylation of CaM kinase I was stimulated by glutamate treatment and the glutamate-induced phosphorylation was inhibited by the NMDA receptor antagonist. Cyclosporin A (CsA) had no effect on glutamate-induced CaM kinase I activation, while CaM kinase IV increased its activity, suggesting that CaM kinase I is not regulated with calcineurin. These results suggest differences in *in situ* regulation of CaM kinases I and IV in neurons.

P02-E-38

Activation of Cdk5 by the activation subunit p39

○Akio Sekigawa, Taro Saito, Yuko Hamazumi, Akiko Asada, Shin-ichi Hisanaga
Dept. of Biol. Sci., Grad. Sch. of Sci., Tokyo Metro. Univ.

Cdk5 is a serine/threonine kinase, structurally related to cyclin-dependent kinases (Cdks) known as cell cycle promoters in proliferating cells. However, Cdk5 activity is primarily detected in differentiated neurons because of the limited expression of the Cdk5-specific activation subunits, p35 and p39. The properties of Cdk5 bound to p35 have been studied well, but little is known about Cdk5 associated with p39. p39 is composed of the N-terminal region specific to p39 and the C-terminal Cdk5-binding and activation regions with a high homology to p35. Mice lacking either or both genes of them have been generated. The comparison of their phenotypes indicates that p35 and p39 are functionally non-redundant. To understand the exact roles of p39/Cdk5, it is important to know how p39/Cdk5 activity is regulated in neurons. Whereas p39/Cdk5 is shown to display the histone H1 kinase activity *in vitro*, only the weak activity has been detected in brains. In this study, we compared the complex formation and activation of p39/Cdk5 with those of p35/Cdk5. His-tagged p35 or p39 was coexpressed with Cdk5 in Sf9 insect cells and purified by a Ni-NTA agarose column. Compared to p35, purified p39 bound the smaller amount of Cdk5, suggested that p39 has a lower affinity for Cdk5 than p35. We also found the p39/Cdk5 complex without the kinase activity. These results were confirmed with p39/Cdk5 purified from rat brains by immunoprecipitation with anti-p39 antibody. Rat brain p39 showed the lower affinity for Cdk5 than p35, and the non-active p39/Cdk5 complex was found. The lower affinity and the kinase inactive complex may be reasons for the weak kinase activity of p39/Cdk5 in rat brains.

P02-E-39

Characterization of a novel protein kinase that associate with Cdk5/p35

○Akiko ASADA, Naoyuki Honma, Sen Takeshita, Eiko Yamakawa, Mariko Enomoto, Taro Saito, Shin-ichi hisanaga

Department of Biological Sciences Graduate School of Science Tokyo Metropolitan University

Cdk5 is a member of the cyclin-dependent kinase family. Although expression of Cdk5 is limited in adult tissues, its kinase activity is limited to neurons. It is due to the limited expression of Cdk5 activator protein p35 in neurons. Cdk5/p35 concentrates at the leading edges of axonal growth cone and regulates neurite outgrowth in cortical neurons in culture. Mice lacking either p35 or Cdk5 exhibit a disrupted cortical lamination pattern. These results suggest that Cdk5/p35 has some function in neurons that migrate long distance during cortical layer formation. Reelin and mDab1 null mouse display nearly identical phenotypes as p35 or Cdk5 null mouse implying that those molecules act in a sequence of pathway during corticogenesis. To identify the factors among them, a yeast two-hybrid screen was performed using p35 and a human embryonic brain cDNA. The partial cDNA fragment with a highly homology to a novel cytoplasmic tyrosine kinase was isolated. According to the result of RT-PCR performed on human brain mRNA, this fragment deduced a homolog of the novel cytoplasmic tyrosine kinase and named p35 binding tyrosine kinase (p35BTK). P35BTK contain tyrosine kinase domain at the N-terminal, PXXP SH3 binding consensus motif, and several SP/TP sequence. P35BTK associated not only with p35 but also with Cdk5 in 293 cells. Cotransfection with both p35 and CDK5 decreased the electrophoretic mobility of p35BTK. This is because of phosphorylation on serine or threonine residue since the mobility is returned after treated with BAP. P35BTK was verified as a substrate of Cdk5/p35 in vitro but association was independent of phosphorylation. Northern blots of mouse tissues showed that p35BTK mRNA was expressed mainly in brain and could be detected from embryo to adult brain. Further investigation is needed to know the function of p35BTK in neuronal cells.

P02-E-40

Prostaglandin I₂ potentiates bradykinin-induced phosphorylation of synapsin I at site-3 in Neuro-2A cells.

○Yuri Matsuo, Keita Torihata, Hirotsugu Kato, Yasuharu Sasaki

Dept. Pharmacol. Sch. Pharmac. Sci. Kitasato Univ.

Inflammatory mediators such as prostaglandins (PGs) are well known to enhance the algesic effects of bradykinin (BK) in sensory neurons, probably by increasing the intracellular Ca²⁺ ([Ca²⁺]_i) and enhancing the neurotransmitter release, however, little is known about the molecular mechanisms of these hyperalgesic responses. Recently, the site specific phosphorylation of synapsin I at site-3 (Ser 603) by Ca²⁺/calmodulin-dependent kinase II (CaMKII) is reported to induce neurotransmitter release. Therefore, in the present study, to reveal molecular mechanisms underlying hyperalgesia, we determined the BK-induced phosphorylation of synapsin I at site-3 and its enhancement by PGI₂ in mouse neuroblastoma Neuro-2A cells, which expressed functional BK B₂ receptor and PGI₂ receptor. Using an antibody against site-3-phospho-synapsin I, we found that BK phosphorylates synapsin I at site-3, which was sensitive to Hoe 140, a bradykinin B₂ receptor antagonist, and thapsigargin, which deplete intracellular store Ca²⁺, but insensitive to des-Arg Hoe 140, a bradykinin B₁ receptor antagonist, and KN62, an inhibitor of CaMKII. Furthermore, this BK-induced KN62-insensitive phosphorylation, but not the 100 mM KCl-induced CaMKII-dependent one, was potentiated by stable PGI₂ analogue carbacyclin, and cAMP elevating agents such as dibutyryl cAMP and forskolin. BK-induced [Ca²⁺]_i rise was also potentiated by carbacyclin. These results suggest the possibility that BK recruits a novel pathway including [Ca²⁺]_i rise from intracellular Ca²⁺ store, besides CaMKII involved one, to phosphorylate the site-3 and which is potentiated by PGI₂ through cAMP elevation in sensory neurons.

P02-E-41

 α CaM kinase II 遺伝子の神経特異的発現

○美馬 佳寿子, 山内 卓
徳島大・薬

カルモデュリン依存性プロテインキナーゼ II (CaM kinase II) は、細胞内情報伝達に関わる代表的なセリン/トレオニンプロテインキナーゼの一つであり、最終の奏功因子として作用すると考えられている。CaM kinase II は、脳の発達過程において神経回路形成のもっとも盛んな時期に一致して発現が増加することが知られているが、その調節については明らかになっていない。CaM kinase II には $\alpha, \beta, \gamma, \delta$ の4つのアイソフォームが存在し、そのうち α と β アイソフォームは脳に特に多く存在する。本研究では、 α アイソフォーム遺伝子の構造と神経特異的発現について明らかにし、さらにプロモーター領域に結合する脳の核タンパク解析した。 α アイソフォーム遺伝子の 5' 上流領域 2.3 kbp の遺伝子配列の解析を行った。この領域は β アイソフォームと相同性はなく、TATA box, v-myb, Myo D などの転写因子結合サイトが存在するが、既知の神経特異的転写因子は存在しない。この遺伝子のルシフェラーゼレポーターを構築し、プロモーター活性を測定した。神経細胞として N18TG2, NG108-15, CAD、非神経細胞として Balb/c3T3, CHO, C6 glioma, HT1080 を用い、トランスフェクション実験を行った。神経細胞では、転写開始部位上流 -199 bp から -100 bp の間で高いプロモーター活性が見出された。しかし、非神経細胞では活性が弱く、神経細胞に比べて100倍から500倍活性は低かった。一方、上流 -275 bp から -199 bp の配列を含むレポーター遺伝子では活性は著しく低下した。この領域に強いサイレンサーが存在し、本遺伝子の強い負の調節を行っていると考えられる。以上のことから、この領域に神経細胞に特異的な強いプロモーターが存在することを明らかにした。さらに、ゲルシフト分析により、プロモーター領域のDNA配列に特異的に結合する脳の核タンパクの存在を見出した。

P02-E-42

Antiapoptotic action of calcineurin via dephosphorylation of Bcl-2

○Hideyuki Yamamoto¹, Takeo Nomura², Hiromitsu Mimata², Miki Shitashige³, Futoshi Shibasaki³, Yoshio Nomura², Eishichi Miyamoto¹

¹Dept. Pharmacol., Kumamoto Univ. Med. Sch., ²Dept. Urology, Oita Med. Univ., ³Mol. Cell Physiology, Tokyo Metro. Inst. Med. Sci.

Calcineurin is the only serine/threonine protein phosphatase under the control of Ca^{2+} and calmodulin, and is most abundant in brain. It is well-known that calcineurin is widely distributed in various tissues and plays a critical role in the regulation of many cellular functions. We found that calcineurin bound to the BH4 region of Bcl-2, and Bcl-2 has been reported to be an *in vitro* substrate for calcineurin. Therefore, we examined whether calcineurin had an antiapoptotic action via dephosphorylation of Bcl-2. When we overexpressed Bcl-2 in COS-7 cells and immunoprecipitated c-Jun N-terminal kinase (JNK), the binding of Bcl-2 to JNK was observed. Among the cell systems examined, taxol treatment of urinary bladder cancer cells (T24 cells) induced the activation of JNK and phosphorylation of Bcl-2 at serine 70 which is one of the critical phosphorylation sites in Bcl-2. Interestingly, phosphorylation of Bcl-2 was strongly enhanced by calcineurin inhibitors, cyclosporin A (CsA) and FK506, with a concomitant loss of calcineurin activity. When the apoptosis was examined by Hoechst 33258 staining and by the measurement of caspase 3 activity, CsA treatment enhanced taxol-induced apoptosis. Therefore, calcineurin may have an antiapoptotic action via the dephosphorylation of Bcl-2 in the case of apoptosis induced by Bcl-2 phosphorylation.

P02-E-43

Direct observation and characterization of gels formed from isolated neurofilaments *in vitro*

○Masashi Kurachi¹, Shinpei Haraikawa², Rei Ono², Jean-Francois Leterrier³, Yoshiaki Komiya¹, Tomoko Tashiro^{2,4}

¹Dept. Mol. Cell. Neurobiol., Gunma Univ. Sch. Med.,

²Dept. Biochem., Aoyama-Gakuin Univ. Coll. Sci. Eng.,

³CNRS, Poitiers Univ., ⁴CREST, JST

Neurofilaments (NFs) confer mechanical stability to the axon by forming highly organized arrays based on NF-NF interaction. To understand how this interaction is regulated, we developed a method in which NF gel formation is directly followed *in vitro* by the Brownian movement of Latex microbeads (0.5 μ m in diameter) added to the solution. Bead movements were tracked with video-enhanced DIC microscope, recorded, and their positions in the XY plane plotted at every 1/30 second interval to give mean square displacement (MSD) values. With sucrose solutions of different concentrations, the slopes of the MSD vs. time plot (Δ MSD/t) were confirmed to be inversely related to the viscosity η . With native NFs extracted from rat spinal cords and purified by sucrose density gradient centrifugation, Δ MSD/t in 0.8M sucrose was dependent on NF protein concentration below 1.5mg/ml. Δ MSD/t in pure sucrose solutions were constant at 37°C, while those in sucrose solutions containing NFs declined with time, indicating that gelation took place. A rapid decline within the first hour after warming was followed by a slower phase of $\sim$ 4 h. When NFs were extensively dephosphorylated with *E. coli* alkaline phosphatase, the initial decline in Δ MSD/t became larger and more rapid, and the final level lower than the control, suggesting that NF gelation is enhanced. Previously, NF viscosity measured with the falling ball viscometer has been shown to decrease by dephosphorylation. The falling ball method measures resistance against mechanical forces, while our method reflects gel formation by the entrapment of beads. The contradictory results of the two methods thus indicate that dephosphorylation enhances gel formation but decreases the mechanical strength of the gel formed.

P02-E-44

A gene product binding to the 3'-untranslated region of *type1 inositol 1,4,5-trisphosphate receptor* mRNA and its involvement in mRNA localization in cerebellar Purkinje cells

○Takatoshi Iijima^{1,2,4}, Yuki Kimura³, Kanako Takatuki⁵, Takao Imai¹, Shigetaka Kawahara⁵, Aran Bernstein³, Yutaka Kirino⁵, Hideyuki Okano^{1,2}

¹Dept. of Physiology Keio Univ. Medical School,

²CREST, JST, ³Samuel Lunenfeld Res. Inst. Mount Sinai Hospital, CAN, ⁴Dept. of Molecular and Cellularbiology, Graduate School of Medicine, Osaka Univ., ⁵Lab. of Neurobiophysics, Graduate School of Pharmaceutical Sciences, Tokyo Univ.

In neuronal cells, specific mRNAs are localized in the dendrites. It has been proposed that the dendritic localization of mRNAs and the locally translational regulation in synaptic region are involved in certain aspects of synaptic plasticity. *Type1 inositol 1,4,5-trisphosphate receptor (IP₃R1) mRNA* is one of dendritically localized mRNAs. *IP₃R1*, an intracellular Ca²⁺ channel, is highly expressed in cerebellar Purkinje cells and required for long-term depression (LTD), a model of synaptic plasticity in cerebellum. However very little has been shown on the mechanisms of the dendritic localization of *IP₃R1 mRNA*. *Here we identified one gene, hzf* (hematopoietic zinc finger), encoding a protein that might be involved in the localization of *IP₃R1 mRNA* into dendrites. *hzf* encodes three C2H2 type zinc-finger motifs. RNA-binding experiment showed that Hzf binds to 3' untranslated region (3' UTR) of *IP₃R1 mRNA in vitro*. Furthermore, in cerebellum, *hzf* was predominantly expressed in Purkinje cells, corresponding to the expression pattern of *IP₃R1*, and its products were localized in the dendrites of Purkinje cells, where *IP₃R1 mRNAs* are localized. Additionally it was revealed that the amount of *IP₃R1 mRNA* in dendrites of Purkinje cells could dramatically reduce in *hzf*-deficient mice. These results suggests the possibility that Hzf could be involved in intracellular localization of *IP₃R1 mRNA* in Purkinje cells. Furthermore, *hzf*-deficient mice exhibit ataxia like symptoms. Then we are investigating the relation between the mRNA localization and cerebellar LTD.

P02-E-45 (MS1-6)

The Roles of WAVES in Filopodia Formation at NG108 Growth Cones

○Shigeaki Miyamoto¹, Motohiro Nozumi¹, Hiroyuki Nakagawa¹, Hiroaki Miki², Tadaomi Takenawa²

¹Dept. Biochem. Eng., Kyushu Inst. Tech., ²Dept. Biochem., Inst. Med. Sci., Univ. of Tokyo

At the neural growth cones, lamellipodia and filopodia formation are promoted by actin-cytoskeleton reorganization as filaments branch formation and bundling. WAVE1~3 molecules, belong to WASP (Wiskott Aldrich Syndrome Protein) family, are thought to play an important role on membrane ruffling and filopodia formation. However, their exact functions remain to be obscure. In this study we paid special attentions to the different roles that WAVE1~3 play in this process. We constructed WAVE1~3-EGFP fusion genes and expressed these to neuroblastoma NG108-15 cells, and observed the localizations and effects on filopodia formation.

【RESULTS】 We induced neurites to NG108-15 cells expressed with WAVE1~3-EGFP by dibutyl-cAMP treatment. WAVE1 molecules were distributed continuously to the leading edge alone of growth cone lamellipodia. WAVE2 localized to the tips of filopodia and stayed during formation and elongation, and disappeared in the retraction. And WAVE3 weakly accumulated to the tips of filopodia. The expression of each WAVE-domain-EGFP fusion constructs show that the localization of WAVES are controlled by SHD(Scar Homology Domain) and the actin bundle elongations are promoted by VCA(Verprolin Homology + Cofilin Homology + Acidic) regions. The localization of WAVE2 to the tips of filopodia may be controlled by another scaffold protein IRSp53, Insulin Receptor Substrate protein 53KDa, which is thought to link Rac and it. The above results suggest that filopodia formation and elongation are promoted by actin polymerization which is controlled by WAVE2 in the downstream of Rac and IRSp53 at the tips.

P02-E-46 (MS1-5)

A role of semaphorin in newronal network formation in cerebral cortex

○Yoshio Goshima, Yukio Sasaki

Dept. Pharmacol. Yokohama City Univ. Sch. of Med.

Semaphorin-3A (Sema3A), a member of class 3 semaphorins, regulates axon and dendrite guidance in the PNS and CNS. In the present study, we used inhibitors and mutant mice and demonstrate that a Src family tyrosine kinase, Fyn and cyclin-dependent kinase 5 (Cdk5) are involved in Sema3A-induced growth cone collapse. Furthermore, biochemical analysis in non-neuronal cells reveals that one of receptor component for Sema3A, plexin-A2 associates with Fyn and Cdk5. Fyn facilitates to activate Cdk5 mediated with Tyr15 phosphorylation of Cdk5. Introduce of Cdk5 mutant converted Tyr15 to Ala to sensory neurons suppresses Sema3A-induced growth cone collapse. sema3A gene shows strong genetic interaction with fyn for apical dendrite guidance in cerebral cortex. Thus, these findings suggest that Fyn and Cdk5 mediate neuronal guidance regulated by Sema3A.

P02-E-47 (MS1-4)

Lysophospholipid signaling in the regulation of neuronal morphology and motility

○Nobuyuki Fukushima

Dept. Biochem. Grad. Sch. Med., Hokkaido Univ.

Lysophosphatidic acid (LPA) is a growth factor-like phospholipid that produces diverse cellular responses. Three subtypes of LPA receptor have been so far identified and shown to be expressed in the developing nervous system. Here I have investigated effects of exogenous LPA on the morphology and underlying actin cytoskeleton of young, postmitotic cortical neurons in primary culture. When treated with LPA, differentiating neurons responded by retracting processes, as expected from studies on neural cell lines, but unexpectedly also showed formation of a novel, retraction fibers that contained radially oriented actin filaments emanating from a single pole of the neuron. This cytoskeletal change utilized Rho, tyrosine kinase and actomyosin pathways. The maximal retraction response was observed at 15 min after LPA exposure, and thereafter neurons reextended process/membranes along with retraction fibers, suggesting that these fibers played a role as scaffolds. I also observed that LPA inhibited neuronal migration from explanted tissues. Taken together, these data implicate LPA as a regulatory signal not only for process outgrowth but also for migration of young, postmitotic neurons. Another aspect of actin regulation by LPA will be also presented. Fukushima et al., *Annu. Rev. Pharmacol. Toxicol.*, (2001) 41, p507, Fukushima et al., *MCN*, in press., Fukushima et al., *The neuroscientist*, in press.

P02-E-48

Identification of fimbrin as a interacting molecule of microglia-specific protein, Iba1.

○Keiko Ohsawa, Yoshinori Imai, Yo Sasaki, Hiroko Kanazawa, Shinichi Kohsaka

Dept. Neurochem., Natl. Inst. Neurosci.

Iba1 is a 17 kDa EF-hand calcium binding protein specifically expressed in microglia among brain cells. We have previously reported that Iba1 has actin-bundling activities and is involved in membrane ruffling and phagocytosis of microglia. In the present study, we isolated Iba1-interacting molecules using a yeast two-hybrid screen and identified an actin-bundling protein, fimbrin.

Iba1 has unique sequences in the N- and C-termini which display no homology with any other peptides. We constructed expression vectors encoding of the N- (a.a. 1-29) or C-terminus (a.a. 114-147) that was fused with EGFP and express the fusion proteins in microglial cell line MG5 cells. The C-terminus protein colocalized with F-actin in membrane ruffles induced by M-CSF, while the N-terminus protein did not. Therefore we screened human spleen cDNA library with the C-terminus as a bait and obtained 8 positive clones. Six clones encoded a part of L- or T-fimbrin. L-fimbrin, one of three fimbrin isoforms, is specifically expressed in hematopoietic cells. By immunocytochemistry of MG5, L-fimbrin was shown to colocalize with Iba1 and F-actin in membrane ruffles induced by M-CSF. To analyze the interaction between Iba1 and L-fimbrin, COS-7 cells were cotransfected with HA-tagged fimbrin and Flag-tagged Iba1 expression plasmids. From the cell lysate, HA-fimbrin was coprecipitated with Flag-Iba1. Purified GST-Iba1 was able to pull down purified His-tagged L-fimbrin, indicating that Iba1 directly binds to L-fimbrin. Furthermore, actin-bundling activity of L-fimbrin was synergistically enhanced by the addition of Iba1. These results indicate that Iba1 forms complex with fimbrin and functions in modulating actin reorganization in membrane ruffling.

P02-E-49

Identification and characterization of drebrin binding protein, Drap1

○Hiroyuki Yamazaki¹, Hideto Takahashi¹, Eiji Hirose², Nozomu Mori², Tomoaki Shirao¹

¹Dept. Neurobiol & Behav., Gunma Univ Sch Med,

²Dept. Molec Genetics., Natl Inst Longevity Sci (NILS)

Drebrin is an F-actin binding protein mainly located in neuron. In the adult cerebral cortex, drebrin is concentrated primarily in the dendritic spines. We have shown that accumulation of GFP-drebrin within a dendritic spine results in elongation of the spine in vitro, possibly via rearrangement of the actin cytoskeleton. However, the mechanism that drebrin regulates the actin cytoskeleton have not been understood. In the purpose of investigating drebrin function in the molecular basis, we have tried molecular cloning of a novel drebrin binding protein. In the course of yeast two-hybrid screen of a rat brain cDNA library using the N-terminal region of drebrin as bait, we isolated many positive-interacting clones. DNA sequence analysis revealed that about 70% of these clones were expressing an identical protein. We named this protein drebrin-associated-protein 1 (Drap1). Computational analysis of Drap1 structure by using the SMART showed that Drap1 was likely to have a transcriptional factor domain. To determine a binding region of the drebrin-Drap1 interaction, we performed a domain analysis with drebrin bait and several fragments of Drap1. The domain analysis showed that the ADF-H domain of drebrin bound to N-terminal region of Drap1 in yeast two hybrid system. It was also indicated in vitro by GST pull down assay. To assay Drap1 properties in cells, we carried out a transfection of GFP-Drap1 in COS-7 cells. In COS-7 cells, GFP-Drap1 exclusively localized in nucleus. However, when GFP-Drap1 and drebrin were coexpressed in COS-7 cells, Drap1 localized not only in nucleus but also in cytoplasm in a large number of co-transfected COS-7 cells. It suggested that the cellular localization of Drap1 might be affected and regulated by drebrin.

P02-E-50

Anterograde axonal transport of endopeptidase 24.15 in rat sciatic nerves

○Masaru Yamamoto¹, Toshiyuki Chikuma², Atsue Yamashita², Mitsune Yamaguchi², Hiroshi Hojo², Md Mahiuddin Ahmed¹, Takeshi Kato¹

¹Grad. Sch. Int. Sci. Yokohama City Univ., ²Dept. Hygien. Chem., Showa Pharmaceut. Univ.

The functional level of a biologically active neuropeptide is regulated, in part, by its degrading-enzymes. Endopeptidase 24.15 (EP24.15) is one of putative neuropeptide degrading-enzymes which degrade a number of neuropeptides in vitro. However, it is not yet known whether EP24.15 involves in the inactivation of these neuropeptides in vivo, because intracellular distribution of EP24.15 exists mainly around nucleus. If EP24.15 plays a role in the metabolism of neuropeptides, it might be anterogradely transported. Thus, in the present study we used a double-ligation technique to quantify the axonal transport of EP24.15 in rat sciatic nerves. At 48 h after the ligations, a significant amount of the axonal transport of EP24.15 activity was found in the proximal segment, while axonal transport of deamidase activity, a lysosomal enzyme, increased both in the proximal and distal segments. Western blot analysis of EP24.15 showed that immunoreactive EP24.15 in the proximal segment was 1.8-fold higher than that in the middle segment. The immunohistochemical analysis of the segments also showed an increase of the immunoreactive EP24.15 in the proximal segment in comparison with that in the middle segment. No significant retrograde axonal transport of EP24.15 was found in the distal segment in all methods examined, indicating that EP24.15 is mainly transported by an anterograde axonal flow. These observations suggest that EP24.15 may involve in the metabolism of neuropeptides in nerve terminals or synaptic clefts.

P02-E-51

Identification of A Novel Neural-Specific Protein Family, BRINP

○Toda Fumiaki, Ueno Satoshi, Mori Takuya, Nakatani Toshiyuki, Matsuoka Ichiro
Dept. Neurosci. Grad. Sch. Pharm. Sci., Hokkaido Univ.

Purpose : Bone morphogenetic protein (BMP) and retinoic acid (RA) are known to play important roles in differentiation of nerve cells. We have previously shown that BMP2 and RA synergistically act on developing sympathetic neurons to induce their responsiveness to neurotrophic factors that act on the initial stage of development of these neurons. In an effort to clarify the mechanism of the actions of BMP/RA, we have identified BRINP1 (BMP/RA-inducible neural-specific protein-1) whose expression is restricted to nervous system from late embryonic to adult stage. In the present study, we identified two homologues of BRINP1.

Results : Following the BLAST search in human genome database, we found DNA fragments in chromosome1 at two neighboring sections which are homologous to BRINP1. Based on sequence of these fragments, we designed PCR primers and obtained RT-PCR products from rat brain. Subsequently, by screening of the rat hippocampus cDNA library, we obtained two cDNA fragments (4.1 kb and 3.0 kb) which are encoding proteins with total amino acid residues of 783 (rBRINP2) and 766 (rBRINP3), respectively. RBRINP2 and rBRINP3 have amino acid identity of 70% and both had amino acid identity of 50-53% with rBRINP1. Like BRINP1, BRINP2 and BRINP3 have no conserved protein motifs except for N-terminal signal peptide and a single coiled-coil domain. RT-PCR experiments indicated that BRINP2 and BRINP3 are predominantly expressed in the CNS and PNS from developmental stage to adulthood. Northern blot and Western blot analysis (using anti-BRINP-peptides antibodies) showed similar tissue selectivity of their expressions. However, individual BRINP gene seems to have distinct regulatory mechanism of their expression within the nervous system. In sympathetic neurons, BRINP2 and BRINP3 expressions were suppressed by BMP and RA, while BRINP1 expression was induced by these factors.

Discussions : On the basis of similarity to BRINP1, we have identified two additional neural specific proteins, BRINP2 and BRINP3, that comprise BRINP family. In nervous tissues, three BRINPs are expressed in partly overlapping and partly complementary manners. We are currently aiming at clarification of the physiological functions of the BRINP family members.

P02-E-52

Transgenic RNA interference in *Drosophila*

○Shunji Ohsako, Yoshiki Takamatsu
Dept. Brain Struc. Tokyo Metropol. Inst. Neurosci.

Since vast genomic sequence information is now available in several organisms, RNA interference (RNAi), the process of which double-stranded RNA (dsRNA) induces the sequence-specific degradation of homologous mRNA, has become an important tool for the study of gene function. In *Drosophila*, injection of specific dsRNA into the early embryo results in interference persisting through the embryonic development. Expression of transgenic inverted repeats (IR) homologous to target genes that form ds hairpin RNAs under the control of the upstream activating sequence (UAS) or the heat-shock (HS) promoter has been reported to trigger RNAi in the embryo as well as in the adult. As it is difficult to manipulate DNA containing IR in *E.coli*, we made a P-element construct in which dsRNA can be transcribed by opposing UAS and HS promoters and established HS-UAS-*hairy* (*h*) transgenic flies to address whether this induces a *h* phenotype. *h* functions as a repressor of the adult sensory organ formation and also as a pair-rule gene during embryonic segmentation. Only one of three lines showed the *h* phenotype in which ectopic sensory organs are created but no segmentation defects, when they were crossed with *hGAL4* driver with *h* pattern. Next we compared its effectiveness with that in UAS-*h*-IR transgenic lines. The six lines showed either weak or no adult *h* phenotype by crossing with *hGAL4*. Finally, we constructed pDUAS+SA vector in which dsRNA is driven by dual UAS promoters. All of six pDUAS+SA-*h* transgenic flies showed adult *h* phenotype when were crossed with *hGAL4*, indicating that this vector becomes a very useful tool to study gene function in combination with various GAL4 driver lines.

P02-E-53

Optimization of water purification technology for biological experiment

○Naoe Ishii¹, Ichiro Kano², Masanori Kanazawa¹

¹Lab water Div., Nihon Millipore K.K., ²Lab water Div., Millipore S.A.

Purified water is as important as reagents which are used in biology domains. Sometimes impurities in water affect failing impact on experimental studies. Many biological experiments require strict reagent preparation.

For example purified water free from RNase is essential for RNA handling. DEPC treatment is often used to inactivate RNase in purified water, however DEPC is known as potent carcinogen and inhibit some enzyme reactions. Introduction of ultrafiltration device at the use point of water purification system delivers RNase free water which allows to be used for RNA handling and treatment. Bottled water guaranteed as RNase free provides also solution but is quickly contaminated once opened.

Sensitive analytical instruments are getting popular in this field recently. Organic contaminants affect seriously LC/MS background. UV photooxidation is a key of water purification process to eliminate organic impurities. When analyses and experiments are getting sensitive to contaminants, water storage in bottle are not any more available because of contaminations such as airborne gas and particle, extraction from bottle and microbial pollution.

P02-E-54

Identification and Characterization of ER-stress sensor IRE1 associated proteins

○Kayoko Oono^{1,3}, Takunari Yoneda^{1,3}, Kazunori Imaizumi², Taiichi Katayama^{1,3}, Masaya Tohyama^{1,3}

¹Dept. Anat. Neurosci., Osaka Univ. Grad. Sch. Med.,

²Dept. struct. cell. bio., NAIST, ³CREST

In our previous study neuronal death by neuropathy, for example Alzheimer's disease, is thought to be caused first in Endoplasmic Reticulum (ER). This neuronal death in ER is caused by accumulating misfolded polypeptides in the lumen of the ER during the ER stress; the any kind of stimulation or the change of environment. The accumulation of misfolded proteins in the lumen of the ER results in activation of the UPR, inducing transcriptional up-regulation of genes encoding molecular chaperones and folding catalysts present in the ER. One of the mediators of the UPR is a transducer of ER stress, the ER-resident transmembrane kinase IRE1. We searched the co-working molecule with IRE1 using yeast two hybrid system, to analyze the particular signal pathway of IRE1. As a result we found some IRE1-binding molecules. We examine more about JAB1, one of them, which reported to be concerning the cell cycle and the JNK pathway, for nothing studied about ER stress. We confirmed the binding between IRE1 and JAB1 by immunoprecipitation using HEK293 T cells overexpressed IRE1 and JAB1. And we make it clear that the strength of this binding becomes weak by ER stress exposure to these cells. In this institution we explain the role and function of JAB1 under ER stress.

P02-E-55

The 5' variants of p270, an integral member of SWI-SNF complexes, in the rat brain.

○Tsuyoshi Yamaguchi¹, Kazuaki Araki², Hiroyuki Nawa², Hiroshi Higuchi¹

¹Dept. Mol. Genetics & Signal Transduction Res. Grad. Sch. Medical & Dental Sci. Niigata Univ., ²Dept Mol. Neurology. Brain Res. Inst. Niigata Univ.

【 Introduction 】 SWI-SNF complexes have a general role in the regulation of gene expression. P270 is an integral member of human SWI-SNF complexes, and shared antigenic specificity with p300 and CREB-binding protein. B120 is also identified as a truncated form of p270. B120 gene products are expressed in 23 neuroblastomas, four of them strongly expressed B120 gene product in both cytoplasm and nucleus. Neuroblastoma cells transfected with B120 expression vector adhered to each other and aggregated through unknown mechanism. The genomic structure of B120 is constituted by 17 exons beyond 20kbp genomic DNA. So far little is known about brain distribution and transcriptional activity of p270. 【 Methods 】 We screened the embryonic rat brain cDNA library with the 0.6kb cDNA fragment of p270 as a probe. Sequence analysis was done by the combination of a ready reaction cycle sequencing kit and the 310 Genetic analyzer. We obtained three clones differed from p270 at 5' region including B120. To investigate transcriptional activity of p270, we transfected NG108-TG2 cells with pNPY-Luci using the calcium phosphate methods and measured luciferase activity using the luciferase reporter assay kit(Promega). 【 Results & discussion 】 In this study, we obtained novel p270 clones different at 5' region. These results showed that 5' variants of p270 exist in the brain and these variants may be physiologically relevant for brain-specific gene expression.

P02-E-56

Localization of prion-protein in cultured mouse neuroblastoma

○Reiko Kamata¹, Yuji Sakasegawa¹, Naomi S Hachiya^{1,2}, Kiyotoshi Kaneko¹

¹Department of Cortical Function Disorders, National Institute of Neuroscience, National Center of Neurology and Psychiatry, ²CREST,JST

'Prion' is a term first used to describe the mysterious infectious agent responsible for several neurodegenerative diseases found in mammals, including Creutzfeldt-Jakob disease (CJD) in humans. Despite of many efforts in dual decade, cellular functions of normal prion-protein have been unknown. Therefore to find prion-protein functions in vivo, we have taken a cell biological approach. To investigate the localization of prion-protein, immunofluorescent microscopy was performed using in cultured mouse neuroblastoma cells. After the fixation, the cells were treated with anti prion-protein antibodies without permeabilization the plasma membrane. In this experiment we found that prion-protein localized on the several regions of plasma membrane. Since this localization pattern was highly similar to that of the micro domain region of plasma membrane, called membrane raft, we examined whether prion-protein co-localize with the membrane raft marker molecule and found that the prion-protein was co-localized on the membrane raft molecule asialo-GM1. Next we observed prion-protein localization under the cell-permeabilized conditions. Interestingly, this result showed that prion-protein localized in the Golgi mainly, and some of them were observed on the plasma membrane as we described above. These finding showed that normal cellular prion-protein was localized on both of the plasma membrane and Golgi apparatus.

P02-E-57

p38 α の新規スプライス産物p38 α 2 の細胞内局在と細胞死の誘導は、そのキナーゼ活性と C 末領域が制御する。

○日下 永子¹, 石川 保幸², 岡田 雅人¹

¹阪大・微研・発癌制御, ²奈良先端科学技術大学院大学

Mitogen-activated protein kinases (MAPKs) は細胞質に存在するセリン-スレオニンキナーゼであり、細胞外からの種々の刺激に対して広く応答する。この MAPKs の一つである、p38 α に対する新規のスプライス産物 (p38 α 2) が存在することを以前発表した。この p38 α 2 は、p38 α の既知のスプライス産物である Mxi2 とは明らかに異ったスプライシングを受けており、そのため、蛋白質レベルにおいて、ユニークな C 末領域が出現していた。P38 α 2 は細胞内でドット状の細胞内局在を示し、細胞死を誘導する。今回我々は、その局在場所がミトコンドリアであることを見いだした。さらに、このような p38 α 2 の細胞内局在性や細胞死の誘導に p38 α 2 のキナーゼ活性や C 末領域が関与しているかどうかを調べるため、変異体や薬剤を用いて調べた。C 末領域のデリーション変異体 (p38 α 2 Δ C)、キナーゼネガティブ変異体 (K53M または p38 特異的阻害剤の SB202190 で処理した場合において、ミトコンドリアへの細胞内局在が見られなくなる上、細胞死も誘導されなかった。これらの結果から、p38 α 2 はそのキナーゼ活性と p38 α 2 に特異的な C 末領域の存在が、細胞内局在性と細胞死の誘導という現象において重要な役割を果たしているということがわかった。

P02-E-58

Determination of fatty acid levels in erythrocyte membranes in patients with chronic fatigue syndrome.

○Zhandong Liu, Dexin Wang, Jian Zhang et al.

Department of Neurology, Binjing Friendship Hospital, Affiliated hospital of Capital University of Medical Science.

Abstract-Chronic fatigue syndrome (CFS) is a condition characterized by impairment of neurocognitive functions, sleep quality and somatic symptoms, such as recurrent sore throat, tender lymph nodes, muscle aches, arthralgias, headaches and postexertional malaise. The patients often felt severe disabling fatigue at least 6 months. Up to now, little is known about the etiology and pathogenesis for CFS. The fatty acids, especially essential fatty acids, in red cell membranes might share certain relationship with their function, and their abnormality might result in the impairment of erythrocyte functions. The changes of the components in red cell membranes might be reflected some alterations in the levels of fatty acids in other biologic membranes.

Objective-To study various biochemical and pathophysiological indices of erythrocyte membranes in chronic fatigue syndrome (CFS).

Methods-Forty-two patients were selected for study on the basis of diagnostic criteria defined by the Centers for Disease Control and Prevention (CDC). After the screening tests of SCL-90 and Hamilton Depression Scale, the patients who have primary depression or somatic symptoms (scores more than 2.5) were excluded. All works were performed by a medical team including experienced, certificated neurologists and psychologists. Thirty-seven age- and sex-matched healthy controls were selected from the medical staffs and volunteers. 5 ml aliquots of whole blood were taken into heparin-containing tubes and the erythrocytes were separated and washed in saline. After lyophilized, the red cell membranes were placed on 4°C. The fatty acids were analysed by gas chromatography. The results were compared to patients and the controls by using independent t-tests.

Results- The concentrations of the arachidonic acid (ARA) and docosahexanoic acid (DHA) were decreased in patients suffered from CFS ($P < 0.05$, both, respectively). There were no significant differences between the other fatty acid profiles of patients and the control group, including palmitic acid, stearic acid, oleic acid, linoleic acid, dihomogamma-linolenic acid, etc.

Conclusions-In this study, the concentrations of the ARA and DHA were decreased in patients of CFS were found. It was suggested that the decreased concentrations of ARA and DHA in erythrocyte membranes might not due to diet malnutrition, but due to some metabolic decompensation, thus resulting in certain clinical symptoms of CFS.

P02-E-59

Activation of p38 MAPK Cascade by the activity-regulated Neuronal Cell Adhesion Molecule arcadlin

○Shin Yasuda¹, Hidekazu Tanaka², Hiroko Sugiura¹, Kyoko Suzuki¹, Takako Takemiya¹, Naomasa Miki², Kanato Yamagata¹

¹Dept. Neuropharmacol., Tokyo Metropolitan Inst. Neurosci., ²Dept.Pharmacol., Osaka Univ.Sch.Med.

We identified an activity-regulated adhesion molecule arcadlin. arcadlin is rapidly and transiently induced in hippocampal granule cells by seizures and by NMDA-dependent synaptic activity in long term potentiation. In this study, we have isolated a protein kinase, which activated p38 MAPK cascade, as a protein associated with the cytoplasmic region of arcadlin by using the yeast two-hybrid system. In HEK 293T cells co-expressing arcadlin and this protein kinase, two molecules were co-localized and specifically associated in the plasma membrane. Homophilic interaction between extracellular domains of arcadlin activated p38 MAPK and induced the internalization of arcadlin in HEK293T cells. This internalization and p38 activation were blocked by overexpression of arcadlin lacking the cytoplasmic region. These findings suggested that arcadlin might play an important role in activity-induced signal transduction.

P02-E-60

Interaction of locally modulated and de novo synthesized cadherins at the activated synaptic membranes

○Hidekazu Tanaka¹, Shin Yasuda², Takahiro Fujimoto¹, Akira Mizoguchi³, Hiroko Sugiura², Kanato Yamagata², Naomasa Miki¹

¹Dept. Pharmacol., Osaka Univ. Sch. Med., ²Dept. Neuropharmacol., Tokyo Metropolitan Inst. Neuroscience, ³Dept. Anat., Mie Univ. Sch. Med.

Synaptic plasticity has been reported to involve the remodeling of synaptic membranes. A synaptic cell adhesion molecule, N-cadherin not only connects pre- and postsynaptic membranes, but also undergoes activity-induced rearrangement without requirement of protein synthesis. Arcadlin is another synaptic adhesion molecule, whose expression is dramatically and transiently induced by synaptic activation. Here, we show that maximal electro-convulsive seizure induces Arcadlin expression and transportation into CA3 excitatory synapse, where N-cadherin is constitutively expressed. By immunoprecipitation, Arcadlin is found to bind to N-cadherin through its transmembrane-cytoplasmic region. Overexpression of an N-cadherin mutant lacking the extracellular domain, which preserves the binding property to Arcadlin and distributes diffusely over the cultured neurons, results in the mistargeting of Arcadlin, suggesting that N-cadherin is essential for the recruitment of Arcadlin. Thus, two different cadherins under distinct regulatory mechanisms may cooperate during the remodeling of synaptic membranes.

P02-E-61

GPI-anchored neural adhesion molecules contactin and NB-3 localized in post synaptic density

○Yasuo Takeda¹, Suni Lee^{1,2}, Vance Lemmon², Kazutada Watanabe³

¹Dept. Cell Recog., Tokyo Metropol. Inst. Gerontol.,

²Dept. Neurosci., Sch. Med., CWRU, ³Dept. BioEng., Nagaoka Univ. Tech.

Contactin and NB-3 are GPI-anchored neural adhesion molecules that belong to immunoglobulin-superfamily. These molecules are known to be recovered in detergent insoluble low density fractions (raft) in which many signaling molecules are co-localized. Our group and Burglund et al have suggested that both molecules are involved in motor functions, by analyzing gene mutant mice. We also reported in the previous meeting that these molecules were dominantly recovered in a crude synaptosomal fraction from rat cerebral cortex. In order to examine whether contactin and/or NB-3 are involved in the synaptic transmission, we performed immunoprecipitation assay of the synaptosomal fraction using antibodies against several adhesion molecules and synaptic markers. Western blot analysis showed that PSD95-like immunoreactivity was detected in both precipitates by anti-contactin and anti-NB-3 antibodies, but little in those by anti-L1 and anti-synaptophysin antibodies as well as in control. These results suggest that GPI-anchored proteins contactin and NB-3 may implicate in post synaptic functions. We are currently trying to confirm that contactin and NB-3 interact with PSD-95 complex using reverse immunoprecipitation and immunocytochemistry.

P02-E-62

Bacterial endotoxin induces IL-20 expression via p38 MAP kinase dependent mechanisms in the glial cells

○toru hosoi, sachiyo wada, yasunobu okuma, yasuyuki nomura

Dept. Pharmacol. Grad. Sch. Pharm. Sci., Hokkaido Univ.

The involvement of IL-20 expression during infection and inflammation has not been elucidated. In the present study, we found that bacterial lipopolysaccharide (LPS) induced IL-20 expression in the mouse primary cultured glial cells. LPS induced an increase in IL-20 mRNA expression at 1 h, which peaked at 2 h and decreased thereafter. The expression of IL-20 was not inhibited by pretreatment with protein synthesis inhibitor puromycin or cycloheximide, indicating that the expression was not dependent on newly synthesized protein. Since p38 MAP kinase inhibitor, SB203580 inhibited LPS-induced IL-20 mRNA expression, it is suggested that p38 MAP kinase is involved in transcriptional regulation of IL-20 gene. Moreover, dexamethasone inhibited LPS-induced IL-20 expression, indicating that the expression of IL-20 is regulated by a negative feedback loop mediated through glucocorticoids. IL-20R α and IL-20R β receptors were expressed in human neuroblastoma cell line SH-SY5Y and human astrocytoma cell line CCF-STTG1. Therefore, it is suggested that IL-20 may play an important role in inflammatory conditions in the brain.

P02-E-63

A novel synaptic protein, ssLRP, with dendritic mRNA

○Tatsuo Suzuki¹, Qing Bao Tian¹, Akira Okano¹, Shoko Miyazawa¹, Nobuteru Usuda²

¹Dept Neuroplasticity, Res Ctr on Aging & Adaptation, Shinshu Univ Sch Med, ²Dept Anatomy, Fujita Health Sci Univ, Sch Med

We have cloned from rat brain a novel gene, which encoded a new member of low density lipoprotein (LDL) receptor family protein. The protein, although shorter in length, resembled the LDL receptor-like protein (LRP) in its structure, possessing a single membrane-spanning domain, NPSY motif in the C-terminal tail, the LDL receptor class A domain, probably a binding motifs for ligands such as amyloid β ($A\beta$) protein, Apolipoprotein E (ApoE), and α 2 macroglobulin. The antibody against carboxyl-terminal portion detected a 72 kDa protein, possibly a proteolyzed C-terminal portion, in the rat brain. Thus, we named the protein as short form synaptic LDL receptor-related protein (ssLRP, GenBank accession No. AB073317). The ssLRP possessed a PDZ motif-binding sequence, SQV, in the C-terminal end and interacted with postsynaptic density (PSD)-95 and synapse-associated protein (SAP)97 but not glutamate receptor-interacting protein (GRIP)1 or Ves1/Homer, via its C-terminal sequence. Interaction with these PDZ domain-containing proteins suggests the association with PSD and coupling to α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptor complex. The C-terminal portion, even after proteolysis, appeared to be complexed with a long N-terminal portion, possibly an extracellular portion. The mRNA, as well as the protein, was localized to the dendrite. These data suggest a unique role of ssLRP in the synaptic transmission and its regulation, the protein being localizing on the synaptic plasma membrane.

P02-E-64

Generation of the SNAP-25 mutant mice carrying a S187A mutation

○Masakazu Kataoka^{1,4}, Reiko Kuwahara², Yoko Nakahara², Rika Suzuki², Yoshiko Motegi², Yuko Kato-Fukui², Minesuke Yokoyama², Masami Takahashi^{3,4}

¹Dept. Env. Sci. Tech. Fac. Eng., Shinsu Univ., ²Mitsubishi Kagaku Inst. Life Sci., ³Dep. Biochem. Sch. Med., Kitasato Univ., ⁴CREST. JST.

SNAP-25 has a central role in neurotransmitter release and axon outgrowth in presynapse through forming a central core complex called SNAREs with syntaxin and synaptobrevin for membrane fusion. As other biological processes, the membrane fusion in presynapse must be regulated by protein phosphorylation. We already identified that serine residue 187 (Ser187) of SNAP-25 was phosphorylated under the protein kinase C pathway, but the biological significance of the phosphorylation for central nervous system remains unclear. To gain insight into the role of SNAP-25 phosphorylation at Ser187 in vivo we substituted serine residue 187 of the *Snap-25* gene with alanine (S187A) by using knock-in gene targeting technology. The DNA region containing the Exon 7 was replaced with mutated mini-gene followed by poly-A signal for the CGK gene, the CGK-promoter and the Neo gene, thus the two mRNAs, for the mutated SNAP-25 and for Neo, should be transcribed from the mutant allele. After generation of chimeric animals by the targeted ES cells injection into C57BL/6J blastocysts, heterozygous *Snap-25*^{S187A} / + animals were obtained. F2 animals having heterozygous alleles were crossed and the offspring was analyzed. Mice heterozygous for the *Snap-25*^{S187A} mutation were robust, fertile and phenotypically indistinguishable from wild-type littermates. The homozygous mutant mice were also obtained and they lived 20 days after birth without any significant difference from other littermates. The ratio of homozygote mutant, heterozygote and wild-type (0.5: 2: 1) was different from the expected Mendelian ratio. In addition, a large part of homozygous mutant mice died during 20~25 days after birth. These characteristics of *Snap-25*^{S187A} / *Snap-25*^{S187A} mutant mice suggests that the phosphorylation of SNAP-25 at Ser187 take important role(s) in higher biological function perspectively in nervous system.

P02-E-65

The binding of CaMKII to syntaxin isoforms

Kazushige Nomura^{1,2,3}, Yoshiaki Komiya³, ○
Michihiro Igarashi¹

¹Div Mol Cell Biol, Grad Sch Med Dent Sci, Niigata Univ, ²Dept Orthopedics, Gunma Univ Sch Med, ³Dept Mol Cell Neurobiol, Gunma Univ Sch Med

Syntaxin 1A is one of the key molecules for the exocytotic molecular machinery in the presynaptic terminal. We have recently demonstrated that clmodulin-depdent protein kinase II (CaMKII) binds to syntaxin 1A in a Ca²⁺-dependent manner, and that this interaction is invovled in the regulation of exocytosis (Ohyama et al., J Neurosci, in press). In nonneuronal cells, there are three other isoforms (syntaxin 2-4) localized in the plasma membrane. We compared the interaction between CaMKII and other those isoforms of syntaxin, with that of CaMKII-syntaxin 1A. The binding affinity of each syntaxin isoform to CaMKII was 1A>2>3>>4. The binding of sytnaxin 2 to CaMKII was also in a Ca²⁺-dependent manner. The CaMKII-binding site of syntaxin 2 was also localized in the linker domain, as well as that of syntaxin 1A. Some experiments using the chimeric molecules of syntaxin isoforms revealed that the linker domain of each isoform determine the binding specificity to CaMKII. Since the amino acid sequence of each syntaxin isoform do not largely differ from each other in the linker domain, we concluded that there are several critical residues which are essential to the interaction with CaMKII.

P02-E-66

NMDA Receptor-dependent Recruitment of AMPA Receptor and Calnexin to Plasma Membrane in Cultured Rat Hippocampal Neurons

○Jun Tsujimura¹, Makoto Itakura², Mariko Sekiguchi³,
Yoshihisa Kudo¹, Masami Takahashi^{2,4}

¹Sch. Life Sci., Tokyo Univ. of Pharm. and Life Sci., ²Dept. Biochem., Kitasato Univ. Sch. Med., ³Mitsubishi Kagaku Inst. Life Sci., ⁴CREST, JST

Regulation of the number of neurotransmitter receptor in postsynaptic plasma membrane is one of the important mechanisms of synaptic plasticity. It was believed that neurotransmitter receptors were incorporated in the plasma membrane through a mechanism of constitutive secretion. However, recent studies have suggested that the process is also regulated by various signaling mechanisms. Previously, we found that AMPA receptor was distributed in various membrane fractions of rat brain homogenate, and the pattern of the distribution was quite similar to that of calnexin, a Ca²⁺ binding protein localized in endoplasmic reticulum (ER) of non-neuronal cells. In order to elucidate the regulatory mechanisms of the number of AMPA receptor in plasma membrane, we biotininated cell surface proteins of cultured rat hippocampal neurons by using a membrane impermeable biotinylation reagent. We found that both AMPA receptor and calnexin were markedly biotininated, and the number of calnexin and AMPA receptor in the surface membrane were changed in NMDA receptor dependent manners. These results suggest that calnexin may play an important role in the regulation of AMPA receptor number in synaptic plasma membrane.

P02-E-67

カイニン酸刺激による興奮性神経活動における海馬ニューロンでの翻訳調節因子の変化

○稲村 直子, 那波 宏之, 武井 延之
新潟大・脳研究所・分子神経生物

シナプスの可塑的变化における長期増強(LTP)などの興奮性神経活動がニューロンでの蛋白質合成を引き起こす可能性が示唆されている。しかし、このときの蛋白質合成の調節を司る因子、即ち翻訳調節因子の動態についてはまだよくわからないことが多い。蛋白質合成は3つの過程(開始・伸長・解離)からなり、様々な翻訳調節因子によって制御されており、その活性の調節は主にリン酸化、脱リン酸化によって行われている。我々はこれらの翻訳調節因子が海馬のニューロンに存在することを報告してきた。今回我々は、カイニン酸刺激により過剰な興奮性神経活動を誘起した際の海馬のニューロンでの翻訳調節因子の動態を、翻訳調節因子のリン酸化抗体でのウェスタンブローディングもしくは免疫組織化学染色を行うことによってしらべた。その結果、カイニン酸刺激によって、海馬ニューロンでの翻訳伸長因子 eukaryote elongation factor 2 (eEF2) の脱リン酸型の免疫反応性の増大、すなわち eEF2 の活性化が観察された。この結果はカイニン酸刺激による興奮性神経活動において、翻訳伸長因子の活性化が見られたことを示し、現在蛋白質合成や他の翻訳調節因子についても検討中である。

P02-E-68 (MS1-1)

Reelin signaling and Cdk5 in the control of neuronal positioning

○Toshio Ohshima¹, Masaharu Ogawa², Katsuhiko Mikoshiba¹

¹Lab. for Developmental Neurobiol., ²Lab. for Cell Culture Development, BSL, RIKEN

Proper neuronal positioning is a fundamental and essential process in the development of the central nervous system (CNS). The analysis of neurological mutant mice has led to dramatic progress in the identification and characterization of genes important for neuronal positioning. Among them, the identification of signal pathways mediated by reelin and Cdk5 kinase provided us a conceptual framework for exploring the molecular mechanisms underlying proper neuronal positioning. Because mutant mice for Reelin and Cdk5 share the similarities and differences of neuroanatomical defects, relation of Reelin signaling and Cdk5 has been discussed. To elucidate the relationship between them, we generated the combined defects of these genes in the mouse line to analyze their patterns of neuronal migrations during brain development. Synergistic effects of gene inactivation of p35 and Reelin/Dab1 were observed in the form of additional defects in neuronal migration, typically seen in the cortical neurons of cerebellum and hippocampus. The deteriorations of neuronal migration were also detected in facial branchiomotor and inferior olive neurons of the hindbrain of the double mutant mice. Biochemical analysis revealed that Dab1 is *in vivo* substrate of Cdk5 kinase. Fyn tyrosine kinase phosphorylates Dab1 and we found its tyrosine phosphorylation of Dab1 was attenuated by prior Cdk5-phosphorylation of C-terminal domain. Its significance in Reelin signaling is currently investigating in neuronal culture.

P02-F-1

Flow cytometric analysis of neural stem cells in the developing and adult mouse brain

○Ayako Murayama^{1,2,3}, Yumi Matsuzaki-Iyoku^{1,3}, Ayano Kawaguchi⁴, Takaki Miyata⁵, Takuya Shimazaki^{1,3}, Hideyuki Okano^{1,3}

¹Dept. Physiol. Sch. Med., Keio Univ., ²Dpt. Cell Biol. and Neurosci. Grad. Sch. Med., Osaka Univ., ³CREST-JST, ⁴Cell asymmetry. Center for developmental biol., RIKEN, ⁵Cell Culture Development. Brain Science Institute., RIKEN

Neural stem cells (NSCs) which can self-renew and generate various types of neurons and glia exist and may function in the central nervous system (CNS) throughout the life of mammals. In recent years, the various methods to identify and isolate NSCs have been developed. Here, we investigated various characteristics of NSCs *in vivo* during CNS development, using FACS to identify the NSCs. We first examined stage-dependent changes in the physical parameters, using forward scatter (FSC) and side scatter (SSC) profiles, of NSCs from the developing striatum. With development, their number decreased in the FSC^{high} fractions but increased in the FSC^{low}/SSC^{high} fraction, whereas NSCs were significantly concentrated in the fraction containing the largest cells (about 20 μ m in diameter). Next, using *nestin*-EGFP transgenic mice (Kawaguchi et al. and 2001), we studied the correlation between the FSC/SSC profile and *nestin*-EGFP expression in neural progenitors, shown that the most of the largest cells exhibited the highest *nestin*-enhancer activity. Furthermore, we examined whether "side population (SP)" defined as the Hoechst33342 low/negative fraction, which is known to be a stem cell-enriched population in bone marrow (Goodell et al., 1996), can be also stem cell population in the CNS. We have found that the embryonic SP are distributed in the *nestin*-EGFP positive fractions containing NSCs, while the adult SP cells were included to some extent in the CD24^{low}/PNA^{low} fraction, where NSCs were exceedingly enriched. In addition, at P2, SP was also enriched for Notch1-positive immature neural cells (about 60%). In contrast to that the ratio of Notch1-positive population in the brain decreases as the development proceeds, that in the SP was constant for all of the developmental stage. These results suggest that the characteristics of NSCs are not uniform and change developmentally.

P02-F-2 (MS3-4)

Interactions between *neccdin* and E2F1 in terminal differentiation and survival of sensory neurons

○Risa Takazaki, Taichi Uetsuki, Hideo Taniura, Kazuaki Yosikawa

Division of Regulation of Macromolecular Functions
Institute for Protein Research Osaka University

Expression of the *neccdin* gene is controlled by genomic imprinting. *Necdin* is expressed only from the paternal allele in the central and peripheral nervous system. We have previously reported that *neccdin* antisense oligonucleotides inhibit terminal differentiation of primary dorsal root ganglion (DRG) neurons treated with nerve growth factor (NGF). This supports our view that *neccdin* is involved in terminal differentiation of neurons. The E2F family proteins are major transcription factors that control cell cycle progression and apoptosis during neurogenesis and in postmitotic neurons. The retinoblastoma gene product (Rb) and *neccdin* bind to E2F1 and suppress E2F1-dependent transactivation of cell cycle-related genes. In this study, we examined whether *neccdin* functionally interacts with E2F1 during neuronal differentiation and apoptosis using DRG neurons. DRG sensory neurons prepared from E12.5 mouse embryo were treated with NGF to differentiate terminally. Antisense oligonucleotides corresponding to Rb and *neccdin* mRNA sequences blocked terminal differentiation of primary DRG neurons, suggesting that both Rb and *neccdin* are essential for negative regulation of E2F1 during neuronal differentiation. When E2F1 was ectopically expressed in these cultures by adenovirus-mediated gene transfer, a marked reduction in the number of differentiated neurons was observed. E2F1 expression also increased the number of apoptotic neurons. Co-expression of *neccdin* significantly increased the population of differentiated neurons and reduced the number of apoptotic neurons in NGF-treated cultures infected with E2F1-expressing adenovirus. These results suggest that *neccdin* antagonizes E2F1-induced de-differentiation of sensory neurons and promotes their terminal differentiation.

P02-F-3

Upregulation of gene expression of PACAP and its receptors during differentiation of the ES cell-derived neural stem cells

○Megumi Hirose¹, Hitoshi Hashimoto¹, Norihito Shintani¹, Naohisa Arakawa¹, Akemichi Baba^{1,2}

¹Lab. Mol. Neuropharmacol. Grad. Sch. Pharm. Sci., Osaka Univ., ²Lab. Mol. Pharmacol. Grad. Sch. Med., Osaka Univ.

A neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP), a member of the VIP/secretin/glucagon family, has been shown to stimulate neuronal growth, differentiation and maturation, indicating a possible function in the development of the nervous system. In the mouse embryo, PACAP and the PACAP-specific PAC₁ receptor are widely expressed in the neural tube as early as day 10.5, and PACAP is an early inducer of cAMP levels and may act in the neural tube during patterning to control cell proliferation and gene expression. As a first step in examining the role of PACAP in early neuronal development, in this study, we have analyzed the expression of PACAP and its receptors in neuronal and glial lineage derived from *in vitro*-differentiated progeny (embryoid bodies [EBs]) of the embryonic stem (ES) cells. ES cell culture was induced into the neural stem cell marker Nestin, the neuronal markers TuJ1, MAP2, and the astrocyte marker GFAP positive culture. Expression of PACAP and its receptor mRNAs and the neuronal and glial differentiation markers were analyzed by RT-PCR. In ES cells, PACAP and PAC₁ receptor mRNAs were slightly expressed. The PACAP and PAC₁ receptor mRNA expressions were increased in neural stem cell-enriched culture, and were markedly upregulated following induction of neuronal and glial differentiation. These results demonstrate for the first time the PACAPergic gene expression in ES cell-derived neuronal and glial culture, and provide a strategy for investigating the role of PACAP during different stages of neuronal development *in vitro*.

P02-F-4

genipin による Neuro2a 細胞の NO-cGMP-PKG 経路から ERK のリン酸化を介した突起伸展

○山崎 眞津美, 大窪 忠利, 千葉 賢三, 毛利 哲郎, 吉藤 茂行

北陸大・薬・生物活性

我々は植物由来の genipin が、PC12h 細胞に対して突起伸展を誘導すること、 β アミロイドによる神経細胞死を抑制することを報告してきた。また、PC12h 細胞に対する突起伸展には、NO-cGMP-PKG 経路を介した ERK の活性化が重要であることも見出した。この NO 依存的な突起伸展は、マウス神経芽細胞腫の Neuro2a 細胞においても観察された。そこで、Neuro2a 細胞における NO-cGMP-PKG 経路および ERK の関与を検討することにより、タイプの異なる神経細胞における genipin の突起伸展シグナルの一般性について考察した。Neuro2a 細胞を血清を含む培地中で 24 h 培養後、試験物を含む無血清培地に交換した。その 48 h 後、画像解析法により突起伸展活性を測定した。ERK のリン酸化は、Western blot 法により検出した。genipin による Neuro2a 細胞の突起伸展は、NO 合成酵素(L-NAME)、可溶性 guanylate cyclase、G kinase および MEK の各阻害剤で著しく阻害された。従って、genipin による Neuro2a 細胞の突起伸展過程に、NO-cGMP-PKG 経路および ERK の活性化が関与していることが示唆された。実際 genipin は処理 30~60 分後に、未処理群より強い ERK のリン酸化をもたらした。さらに、genipin によるこの ERK のリン酸化の増強は、L-NAME で著しく抑制されたが、その不活性体では抑制されなかった。このことは、NO-cGMP-PKG 経路の下流で ERK が活性化されることを示唆し、PC12h 細胞で見られたメカニズムと同様であった。従って、genipin は PC12h 細胞と共通のメカニズムによって、Neuro2a 細胞に対して突起伸展作用を示すことが明らかになり、genipin は種々の神経細胞に対して突起伸展作用を含めた神経栄養因子様作用を発揮することが期待された。

P02-F-5

CYSTATIN C INDUCES ASTROCYTE DIFFERENTIATION BY INHIBITION OF CYSTEIN PROTEASES

○Akiko Hasegawa^{1,2}, Yasuno Iwasaki¹, Kazuhiro Ikenaka¹

¹National Insitutue for Physiological science, ²The Graduate University for Advanced Studies

CysC is an endogenous inhibitor of cystein protease and is known to be produced by mature astrocytes. We previausly showed that CysC added into culture medium induces astrocyte differentiation. We also demonstrated that other cystein protease inhibitor, such as E64 or Egg Cystatin (non-glycosylation cystein protease inhibitor), can induce astrocyte differentiation. Thus, astrocyte differentiation can be inducing by inhibition of cystein proteases. In Primaly culture cells from E12-E16 mouse cortex. The inhibitory activity of conditioned media increased day by day, and was dependent on the stage of development. The Cystatin C antibody blocked the inhibitory activity of conditioned media, indicating that primary cells release cystein protease inhibitor in extracellulaly, and it is mainly Cystatin C or substances closely related to it.

P02-F-6

Negative regulation of neural progenitor cell proliferation by necdin

○Hideo Taniura, Noriko Fukunaga, Taichi Uetsuki, Kazuaki Yoshikawa

Div. Reg. Macromol. Func. Inst. Prot. Res., Osaka Univ.

Necdin is a potent growth suppressor expressed predominantly in postmitotic neurons. Necdin binds to viral oncoproteins and the transcription factors E2F1 and p53. Necdin suppresses these transcriptional activities. The necdin gene is maternally imprinted and transcribed only from paternal allele. The necdin gene is implicated in Prader-Willi syndrome pathogenesis, a neurogenetic disorder related to genomic imprinting. To study the involvement of necdin in early neural development, we examined the growth and differentiation of primary cortical progenitor cells prepared from E12.5 Ndn M+/P- mutant mice, which lacks only paternal allele of the necdin gene, and wild-type M+/P+ control. The most of control proliferative progenitors expressed nestin and could be subcultured in the presence of FGF. We found the significant expression of necdin protein in proliferative progenitors, whereas no necdin expression was detected from mutant mice. We compared the in vitro differentiation potential of progenitors between mutant and control. No significant difference was observed in the number of MAP2-positive neurons or in the number of GFAP-positive astrocytes. We next analyzed progenitor cell proliferation and a significant increase in BrdU-labeled nuclei was observed in mutant progenitors. Using the neurosphere cultures, we found a greater average diameter of the spheres and an increased number of cells in mutants, indicating that increases in sphere size can be attributed to increased cell proliferation. When neurospheres from primary cultures were dissociated and repropagated, the secondary mutant spheres also had larger diameters. These results suggest that necdin negatively controls proliferation of neural progenitors.

P02-F-7

Involvement of small GTPase RhoA to STAT3 signaling pathway during astrocytic differentiation of rat C6 glial cells

¹Hiromi Takanaga¹, Tatsumi Adachi², Manabu Unimoto¹

School Pharm. Sci., Kitasato Univ., ²Dept. Basic Med. Sci., Natl. Inst. Minamata Dis.

We previously reported that the activation of dioxin receptor, AhR (aryl hydrocarbon receptor), inhibited the astrocytic differentiation of C6 glial cells. The inhibitory effect was observed in the cellular morphological change and the inhibition of cAMP-dependent induction of astrocyte marker, GFAP (glial fibrillary acidic protein). Recently, we found that activation of AhR downregulated the expression of small GTPase RhoA. Moreover, by the treatment of cells with Rho-kinase inhibitor Y27632, cAMP-induced GFAP expression was attenuated. Therefore it seemed that the AhR-dependent inhibition of astrocytic differentiation was done by inhibiting RhoA signaling pathway. To investigate the downstream signals of RhoA for the differentiation, the phosphorylation of STAT3 (signal transducers and activators of transcription) was examined. STAT3 is a transcription factor that is reported to be crucial for differentiation of astrocyte, and promoter region of GFAP possesses STAT3 binding site. Phosphorylation on Tyr-705 of STAT3 is a prerequisite for dimer formation, nuclear translocation, binding to its cognate DNA sequences, and regulation of the target gene transcription. By the treatment of cells with Y27632, cAMP-induced phosphorylation of Tyr-705 was reduced significantly. This inhibition was also observed by the treatment of cells with AhR ligand 3-methylcholanthrene. These results suggest that the AhR activation could inhibit the kinase activity of Janus kinases (JAKs) through RhoA signaling cascade, then leads to the attenuation of the astrocytic differentiation of C6 glial cells.

P02-F-8

Roles of Gab1 in the maintenance and differentiation in neural stem cells

¹Yoshika Hayakawa^{1,3}, Keigo Nishida², Toshio Hirano², Takuya Shimazaki^{1,3}, Hideyuki Okano^{1,3}

¹Dept. Physiol. Sch. Med., Keio Univ., ²Dept. Mol. Onco. Grad. Sch. Med., Osaka Univ., ³CREST, JST

Neural stem cells (NSCs) are defined by their ability to self-renew and generate a range of different central nervous system (CNS) cells. NSCs may play a major role in mammalian CNS development and continue to function throughout adulthood. It has been shown that a number of cytokines and growth factors regulate the maintenance, proliferation and differentiation of NSCs. However, the detailed mechanisms of these regulations through the complex signal transduction pathways remain to be elucidated. Recently, it was reported that gp130-mediated signaling, activated by the IL-6 family cytokines, supports the maintenance of NSCs and differentiation of glia. Here, we focused Grb2 associated binder 1 (Gab1) which is a downstream signaling mediator in the gp130-mediated signaling and in epidermal growth factor (EGF) receptor-mediated signaling known as a mitotic signal for NSCs. To investigate the role of Gab1 in the maintenance and/or differentiation of NSCs, we studied CNS development in *gab1*-deficient mice. Since *gab1*^{-/-} embryos died at mid-to-late gestation date, we first analyzed the number of NSCs in the striatum at the embryonic day 14 by neurosphere formation assay. The lack of Gab1 didn't affect the number of NSCs, but the loss of their responsiveness to EGF was observed. However, the number of NSCs in periventricular area of the lateral ventricle in the adult *gab1*^{+/-} mice was increased. On the other hand, in the embryonic spinal cord, the number of oligodendrocyte precursor cells, assessed by immunostaining of Olig2, was decreased in *gab1*^{-/-} and *+/-* embryos. These results suggest that Gab1 suppress the maintenance of NSCs after birth and is essential for the differentiation, proliferation or survival of the oligodendrocyte precursor cells.

P02-F-9

Roles of Musashi Family in mammalian nervous development

○Shinsuke SHIBATA^{1,3}, Shin-ichi SAKAKIBARA², Takuro KOJIMA^{1,3}, Hideyuki OKANO^{1,3}

¹Dept. Physiol., Sch. Med., Keio Univ., ²Dept. Histol. Neurobiol., Sch. Med., Dokkyo Univ., ³CREST-JST

Musashi (Msi) is an evolutionarily conserved RNA-binding protein among different species. We have reported *Drosophila*-Msi regulates asymmetric division of sensory organ precursor cells in neural development of *Drosophila*. In mammalian CNS, we identified two members of Msi family, Musashi1 (Msi1) and Musashi2 (Msi2). They are strongly co-expressed in neural precursor cells including CNS stem cells. We supposed Msi family regulates asymmetric division or maintenance of immaturity of neural stem cells in mammalian nervous systems through the post-transcriptional regulation. To reveal the function of Msi family *in vivo*, we generated Msi1 and Msi2 deficient mice.

Msi1 deficient (Msi1 ^{-/-}) newborn mice frequently died of obstructive hydrocephalus with abnormal proliferation of ependymal cells surrounding the cerebral aqueduct. Msi2 deficient (Msi2 ^{-/-}) mice show poor body gain. Both Msi1 and Msi2 deficient (Msi1 ^{-/-}, Msi2 ^{-/-}) newborn mice showed severe neurological defects. They died a few hours after birth and have severe disturbance of the lamination of the cortex and hippocampus. These results suggest that Msi proteins play important role cooperatively in the development of mammalian CNS.

P02-F-10

Inhibition of Oligodendrocyte Differentiation by Wnt signaling

○Takeshi Shimizu¹, Tamaki Wada¹, Tetsushi Kagawa¹, Shinji Takada^{2,3}, Yuko Muroyama^{2,3}, Kazuhiro Ikenaka¹

¹Nat. Inst. Phys. Sci., Okazaki. Nat. Res. Inst., ²Cent. Integ. Biosci., Okazaki. Nat. Res. Inst., ³ERATO. Kondoh. Diff. Signal. Proj

Multiple steps of oligodendrocyte development were inhibited by yet unidentified factors secreted from the dorsal spinal cord (Wada et al, Dev. Biol. 2000). We have been trying to identify the inhibitory factors. We reported previously that the developmental profile of wnt-1/3a gene expression was well correlated with that of the dorsal inhibitory activities. The conditioned medium of L cells that stably expressed Wnt3a decreased the number of O4+ oligodendrocyte. Here we report that extra cellular domain of Xenopus frizzled8 (ECD8) and Dickkopf-1 (Dkk-1), secreted inhibitors of Wnt signaling, antagonize the reduction of the O4+ immature and O1+ mature oligodendrocytes by Wnt-3a supernatant. These results confirmed the effects of Wnt-3a on oligodendrocyte lineage cells. Thus Wnt-1/3a is a potent candidate for the dorsal factors that inhibit oligodendrocyte development. There may exist β -catenin signaling pathway in oligodendrocyte progenitors as a down stream effector of Wnt-3a. Wnt-1/3a knockout mice are now under investigation.

P02-F-11

The Wnt/calcium pathway activates NF-AT and promotes ventral cell fate in *Xenopus* embryos.

Takeo Saneyoshi¹, Shoen Kume¹, ○Katsuhiko Mikoshiba^{1,2,3}

¹Dept. Molecular Neurobiology, The Inst. of Med. Sci., U. of Tokyo, ²Mikoshiba Calciosignal Net Project, ERATO and Calcium Oscillation Project, ICORP, JST, ³RIKEN Brain Sci. Inst.

The involvement of IP₃-Ca²⁺ signaling in dorsoventral axis formation in *Xenopus* embryo has been proposed, but the immediate target of free Ca²⁺ is not well understood. The secreted Wnt protein family comprises two functional groups, canonical Wnt and Wnt/Ca²⁺ pathways. Despite the finding that the Wnt/Ca²⁺ pathway interfered with the canonical Wnt pathway, the underlying molecular mechanism is poorly understood. We cloned the cDNA encoding *Xenopus* homolog of the nuclear factor of activated T-cell (XNF-AT). Gain-of-function XNF-AT mutant (CA XNF-AT) inhibited anterior development of the primary axis as well as Xwnt8-induced ectopic dorsal axis. Loss-of-function XNF-AT mutant (DN XNF-AT) induced an ectopic dorsal axis and expression of the canonical Wnt signaling target molecules, *siamois* and *Xnr3*. Xwnt5A induced translocation of XNF-AT from the cytosol to the nucleus. These data strongly suggest that XNF-AT functions as a downstream target of Wnt/Ca²⁺ and IP₃-Ca²⁺ pathways and plays an essential role in mediating ventral signals in the *Xenopus* embryo by suppressing the canonical Wnt pathway.

P02-F-12

Expression of a glutamine-rich forkhead transcription factor in the developing central nervous system.

○Hiroki Iwanishi, Yoshihiro Morikawa, Emiko Senba
Dept. Anat. and Neurobiol., Wakayama Medical Univ.

The forkhead gene family belongs to the winged helix class of DNA-binding proteins. This DNA binding domain was first identified as a region of homology between the *Drosophila* forkhead protein and the rat hepatocyte nuclear factor (HNF) 3β. This domain is known to be highly evolutionary conserved. Forkhead family is involved in the somitogenesis and tissue-specific gene expression in liver, lung, and adipose tissue as a developmental regulator. Recently, it has been reported that some forkhead transcription factors are associated with the development of the central nervous system (CNS) during the embryogenesis. We isolated a novel forkhead transcription factor that contained glutamine-rich domain and was predominantly expressed in the brain and the lung of adult mice by Northern blot analysis. We used in situ hybridization technique to explore the expression pattern of this gene in the developing CNS. From embryonic 9.5 days, this gene was expressed in the ventral horn of the developing spinal cord and its gene expression was similar to Lim1 expression pattern. From the late-stage of development within a few weeks after birth, strong gene expression was observed in caudate-putamen, neocortex, cerebellum and CA1 region of hippocampus. During embryogenesis, few signals were seen in neuroepithelial cells. Our results suggest that this novel forkhead transcription factor is required for the maturation of postmitotic neurons, mainly in motor system, not for neurogenesis.

P02-F-13

Kohjirin, A novel bone morphogenetic protein-4 antagonist derived from astrocytes.

○Kanna Kuriyama¹, Takatoshi Ueki¹, Masamitsu Tanaka², Naoyuki Miura³, Haruhiko Sugimura², Kohji Sato¹

¹Dept. Anat. & Neurosci. Sch. Med., Hamamatsu Univ.,

²Dept. Patho.1 Sch. Med., Hamamatsu Univ., ³Dept. Biochem. Sch. Med., Hamamatsu Univ.

Chordin and other Chordin-like proteins are characterized by their cysteine rich domains, and those secreted proteins are known to act as inducible factors of neuralization or dorsalization in mammalian embryos by antagonizing the activity of bone morphogenetic proteins (BMPs). Although the roles of Chordin-like proteins in early development have been well established, their roles and those of BMPs, plentifully expressing in mature brain, are scarcely elucidated. In the present study we report Kohjirin, a novel BMP-4 antagonist derived from rat mature astrocytes. Kohjirin (Kjr) is a 521 amino acid protein containing N-terminal signal peptide and 3 cysteine rich domains to which BMP-4 is bound. Kjr is ubiquitously expressed through development of brain, and intriguingly it was revealed that Kjr is abundantly expressed by astrocytes in adult brain. *in vitro* astrocytes express Kjr increasingly in parallel with their maturation, and less express it while proliferating. Kjr antagonized BMP-4 and induced dorsalization in early embryos of *xenopus*. Our findings raise the possibility that astrocytes may be involved in the neural development even in the adulthood. Kjr secreted by astrocytes may play an important role in neurogenesis and brain development.

P02-F-14 (MS3-1)

Mechanisms of riluzole-induced brain-derived neurotrophic factor synthesis in the rat hippocampus

○Ritsuko Katoh-Semba, Kanefusa Kato

Inst. Dev. Res. Aichi Human Service Center

We have reported that an intraperitoneal injection of riluzole causes a marked elevation of brain-derived neurotrophic factor (BDNF) levels selectively in the rat hippocampus (particularly in the dentate gyrus) and entorhinal cortex. In the present study, we examined about pathways of the riluzole-induced BDNF production. Repeated injections of riluzole not only maintained at a high level of BDNF but also decreased NT-3 levels in the hippocampus. This condition was similar to that of long term potentiation (LTP). Therefore, we investigated effects on riluzole-induced BDNF production of compounds that regulate induction of LTP in the rat hippocampus. An injection of apyrase, a nucleotidase of ecto-ATP/ADP, or CCPA, an agonist of adenosine, showed a tendency to reduce a riluzole-induced elevation of hippocampal BDNF. In contrast, DPX, an antagonist of adenosine, not only enhanced BDNF levels by itself but also augmented riluzole effects. CgTX GVIA, a blocker of N-type voltage-dependent calcium channels, markedly inhibited the increase in BDNF levels by riluzole. A low level of adenosine in the hippocampus is shown to induce LTP similar to that seen in tetanus stimulation. The present results suggest that hippocampal BDNF production in riluzole-injected rats is regulated through adenosine receptors. Since riluzole is a blocker of voltage-dependent sodium channels, it is likely that the drug reduces a corelease of ATP from cholinergic neurons, resulting in low levels of extracellular adenosine.

P02-F-15 (MS3-2)

bFGF Evokes a Rapid Glutamate Release through Activation of the MAPK Pathway in Cultured Cortical Neurons.

○Tadahiro Numakawa^{1,2,3}, Daisaku Yokomaku^{1,2}, Kazuyuki Kiyosue², Naoki Adachi¹, Tomoya Matsumoto^{1,2}, Yumiko Numakawa^{1,2}, Takahisa Taguchi², Hiroshi Hatanaka¹, Masashi Yamada¹

¹Division of Protein Biosynthesis, Institute for Protein Research, Osaka University, ²Neuronics R.G. Division for Human Life Technology National Institute of Advanced Industrial Science and Technology (AIST), ³a JSPS Postdoctoral Fellow

Neuronal transmissions require mechanisms that modulate the balanced interaction of multiple factors, which control functional maintenance and plasticity in the central nervous system (CNS). Many factors including growth factors and the excitatory amino acid glutamate control neuronal functions. In particular, brain-derived neurotrophic factor (BDNF, one of the neurotrophins) plays a fundamental role in neuronal transmission and plasticity. We previously reported that rapid glutamate release was induced by BDNF through the glutamate transporter in cultured cerebellar and cortical neurons (Numakawa et al., 2001, 2002). However, very little study has been done as concerns the roles of other neurotrophic factors in the acute modulation of CNS and peripheral nervous system (PNS) functions. We examined the possibility that basic fibroblast growth factor (bFGF) is involved in synaptic transmissions. We found that bFGF rapidly induced the release of glutamate and an increase in the intracellular Ca^{2+} concentration through voltage-dependent Ca^{2+} channels in cultured cerebral cortical neurons. bFGF also evoked a significant influx of Na^+ . Tetanustoxin inhibited the bFGF-induced glutamate release, revealing that bFGF triggered exocytosis. The mitogen-activated protein kinase (MAPK) pathway was required for these acute effects of bFGF. We also found that pretreatment with bFGF significantly enhanced high K^+ -elicited glutamate release also in a MAPK activation-dependent manner. Therefore, we propose that bFGF exerts promoting effects on excitatory neuronal transmission via activation of the MAPK pathway.

P02-F-16 (MS3-3)

change of the expression of glial cell line-derived neurotrophic factor (GDNF) in the rat sciatic nerve after its transection

○Yoshihisa Yamada¹, Atsumi Nitta², Katsuji Shimizu¹, Shoei Furukawa²

¹Dept. of Orthop. Surg., Gifu Univ. school of Medicine,

²Lab. of Mol. biol., Gifu Pharm. Univ.

GDNF is one of the most potent motoneuron survival factors that has been identified, and may be involved in the nerve regeneration processes in the peripheral nervous system. In order to study the role of GDNF during nerve regeneration processes, we examined the changes of GDNF protein and mRNA levels in the dorsal root ganglia, peripheral nerve trunk, and muscle following rat peripheral nerve transection using the enzyme immunoassay (EIA) system and reverse-transcription polymerase chain reaction (RT-PCR) method, respectively. GDNF protein level started to increase in only the distal segments of the transected sciatic nerve within 12 hr after the injury, peaked 3 day post operation. Following transection of the sciatic nerve GDNF and its receptor, GDNF family receptor α 1 (GFR α 1) mRNAs were also upregulated rapidly within 3 hr in the denervated distal nerve segments adjacent to the transection site. Both upregulation peaked 1 week after operation. The change of GDNF protein level paralleled with that of expression of GDNF mRNA. These findings suggest a long-lasting de novo GDNF synthesis in the segments distal to the injury site. Immunohistochemical study localized GDNF-immunoreactivity in Schwann cells of the sciatic nerve irrespective of its transection. All results obtained using the EIA, RT-PCR, and immunohistochemical technique consistently provided evidence for the upregulation of GDNF synthesis in Schwann cells in distal side of the sciatic nerve after transection. These results suggest that the Schwann cells of the sciatic nerve respond to denervation or loss of axonal contact, and upregulate their expression of GDNF. The accumulation of GDNF protein in the distal stumps may have an important impact on the success of peripheral nerve regeneration.

P02-F-17

cAMP-dependent protein kinase phosphorylates the p75 receptor and regulates its localization to lipid rafts

○Haruhisa Higuchi, Toshihide Yamashita, Masaya Tohyama

Dept. Anat. and Neurosci. Grad. Sch. Med., Osaka Univ., CREST, JST

Neurotrophins mediate the survival, differentiation, growth and apoptosis of neurons by binding to two types of receptors, the Trk tyrosine kinases and the p75 neurotrophin receptor (p75^{NTR}). Although p75^{NTR} modulates the affinity of Trk receptors to neurotrophins and increases ligand specificity in cells that express both Trk and p75^{NTR}, it transduces its own signal in cells that express only p75^{NTR}. The most prominent biological function of p75^{NTR} may be that it induces cell death, as it contains a death domain sequence similar to the Fas and TNF receptors. Other p75^{NTR}-mediated activities have been proposed, including enhancing axonal outgrowth. Although a large number of studies have been made on the diverse effects mediated by p75^{NTR}, little is known about the molecular mechanisms by which p75^{NTR} initiates intracellular signal transduction. We identified a variant of the β catalytic subunit of cAMP-dependent protein kinase (PKAC β) as a p75^{NTR} interacting protein, which phosphorylates p75^{NTR}. Intracellular cAMP in cerebellar neurons was transiently accumulated by ligand binding to p75^{NTR}. Activation of cAMP-PKA is required for translocation of p75^{NTR} to lipid rafts, and for Rho inactivation, which is one of the downstream signals of p75^{NTR}. Lipid rafts are cholesterol and sphingolipid-rich lipid microdomains in eukaryotic cell membranes, and are believed to function in cellular signaling by concentrating or separating specific molecules in a unique lipid environment in neurons. Proper recruitment of activated p75^{NTR} to lipid rafts is of fundamental importance in determining p75^{NTR} bioactivity.

P02-F-18

Responsiveness of embryonic striatal neurons to bone morphogenetic protein and neurotrophic factors

○Kikuchi Saya¹, Nakamura Takeshi², Mori Nozomu², Matsuoka Ichiro¹

¹Dept. Neuroscience, Grad. Sch. Pharm. Sci., Hokkaido University, ²Dept. Mol. Gen., Nat. Inst. for Longevity Sci.

Bone morphogenetic protein (BMP) is known to promote neural differentiation of cells in subventricular zone into the both neuronal and glial lineages. We have previously shown that BMP2 and retinoic acid (RA) synergistically act on developing sympathetic neurons to induce their responsiveness to specific neurotrophic factors (NT3 and GDNF) and expression of the novel protein family, BRINP (BMP/RA-Inducible Neural specific Protein). In the present study, we examined the responsiveness of striatal neurons to BMP and neurotrophic factors. Striatal neurons were prepared from E17 Wistar rats and cultured in the B27-supplemented Neurobasal medium together with BMP2 (10 ng/ml) or neurotrophins (10 ng/ml). Total RNA was prepared from cultured striatal neurons by AGPC method and expression levels of mRNA for various neural genes were analyzed by quantitative RT-PCR. At first, we found that BMP2 to increases specifically the levels of mRNA for TrkC, high affinity tyrosine-kinase receptor for NT-3 and BRINP2. RA promoted slightly the action of BMP2 on the upregulation of BRINP2. The levels of mRNA for TrkA, TrkB, BRINP1 and BRINP3 remained unchanged by the treatment with BMP2 and/or RA. On the other hand, it was found that NT-3 increases specifically the levels of mRNAs for BRINP3 and N-Shc, a neural-specific adapter molecule of the Shc-family which functions at downstream of Trk receptors. The levels of mRNA for other BRINPs and Shc family members did not change significantly by the NT-3 -treatment. BDNF had similar effects as NT3, but was less potential. These results suggest that during the course of physiological development of striatal neurons, BMP acts on the differentiation of these neurons to induce responsiveness to particular neurotrophic factors such as neurotrophins (NT3 and BDNF) by regulating their receptor expressions, then, neurotrophins act on the induction of N-Shc that plays roles in the functional differentiation of striatal neurons. It is also suggested that each member of the BRINP family has distinct roles in different aspects of the development of these neurons.

P02-F-19

Autocrine action of BMP regulates GDNF expression in Schwann cells

○Emi Kinameri, Ichiro Matsuoka

Laboratory of Neuroscience, Graduate School of Pharmaceutical Sciences, Hokkaido University

Schwann cells play important roles in the neural development as well as in the regeneration after nerve injury by providing neurotrophic supports. We have identified various mechanisms in Schwann cells regulating the expression of neurotrophic factors of the neurotrophin and neurokinin families. Recently, we found that bone morphogenetic protein (BMP) and retinoic acid (RA) promote differentiation of sympathetic neurons and induce their responsiveness to NT3 and GDNF. In the present study, we examined whether BMP and RA act also on the neurotrophic factor expression of Schwann cells. Schwann cells were prepared from sciatic nerves of postnatal 2-3d rats and were stimulated with various factors and the expression levels of mRNA for various neurotrophic factors were measured. First, we found that cultured Schwann cells expressed the receptors for both BMP2 (type I and II) and RA (RAR type α). When stimulated with BMP2 (10 ng/ml) or RA (100 nM) alone, the expression levels of mRNA for NGF, BDNF or GDNF in Schwann cells did not change significantly. However, combination of BMP2 and RA synergistically and specifically increased GDNF-mRNA (4-fold). Then, we examined the effects of various inhibitors of intracellular signal transduction machineries to find a possible involvement of the activation of PKC in the induction of GDNF-mRNA by BMP/RA. Ionomycin, a Ca^{2+} ionophore, also remarkably increased GDNF-mRNA (10-fold). Interestingly, induction of BMP2-mRNA was observed in the ionomycin-stimulated Schwann cells as well as in the sciatic nerves transferred to explant culture. These results suggest that, with a possible help of RA, autocrine action of BMP2 regulates the GDNF expression in Schwann cells during development and regeneration of the PNS

P02-F-20

Levels of mRNA for Brain Derived Neurotrophic Factor are Up-regulated in the Urethra of Diabetic Rats

○Rintaro Machino¹, Ichiro Matsuoka², Hidehiro Kakizaki¹, Takashi Shibata¹, Tomohiko Koyanagi¹

¹Dept. Urology, Grad. Sch. Med., Hokkaido Univ.,

²Dept. neurosci., Grad. Sch. Pharm. Sci., Hokkaido Univ.

PURPOSE : Diabetic neuropathy is often accompanied by neurogenic voiding dysfunction, which is caused by peripheral neural impairment of the bladder(diabetic cystopathy) as well as the urethra. However, diabetic cystopathy has been the main focus of investigations and few studies have been done on the etiology of diabetic urethral dysfunction. In the present study, to elucidate the molecular mechanisms of diabetic urethral dysfunction, we examined neurotrophic changes in the urethra of diabetic rats. **METHODS :** Male Sprague-Dawley rats were made diabetic with streptozotocin at the age of 8 weeks. Vehicle treated rats fed with and without 5% sucrose water from age of 8 weeks were also obtained as control. Rats were sacrificed to extract total RNA from the urethra after 2, 4, 6 and 8 weeks of diabetic induction. RNase protection assay was employed to quantitate the levels of mRNA for NGF, BDNF, GDNF, CNTF, NT-3 and NT-4. **RESULTS :** NGF-, CNTF- and NT3-mRNA levels were up-regulated at 2 weeks of diabetic induction and then, down-regulation was observed at 8 weeks, but these changes remained in rather small range (10-50% of control groups). GDNF- and NT4-mRNA levels were very low and no obvious changes were observed. On the other hand, BDNF-mRNA levels are up-regulated dramatically in diabetic group from 6 weeks. Their levels reached 9 times as much as those of control groups at 6 weeks and 12 times at 8 weeks. **DISCUSSION :** Up-regulation of BDNF-mRNA, and concomitant down-regulation of NGF- and NT3-mRNA in skeletal muscle of diabetic rats were reported previously elsewhere. Our results are compatible with these reports, because substantial part of the male rat urethra is composed of striated muscle. The changes in urethral neurotrophic factors observed in this study may represent an endogenous protective and/or reparative response of the muscle induced by diabetes mellitus. Our study demonstrates novel urethral neurotrophic changes in diabetic rats that may be relevant to the molecular mechanisms of the development of neurogenic urethral dysfunction seen clinically in diabetic patients.

P02-F-21

Accumulation of nerve growth factor protein at both rostral and caudal stumps in the transected rat spinal cord

○Yoshiko Furukawa, Yutaka Murakami, Atsumi Nitta, Shoei Furukawa
Lab. Mol. Biol. Gifu Pharm. Univ.

Nerve growth factor (NGF) is a neurotrophic factor for small-diameter sensory neurons, sympathetic neurons in the peripheral nervous system and some neuronal populations in the central nervous system such as basal forebrain cholinergic neurons. Whereas information concerning the intraspinal NGF is scant. In this study changes in the NGF content in the rat spinal cord during development or after traumatic spinal cord injury were examined by using a two-site enzyme immunoassay system and an immunohistochemical technique. From embryonic day (E) 14 to postnatal day (P) 70, the spinal cord contained 200–300 pg NGF/g of wet tissue evenly in all regions tested. After complete spinal cord transection of P49 rats, the NGF level started to increase in the rostral and caudal stumps nearest to the injury site at 2 and 4 days, respectively. The NGF level of the caudal side returned to the original level by 2 weeks, but that of the rostral side remained high even 3 weeks, after the injury. At 4 days after the injury, NGF-like immunoreactivity in both stumps was predominantly localized in the axon-like structures of the white matter and in cells morphologically resembling immune cells. These observations suggest that the NGF was transported within the spinal tracts, and that NGF secreted from immune cells that had invaded into the injured spinal cord had accumulated around the transection site. Increased NGF at the injury site may be advantageous for injured neurons and involved in mechanisms directing to axonal regeneration of the injured spinal cord.

P02-F-22

Effect of neurotrophins on the expression of bHLH transcription factors

○Hisanori Ito, Aki Nakazima, Shoei Furukawa
Lab. of Mol. Biol., Gifu Pharmaceut. Univ.

Neural stem cells are defined as self-renewal multipotent progenitors that give rise to both glial and neuronal lineages. The basic helix-loop-helix (bHLH) factor such as Mash1, Math1 and Neurogenin promote determination and/or differentiation of neurons. In contrast, Hes1 and Hes5 negatively regulate neuronal differentiation, and directly represses Mash1 expression. However, the factors that regulate the expression of bHLH transcription factors are not clear except for Notch signaling. Since neurotrophic factors have been reported to induce neuronal differentiation from neural stem cells, we examined influences of neurotrophic factors, especially NGF, BDNF or NT-3, on the expression of bHLH factors. Neural stem cells were cultured from the telencephalon of mouse embryos on embryonic day 14, treated with 100 ng/ml NGF, BDNF or NT-3, and expression of bHLH transcription factor was evaluated mRNAs using RT-PCR. When the cells were treated with neurotrophins under the growth conditions containing FGF-2, the expressions of Mash1, Math1 Neurogenin2 were significantly increased. Neurotrophins did not have influence on expression of Hes1, Hes5 and NeuroD. Furthermore, neural stem cells treated with respective neurotrophins normally proliferated and formed neurosphere in the presence of FGF-2. When the cells were treated with all neurotrophins in combination, Mash1, Math1 Neurogenin2 mRNA levels were higher than those of the cells treated with each neurotrophin alone. The expression of NeuroD was accelerated much earlier in neurotrophin-treated cells than in non-treated cells after deprivation of FGF-2. These results suggest that neurotrophins stimulate expression of bHLH factors such as Mash1, Math1 and Neurogenin2, and promote determination of neuronal lineages.

P02-F-23

Shp-2 Tyrosine Phosphatase Regulates Trophic Effect of BDNF through Activation of the PI3-K and MAPK Pathways

○Satomi Takai, Masato Okada

Dept. Oncogene Res., Ins. Microbial Diseases, Osaka Univ.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophins, promotes differentiation and survival, and regulates synaptic plasticity of several types of neurons. BDNF binds to and activates TrkB, a receptor tyrosine kinase, initiating the intracellular signaling that exerts the functions of BDNF. Shp-2, a Src homology 2 domain-containing cytoplasmic tyrosine phosphatase, is involved in neurotrophin-stimulated intracellular signaling in PC12 cells and cultured cerebral cortical neurons. To examine the roles of Shp-2 in BDNF-exerted trophic effect on cultured dopaminergic neurons from rat substantia nigra, we ectopically expressed the wild type, phosphatase-inactive (C/S) mutant, phosphatase domain-deficient (delta P) mutant and constitutively active (D61A and E76A) mutants of Shp-2 in cultured dopaminergic neurons using recombinant adenovirus vectors. We found that the wild-type Shp-2 enhanced BDNF-promoted survival of the cultured dopaminergic neurons. In contrast, the C/S and delta P mutants of Shp-2 did not affect cell survival. In addition, the constitutively active D61A and E76A mutants mimicked BDNF and promoted survival. Inhibitor of PI3-K and MAPK blocked the enhancement effects of Shp-2 on BDNF-promoted survival of dopaminergic neurons. These results indicate that Shp-2 positively regulates BDNF-exerted trophic effect on cultured dopaminergic neurons through activation of the PI3-K and MAPK pathways. Further studies are necessary to understand the molecular mechanisms by means of which Shp-2 regulates downstream signaling and neuronal survival.

P02-F-24

Autocrine action of acidicFGF to stimulate cholesterol metabolism and apoE secretion of rat astrocytes

○Jinichi Ito, Yuko Nagayasu, Shinji Yokoyama

Dept. Biochem.1. Nagoya City Univ. Med. Sch.

Production and secretion of apoE and cholesterol are highly upregulated in the astrocytes (M/W cells) prepared by one-week secondary culture after one-month primary culture of rat fetal brain cells in comparison to the cells (W/W cells) obtained by a conventional method of one-week primary and one-week secondary culture. We have found in the previous investigation that aFGF stimulates biosyntheses and secretion of cholesterol and apolipoprotein (apo) E of the W/W cells and that the conditioned medium of the one-month primary culture of brain cells (4W-CM) has an aFGF-like activity to enhance secretion of cholesterol and apoE also. We studied what neuronal cells produce aFGF-like factor in long-term primary culture of rat fetal brain cells. The expression of aFGF mRNA was markedly increased during primary culture of the brain cells for three weeks. There was hardly any neuron in over two-weeks primary culture, suggesting that aFGF was produced by astrocytes themselves. Not only the 4W-CM but also the conditioned media of the M/W cells and the cells (W/M cells) prepared by one-week primary culture and one-month secondary enhanced markedly the release of apoE and cholesterol from the W/W cells. The conditioned medium of W/W cells has no activity. The aFGF-positive cells were observed much more in the M/W cells than the W/W cells by immunocytochemistry using anti-aFGF antibody. The mRNA expression of aFGF in W/M and M/W cells was extremely greater than that of W/W cells. The heparin-binding protein contained in the cytosol of M/W and W/M cells cross-reacted to anti-aFGF antibody. These findings suggested that an aFGF-like trophic factor is produced by astrocytes and plays an important role in intercellular cholesterol transport in the central nervous system by upregulation of biosynthesis and secretion of the apoE/HDL of astrocytes through the autocrine action.

P02-F-25

A brain-specific EGF-like family protein, NELL2, is a novel survival factor for neurons

○Masato Horie¹, Koutoku Aihara¹, Katsuyuki Tanizawa², Shun'ichi Kuroda²

¹Second Institute of New Drug Discovery, Otsuka Pharmaceutical Co., Ltd., ²Department of Structural Molecular Biology, Institute of Scientific and Industrial Research, Osaka University

Background: Neurotrophic factors have generated considerable excitement for their potential as therapy for a wide variety of neurodegenerative disorders, such as Alzheimer's disease. Thus, identification of novel trophic factors for neuronal cells is of great interest to many, from academic researchers to pharmaceutical companies. The NELL2 has been identified as a brain-specific gene that encodes a protein containing an N-terminal thrombospondin-1-like module, five von Willebrand factor C domains, and six epidermal growth factor (EGF)-like domains. NELL2 is secreted from the cells as homotrimeric glycoproteins. **Objective:** Although the involvement of NELL2 in neural functions has been inferred from its expression and biochemical profiles, biological effects of NELL2 remain uncertain. This study aims to examine trophic effects of NELL2 on neurons. **Methods:** Cortical and Hippocampal neurons were obtained from cerebra removed from Sprague-Dawley rat fetuses on the 18th gestational day. These primary cultured neurons were treated with a recombinant NELL2 protein to evaluate biological and signaling properties of NELL2. **Results:** We evaluated the survival effect of NELL2 using primary cultured neurons from rat fetal brain following treatment with a recombinant NELL2 protein. NELL2 increased survival of neurons of the cerebral cortex and hippocampus. To understand signaling properties underlying this survival effect, we studied activation of mitogen-activated protein kinases. We found that treatment of primary cultured cells from the hippocampus with NELL2 enhanced phosphorylation of c-jun N-terminal kinase (JNK). **Conclusion:** NELL2 is a novel class of survival factor for neurons activating JNK.

P02-F-26

Changes of the gene expression in the injured site of the rat spinal cord

○Manabu Hashimoto, Takuya Hori, Shoei Furukawa
Lab. of Mol. Bio., Gifu Pharm. Univ.

[PURPOSE] To identify the genes that participate in the regeneration of the central nervous system, we searched the genes whose expression changed in the injured site of the spinal cord, and also examined the expression of neurotrophins and their receptors. **[METHODS]** Rat spinal cords were completely transected at the position T10. The tissues adjacent to the transected site were collected, and examined about mRNA expression of neurotrophins and their receptors with RT-PCR. To search other genes that change the expression, we performed cDNA subtraction method. **[RESULTS AND CONCLUSION]** NGF and NT-4/5 mRNAs were increased, and NT-3, TrkA, TrkB and p75 mRNAs were decreased in the injured site. Immunoblot analysis showed the decrease of NT-3 protein in the injured site, whereas potent immunostaining signal was found in the neurites adjacent to the injured site. Further, we found three genes that changed the expression in the injured site by cDNA subtraction. These included MCP-1, a chemokine, NAP-22, a molecular similar character to GAP-43, and an unidentified gene. The RT-PCR for these genes showed upregulation of MCP-1 and NAP-22 mRNAs, and downregulation of the unknown gene mRNA after injury. The immunohistochemical staining of MCP-1 demonstrated that motoneuron-like cells and macrophage-/microglia-like cells were positive. We further investigated the expression of MIP-1 alpha, MIP-1 beta, MIP-2 or RANTES, a family of chemokines resemble to MCP-1, and found their all upregulations in the injured site. These results suggest that downregulation of NT-3, TrkA, TrkB and p75, and upregulation of chemokines attribute to the unsuccessful nerve regulation after spinal cord injury, although upregulation of NGF, NT-4/5 and NAP-22 may to some extent compensate these.

P02-F-27 (MS1-7)

Neurite outgrowth effects of withanolide A, withanoside IV and withanoside VI in A β 25-35-induced atrophy of neurites

○Tomoharu Kuboyama, Chihiro Tohda, Katsuko Komatsu
Res. Ctr. Ethnomed., Inst. of Natural Med., Toyama Med. & Pharm. Univ.

We previously found that withanolide A, withanoside IV and withanoside VI isolated from *Ashwagandha* (roots of *Withania somnifera* Dunal) had neurite outgrowth activities. Withanolide A-treatment predominantly extended axons in normal rat cortical neurons, whereas withanosides IV- and VI-treatment extended more dendrites than axons. In the present study, we investigated the neurite outgrowth effects of these three compounds on damaged neurons. Cortical neurons of rats (SD, E18) were treated with A β 25-35 (a partial peptide of β -amyloid, 10 μ M) for 3-4 days. Approximately 30% of neurons were dead, and extension of MAP2-positive (dendrites) and phosphorylated NF-H-positive (axons) neurites were inhibited in remaining neurons. Addition of withanolide A, withanoside IV or withanoside VI (1 μ M each) simultaneously with A β 25-35 increased extensions of dendrites and axons over to the control level. When added each 3 compounds 4days after the treatment of A β 25-35, outgrowths of dendrites and axons were enhanced compared with vehicle-added neurons, but it did not reach the control level. The most potent elongation of axons was shown by withanolide A-treatment, whereas dendrites tended to be extended by withanosides IV- and VI-treatment than by withanolide A-treatment. A β 25-35-induced neuronal death was not protected by these 3 compounds. These results suggest that treatment with withanolide A, and withanosides IV and VI induce predominant outgrowths of axons and dendrites, respectively in A β 25-35-induced atrophy of neurites, even after the impairment has already progressed.

P02-F-28 (MS1-8)

Expression of tau isoforms containing exon2 and 3 during synaptogenesis *in vivo* and *in vitro*

○Chikara Kitamura, Yuta Wakayama, Masaki Masuno, Miyuki Noro, Tomoko Tashiro
Dept. Biochemistry, Coll. Science and Engineering, Aoyama-gakuin Univ.

Tau is one of the major neuronal microtubule-associated proteins enriched in the axon. In the CNS, 6 tau isoforms are produced from a single gene by alternative splicing of exons 2, 3 and 10. Among these, exon 10 contributes an additional microtubule (MT)-binding repeat, and confers higher MT-binding affinity to isoforms containing this exon compared with those without this exon. To elucidate the physiological significance of exons 2 and 3 which remains poorly understood, we examined the expression of these exons at mRNA and protein levels during early postnatal development of the rat cerebellum (*in vivo*) as well as in cultured rat cerebellar cortical neurons (*in vitro*). Isoforms of mRNA were examined by RT-PCR whereas protein isoforms were detected using exon-specific, anti-peptide antibodies. Synaptogenesis monitored by immunostaining with anti-synapsin I antibody took place *in vivo* between postnatal days 10 (P10) and 14. With a primer pair selected in exons -1 and 4, only the shortest mRNA without exons 2 and 3 was detected up to P4. Significant amounts of the two longer mRNA isoforms containing exon 2 and exons 2+3 were detected at P11 and rapidly increased relative to the shortest isoform between P14 and P21, reaching the adult isoform composition at P21. Protein isoforms containing exon 2 or 2+3 were first detected at P7, and increased between P10 and P21. With cultured cortical neurons obtained from E18 rats, synaptogenesis took place between 6 and 8 days in culture (DIV). Tau isoforms containing exon 2 or 2+3 first increased within this time interval, which was followed by a second rapid increase between DIV10 and 13. Tau exons 2 and 3 are thus expressed and up-regulated at the time of synaptogenesis, suggesting their role in the maturation of the axon.

P02-F-29 (MS1-3)

Dynamic morphological changes of migrating neurons during the cortical development

○Hidenori Tabata¹, Kazunori Nakajima^{1,2}

¹Dept. Mol. Neurobiol., Inst. DNA Med., Jike Univ. Sch. Med., ²PRESTO, JST

During the development of cortical plate, neurons generated in the ventricular zone migrate toward the pial surface of telencephalic wall and terminate the migration beneath the marginal zone. These migrating neurons had long been thought to have a thick leading process and a thin trailing process and to use the radial fibers as the scaffolds of migration. Recently, another mode of migration, somal translocation, was directly observed, in which migrating neuron has a long process reaching the pial surface and contracts the process as the cell soma translocates toward the marginal zone. We recently established a gene-transfer system to mouse embryos *in utero* based on *in vivo* electroporation. When we introduced the green fluorescent protein (GFP) expression vectors to the embryonic brains and examined a few days later, we observed that the majority of GFP-expressing cells in the intermediate zone had multipolar morphology. This morphology was completely different from those of the classical type migration or somal translocation. We made time-lapse observations of the migrating neurons on the cortical slices of the brains that were transfected with the DsRed expression vector. We found that the multipolar cells were extending and retracting thin processes in various directions very dynamically, advancing slowly toward the pial surface. We named this movement as multipolar migration. We are now investigating the origin and fate of these cells.

P02-F-30

Lateralization of thalamofugal pathway before light exposure in developing chicken visual pathway

○Mamiko Koshiba^{1,2}, Tateki Kikuchi², Masfumi Yohda¹, Shun Nakamura²

¹Tokyo Univ. A&T, ²Natl. Inst. Neurosci.

The light exposure to one eye induces functional lateralization in developing chick visual pathway. In the normal developmental configuration, the right eye is exposed to light illumination, leading to the dominance of the anatomical development of the left thalamofugal pathway. We have shown that this lateralization can be inverted by the right eye-occlusion using retrograde tracers (Koshiba M., et al., Neurosci. Lett., 318, 113-6, 2002). Then, we asked when this lateralization starts. To address this question, we introduced a histochemical staining of a subunit of nicotinic acetylcholine receptor ($\alpha 8$) with a monoclonal antibody (Mab305). This staining revealed high neuropiles expression of the receptor in the dorsolateral anterior thalamus (DLA) and the anatomical lateralization within DLA. Thus, it could be a nice landmark of the development of the lateralization. Surprisingly, we found that the left DLA is already dominant before the critical period of this light-inducing lateralization in the dark-reared embryos (E18, n=6), though a few exceptions were found. Furthermore, this dominance is morphometrically obvious without the antibody staining. The right eye occlusion inverted the lateralization revealed by Mab305 or morphometry 3 more days after hatching. These data suggest that the thalamofugal visual pathway develops to be left dominance without light exposure and is instructed to be either left- or right dominance in depend on the light experience during the critical period (e19-P2). Next, we asked a mechanism of the anatomical lateralization revealed by the retrograde tracer experiment. The results using Tunel and BrdU staining methods suggest that the cell death or growth is unlike. Then, another possibility like as neurites extension and synaptic formation should be considered.

P02-F-31

Is Otx1 essential for the establishment of subcortical projections from the visual area?

Koichi Ando¹, Chikara Kubota¹, Hideshi Yagi¹, Takashi Nagano¹, Shinichi Aizawa², Toshio Terashima³, [○]Makoto Sato¹

¹Dept. Anat.2, Fukui Med. Univ., ²CARD, Kumamoto Univ., ³Dept. Brain Sci., Grad. Sch. Med., Kobe Univ.

Cortical pyramidal neurons of layer 5 innervate many of their targets by collateral axon branching in a area-specific manner: those in the motor area project toward the pontine nuclei in addition to the spinal cord, while those in the visual area extend toward the superior colliculus in addition to the pontine nuclei. These apparent cortical area-specific sets of projections are established from the unique prototype of projection that is formed transiently during development. Cortical axons extend toward the spinal cord as well as the pontine nuclei and the superior colliculus in this prototype. Later in the development, some of the collaterals are pruned resulting in the adult type of mature area-specific patterns of projections. The homeobox transcription factor Otx1 is expressed in the subcortical projection neurons in the visual area of cortex, suggesting that Otx1 is involved in area-specific formation of cortical projections. Actually, Weimann et al. has reported that Otx1 is required for the refinement of exuberant axonal projections (Neuron, 24: 819, 1999). In this study, we explore a potential role of Otx1 for the establishment of subcortical projections then reveal that it is unlikely that Otx1 plays a pivotal role for the establishment of visual cortex-specific patterns of connectivity despite the previous report. In the Otx1 null mice, cortical areas shifted rostro-medially resulting in a shift of projection neurons extending toward the spinal cord within the cortex. Details will be discussed at the meeting.

P02-F-32 (MS1-2)

CRMP-2 and axon formation

[○]Yuko Fukata, Kozo Kaibuchi

Dept. Cell Pharmacol. Grad. Sch. Med., Nagoya Univ.

Collapsin response mediator protein-2 (CRMP-2) is a mammalian homologue of *Caenorhabditis elegans* UNC-33 whose mutations lead to abnormal axon guidance. We recently showed that CRMP-2 is highly concentrated in growing axons of hippocampal neurons, that overexpression of CRMP-2 in cells induces the formation of multiple axons and that expression of truncated CRMP-2 mutants suppressed the formation of primary axon. Thus, CRMP-2 appears to be critical in establishment and/or maintenance of neuronal polarity. However, it remains largely unknown how CRMP-2 exerts its function. Here, we identified α - and β -tubulin as CRMP-2-interacting proteins by using CRMP-2 affinity chromatography. Alpha- and β -tubulin were coimmunoprecipitated with CRMP-2 from developing rat brain extracts. CRMP-2 directly bound to tubulin heterodimer, formed a heterotrimer comprised of one CRMP-2 and one tubulin dimer, and co-polymerized with tubulin dimers into microtubules. On the other hand, CRMP-2 weakly bound to taxol-stabilized microtubules as compared with a well-known microtubule associated protein (MAP), tau. Dark field video microscopy revealed that CRMP-2 promoted the assembly of microtubules *in vitro* and that the region responsible for this activity was determined to be 323-381 amino acids within CRMP-2. Studies in N1E-115 neuroblastoma and primarily cultured neuron revealed that CRMP-2 promoted neurite formation or axon elongation acting on microtubule assembly. Taken together, our results indicate that CRMP-2 regulates microtubule polymerization dynamics in a different fashion from well-known MAPs, and that CRMP-2 plays an essential role in axon formation through the regulation of microtubule assembly.

P02-F-33

Conformational changes of Pur α concomitantly increase in ssCRE binding activity

○Che-Hui Kuo, Ling-Hui Zeng, Naomasa Miki
Dept.Pharmacol. Osaka Univ. Med. School

Accumulated evidence indicates that Pur α , a single stranded (ss) DNA binding nuclear protein, seems to be a multi-functional protein in terms of affecting on the replication, transcription, cell cycle and cell growth.

We have reported that 1) Pur α is an abundant protein in the brain, 2) Pur α binding to the ssCRE is enhanced by a heat stable endogenous activator, and 3) the activator was identified as a calmodulin (CaM) by the purification.

During the study on the interaction between Pur α and CaM in the presence of ssCRE, we found conformation change of GST-Pur α . It is easily detected on the non-denature gel by the silver/Coomassie brilliant blue staining or by the non-denature gel/ western blot analysis with anti-Pur α antibody. The Pur α conformation change induced by ssCRE reveals as an increase of intensity of Pur α staining or increase of immunoreactivity of Pur α against anti-Pur α antibody. Endogenous Pur α in soluble fraction from mouse brain and in extract from microsomal fraction by NaCl treatment was also analyzed. The conformational change of GST-Pur α /endogenous Pur α was depended on the ssCRE concentration in a sequence-specific manner and was further enhanced in the presence of CaM in a dose-dependent manner in parallel with the binding activity of Pur α to the ssCRE estimated by a band shift assay.

From these results, we concluded that Pur α conformation change induced by ssCRE/CaM is closely associated with its ssDNA binding activity.

P02-F-34

Cytoplasmic p21^{Cip1/WAF1} regulates neurite remodeling by inhibiting Rho-kinase activity

○Hiroyuki Tanaka, Toshihide Yamashita, Masaya Tohyama

Dept. Anatomy and Neuroscience. Grad. Sch. Med., Osaka Univ., CREST, JST

p21^{Cip1/WAF1} has the cell-cycle inhibitory activity by binding to and inhibiting both cyclin/Cdk kinases and proliferating-cell nuclear antigen (PCNA). Here we show that p21^{Cip1/WAF1} is induced in the cytoplasm during the course of differentiation of chick retinal precursor cells. Ectopic expression of p21^{Cip1/WAF1} lacking the nuclear localization signal in N1E-115 cells and NIH3T3 cells affects the formation of actin structures, characteristic of inactivation of Rho. p21^{Cip1/WAF1} forms a complex with Rho-kinase and inhibits its activity in vitro and in vivo. Neurite outgrowth and branching from the hippocampal neurons are promoted if p21^{Cip1/WAF1} is expressed abundantly in the cytoplasm. These results suggest that cytoplasmic p21^{Cip1/WAF1} may contribute to the developmental process of the newborn neurons that extend axons and dendrites into target regions.

P02-F-35

活動抑制による細胞死に先行して起こるシナプス廃止

○小倉 明彦^{1,2}, 橋本 典和¹, 富永(吉野) 恵子^{1,2}¹阪大・院・理, ²阪大・院・生命機能

仔ネコの片眼閉鎖実験などから、シナプスは活動によって発芽・新生し、不活動によって廃止される競争下にあるとされる。いっぽう、小脳顆粒細胞の脱分極培養実験などから、神経細胞自体の生存も活動依存的であるとされる。しかし、シナプスの維持と細胞の生存とがどのような因果関係にあるのか、機構上の関連があるのか、についてはほとんどわかっていない。この問題に手がかりを得る目的で、種々のラット海馬培養標本にテトロドトキシン (TTX) を添加して、自発活動を抑制した後の細胞構造の変化を追跡した。切片培養にTTXを加えると、細胞死に先行して樹状突起の退縮が起こった。グリア島上の単一神経細胞培養についてのタイムラプスビデオ観察でも同様の経過をたどった。分散培養について、培養1週経過後からTTXを投与すると (1週までは差なし)、シナプス部位の減少→樹状突起の減少→細胞体のアポトーシスの順で変化が起こった。投与2日以内にTTXを除くと前二者は回復し、細胞生存率曲線は対照群と平行に戻った。さらに、シナプス部位について詳しく観察すると、PSD95陽性スポット (シナプス後構造) が、シナプトフィジン陽性スポット (シナプス前構造) に先行して消失した。これらの結果は、細胞の廃用退行に一定の順序があり、シナプス廃止の極限形として細胞死があることを示唆する。

P02-F-36

Dopaminergic neurons derived from mouse midbrain maintain synchronous oscillation of intracellular calcium in primary culture

○Fumie Yasumoto¹, Takayuki Negishi^{2,4}, Yoshiyuki Ishii², Shigeru Kyuwa², Yoichiro Kuroda^{3,4}, Yasuhiro Yoshikawa^{2,4}¹Dept. Biomed. Sci., Faculty of Agri., The Univ. of Tokyo., ²Dept. Biomed. Sci., Grad. School of Agri. and Life Sci., The Univ. of Tokyo., ³Dept. of Mol. and Cell. Neurobiol., Tokyo Metropolitan Inst. for Neurosci.,⁴CREST/JST

It is known that primary cultured rat cortical neurons burst synchronously and periodically without any stimulation, which is caused by glutamatergic neurons. This phenomenon can be visualized as the periodic changes of intracellular Ca^{2+} concentration (synchronous oscillation). In the present study, we first observed synchronous oscillation in cultured mouse midbrain neurons, and confirmed participation of endogenous dopamine in this neural network.

Midbrain and cerebral neurons were prepared from 17 days old fetal ICR mouse. We detected a few TH-positive (dopaminergic) neurons by immunocytochemistry. Synchronous oscillation in midbrain neurons was abolished by the application of tetrodotoxin, voltage dependent Na^+ channel blocker, suggesting that these neural bursts in synchronous oscillation in midbrain neurons were accompanied with action potentials as well as that of cerebral neurons. Synchronous oscillation in midbrain neurons was also abolished by the application of R(-)-2-amino-5-phosphonopentanoic acid and 6-cyano-7-nitroquinoxaline-2,3-dione which are NMDAR and non-NMDAR antagonist, respectively, suggesting that it was generated by glutamate. R(-)-SCH-12679, D1R antagonist, stopped synchronous oscillation in midbrain neurons. This suggests that endogenous dopamine maintained this phenomenon. On the other hand, SCH-12679 could not stop synchronous oscillation in cerebral neurons. We are now investigating the effects of agonists and antagonists for various DAR subtypes.

Synchronous oscillation of midbrain neurons would be useful tool for *in vitro* researches in various neural disorders directly and/or indirectly caused by dopaminergic neurons.

P02-F-37

Disparity of cell death and mossy fiber sprouting in kainic acid-treated hippocampal slice cultures

○ Chunxi Han¹, Yuriko Furukawa^{1,2}, Keiichi Torimitsu^{1,2}

¹NTT Basic Research Lab., ²C R E S T

Hippocampal cell death and mossy fiber sprouting typically arise in experimental animals after repeated seizures and may contribute to the hyper-excitability of the epileptic brain. It is unclear, however, whether kainic acid (KA)-induced mossy fiber sprouting results from neuronal cell death or the seizure activity. In order to examine the relationship between cell death and the mossy fiber sprouting response, the entorhinal cortex attached and unattached to the hippocampus was cut from brains of wistar rat pups (postnatal day 10-12) and slice cultures were prepared. KA (total concentration 6 μ M) diluted in the culture medium was applied to cultures for 24-48h. After KA treatment, cultures were transferred to a fresh medium washing-out residual KA and further cultured for 2-4 weeks. The degree of mossy fiber sprouting and cell death were assessed from Timm staining and cellular uptake of the fluorescent dye propidium iodide (PI) respectively. Significant pyramidal cell death occurred in the CA3a/b, but no significant cell loss was observed in the CA1 pyramidal cell layer after KA treatment. The cell death was accompanied by considerable mossy fiber sprouting in the inner molecular layer of the dentate gyrus. Interestingly, some of the cultures without KA treatment also arose slightly mossy fiber sprouting in the case of cutting hippocampus afferent axons from the entorhinal cortex. These results suggest that sprouting of mossy fiber was induced by cell death, repeated seizures and loss of afferent control in the slice cultures of rat hippocampus.

P02-F-38

細胞形態測定装置を用いた神経細胞の突起伸展開評価

○田中 史子, 清水 博喜, 隠岐 徹, 長谷川 嘉一
エーザイ (株) 筑波研究所

【目的】 従来、神経細胞の突起伸展開評価は、主に顕微鏡下での目視観察により行われてきた。この方法は、客観的な数値化が困難であり、多大な労力を用するため多検体評価には向かない。そこで我々は、神経細胞の突起伸展開作用を促進する化合物をスクリーニングするために、多検体処理、数値化が可能なELISA法を構築した。しかし周知のとおりELISA法は細胞固定、抗体反応、washなど、ステップ数が多く操作が煩雑であるというデメリットがある。近年、特定の細胞形態を抽出し数値化・定量化する機能を有する細胞形態スクリーニング装置HTS-50 ANAX (九州松下電器) が開発された。我々はこのANAXを使用して神経細胞の突起伸展開を評価し、ELISA法および従来の目視観察との比較検討を行った。【方法】 PC12細胞において、神経成長因子 (NGF) による突起伸展開作用をスケールとした目視観察により、検体の作用をスコアリングした。同じくPC12細胞のELISAでは、突起部位の主要マーカー (リン酸化ニューロフィラメント) を抗体で認識させ、突起伸展開量を定量した。またラット脊髄後根神経節 (Dorsal Root Ganglion) 細胞は、目視観察により突起保持細胞数をカウントした。一方ANAXによる解析では、PC12細胞、DRG細胞の各々において突起長、細胞部面積を測定した。【結果・考察】 ANAXによる測定結果は、PC12細胞における目視観察、ELISAによる測定結果およびDRG細胞における目視観察の結果、いずれとも良く相関するというデータが得られた。これにより、神経細胞の突起伸展開評価において、操作の簡略化、目視に頼らない数値化、多検体評価の自動化が可能となった。さらにANAXは固定や染色を必要としないため、生きたままで同一細胞の形態変化を経時的・定量的に追跡することも可能である。また蛍光観察機能も有しており、細胞形態解析以外への応用も期待でき、現在検討中である。

P02-F-39

Transglutaminase expression and its implication in goldfish optic nerve regeneration

○Kayo Sugitani^{1,2,3}, Toru Matsukawa^{2,3}, Keiko Homma^{1,2}, Satoru Kato^{2,3}

¹Dept. Lab. Sci., Fac. Med., Kanazawa Univ., ²Dept. Mol. Neurobiol., Grad. Sch. Med., Kanazawa Univ.,

³CREST

In contrast to the mammalian optic nerve, the fish optic nerve can regenerate after transection. A coarse retinotectal connection is re-established by 20-40 days after axotomy. We have used this regenerating goldfish optic nerve system to search for factors involved in this process. Transglutaminase (TG) is one of enzymes which are induced after nerve injury. To investigate the molecular mechanism of fish TG in optic nerve regeneration, we examined enzyme activity, protein and gene expression during regeneration. The peak of retinal TG activity was 20-30 days after optic nerve transection. We further isolated a cDNA clone for TG from a goldfish retinal cDNA library. The predicted TG protein comprised 679 amino acids that showed 65% homology to the human tissue type of TG. We made a recombinant protein and thereby obtained an antiserum against TG. Western blot analysis identified the TG protein of 70 kDa in the retinal extract. Immunohistochemical experiments showed that the most significant change during optic nerve regeneration was an increased staining only in the ganglion cells and their axons 15-20 days after axotomy. The specific time course and cell localization in the regenerating retina was confirmed by northern blotting and in situ hybridization. Using an explant culture system, neurite outgrowth from mature retina was clearly inhibited by adding anti-TG serum. These results suggest that TG activation is necessary for optic nerve regrowth (regeneration). It is also important to identify endogenous substrates for TG during optic nerve regeneration. We are now investigating possible candidate proteins in the retina.

P02-F-40

A member of collapsin response mediator protein(CRMP) family has a potent neurite elongation activity in nerve regeneration

○Yasuhiro Suzuki^{1,2}, Saya Nakagomi¹, Naoyuki Inagaki³, Kozo Kaibuchi⁴, Hiroshi Kiyama¹

¹Dept.Anat.Grad.Sch.Med.Osaka-City Univ.,

²Dept.Med.Asahikawa Med.Coll.,

³Div.Sig.Trans.NAIST, ⁴Dept.Cell Pharm.Nagoya Univ.grad.Sch.Med.

The collapsin response mediator protein (CRMP) family consists of five members, CRMP-1 to -5. Their functional consequence during embryonic development particularly in neurite elongation is demonstrated, however their implication in nerve regeneration is still obscure. We examined the functional significance of CRMP family members during hypoglossal nerve regeneration. Among the members, CRMP-1, -2 and -5 mRNA expressions were increased after nerve injury, whereas CRMP-3 and CRMP-4 mRNA didn't show any significant changes. We next examined CRMP family mRNA expression using NG108 cells, which elongate neurites after forskolin treatment. During the nerve elongation, only CRMP-2 mRNA level was transiently increased, whereas CRMP-1 and -5 mRNA levels were transiently decreased. The other members did not show any altered expressions. Taking those results into the consideration, it would be plausible that CRMP-2 is the most potent among the members in terms of neurite elongation activity after nerve injury. We therefore examined whether over-expression of CRMP-2 in the injured hypoglossal motor neurons by means of adenovirus vector accelerates the nerve regeneration. A retrograde-tracer, Fluoro-Gold was used to evaluate the number of re-projecting motor neurons. CRMP-2-overexpressing motor neurons demonstrated accelerated re-projection in speed. The present study suggests that CRMP-2 has a potent neurite elongation activity in nerve regeneration in vivo.

P02-F-41

神経周膜の修復・端側縫合移植モデルにおける神経再生

○岡島 誠一郎¹, 小田 良¹, 平川 永徳¹, 小橋 裕明¹, 久保 俊一¹, Julia Terzis²

¹京府医大・整形, ²バージニア医学校

【目的】末梢神経損傷の治療成績はマイクロサージャリーの導入により飛躍的に改善した。今回われわれは末梢神経部分損傷の一形態であるperineurial windowモデルを作製し、その修復過程を電子顕微鏡を用いて観察した。このperineurial windowモデルに端側縫合による自家神経移植を追加し移植神経に再生神経が形成されるかどうか検討した。【方法】SD系ラット（雄、200～250g）の左坐骨神経を顕微鏡下にマイクロ用剪刀を用いV字形に神経周膜および神経の一部を部分切除した（perineurial windowモデル）。損傷後6時間、3,7,14,21日後（2匹ずつ、計10匹）に2%パラホルムアルデヒド・2.5%グルタルアルデヒド溶液にて灌流固定を行った。このperineurial windowに同側の遊離伏在神経（約1.5 cm）を縫合し手術後1,2,6,12および24日に同様に灌流固定した。これらの組織を2%オスミウム酸による後固定、アルコール脱水を行い、エポン包埋ののち準超薄切片を作成しトルイジンブルー染色を行い光学顕微鏡にて観察した。超薄切片を作成し、電子顕微鏡にて観察した。また右側の坐骨神経を展開し外科的処置を行わずそのまま閉創しsham operation群とした。【結果】perineurial windowモデルでは損傷後6時間では損傷部は陥凹しており損傷部に線維芽細胞の集積、膠原線維の増生が観察された。損傷後3日目には髄鞘の変性とともに関与近位部より再生神経と考えられる神経線維が観察された。損傷後21日目においては膠原線維の増生を伴う神経周膜の再生が観察された。端側縫合を行った実験においては術後24時間ではドナー神経内に再生神経が観察されたが移植神経には再生神経は見られなかった。術後48時間では移植神経の基部に少数の再生神経が観察された。術後12日ではいずれの損傷群においてもシュワン細胞の細胞質に囲まれた再生無髄神経が移植神経内に観察された。術後24日では薄い髄鞘を持つ再生有髄神経が移植神経内に観察された。

P02-F-42

Damage Induced Neuronal Endopeptidase (DINE/ECEL) Expression Is Regulated by LIF and a Deprivation of NGF

○Ryuichi Kato^{1,2}, Sumiko Kiryu-Seo², Hiroshi Kiyama²

¹Dept. Urol. Sch. Med., Sapporo Med. Univ., ²Dept. Anat. Grad. Sch. Med., Osaka City Univ.

The expression profile of DINE (Damage-induced neuronal endopeptidase) mRNA, which was isolated as a nerve-injury associated molecule, was characterized in the dorsal root ganglia (DRG) after sciatic nerve injury. A substantial increase of the DINE mRNA expression was observed in relatively smaller-sized DRG neurons after nerve injury. This expression was mainly observed in TrkA positive and IB4 negative neurons, and the expression profile is fairly similar to that of the neuropeptide, galanin. More than 80 % of DINE mRNA positive neurons simultaneously showed galanin immunoreactivity after nerve injury. In cultured DRG, the DINE mRNA expression was enhanced by leukaemia inhibitory factor (LIF), but not by other growth factors and cytokines. LIF treatment to rat sciatic nerve induced the DINE mRNA expression in DRG, and conversely the intra-nerve injection of anti-gp130 antibody after the sciatic nerve injury significantly inhibited the up-regulation of DINE mRNA in the DRG. Furthermore the nerve growth factor (NGF) deprivation, which could induce the galanin expression, also enhanced the DINE mRNA expression in vitro and in animal. Both LIF application and NGF deprivation additively enhanced DINE expression in vitro. These results suggest that the gene expression of DINE is regulated by both LIF and a deprivation of NGF separately, and this regulation pattern is pretty similar to that of galanin gene expression.

P02-F-43

***in vitro* selection of brain-migrating cells from immature bone marrow cells**

○Kenji Ono^{1,2}, Ken Yoshihara^{1,2}, Kenji Tanaka¹, Takemasa Takii², Kikuo Onozaki², Makoto Sawada¹

¹JRDTAID, Inst. Comp. Med. Sci., Fujita Health Univ.,

²Dept. Mol. Health. Sci. Grad. Sch. Pharm. Sci., Nagoya City Univ.

We have reported that a part of bone marrow (BM) cells migrated into the brain parenchyma and that they expressed ER-MP12 antigen, a marker for the immature BM Cells, which express part of microglia but not monocytes/macrophages. In this study, we investigated for a selection of brain-migrating cells from immature BM cells in culture. BM cells showed similar properties to macrophages in the surface antigen expression such as CD45 and Mac-1 when co-cultured with hepatoma cells. However, it showed different properties from macrophages in the surface antigen expression and proliferation when co-cultured in the mixed brain cell culture (MBC). BM cells in MBC preserved to express ER-MP12 antigen and grew about ten times as many as macrophages in the cell number for 2 weeks. Furthermore some BM cells in MBC had a potential to migrate into the brain and still expressed ER-MP12 antigen in the brain parenchyma. These properties of brain-migrating activity and expression of ER-MP12 antigen were observed when BM cells were cultured with the conditioned medium from astrocytes for 4 days, but not with that from hepatoma cells. A fraction of ER-MP12-positive BM cells by MACS preserved the brain-migrating activity and decreased the peripheral tissue-migrating activity. These results suggested that ER-MP12 antigen might be a key marker for the selection of brain-migrating cells from immature BM.

P02-F-44

Challenge to raise monoclonal antibodies against galectin-1

○ Koichiro Hori¹, Kayo Fujimaki¹, Kazunori Yoshimura¹, Toshihiko Kadoya², Hidenori Horie³, Masahiko Nomura¹

¹Dept. Physiol., Saitama Med Sch., ²R&D Center, Prod. Dept., Pharmaceut. Div., Kirin Brewery Co. Ltd.,,

³Adav. Res. Center, Biol. Sci., Waseda Univ.

We have no sure and rigid method to promote nerve regeneration even for peripheral nerve, although there have been many investigations to regenerate various type of injured nerve such as axotomy and nerve degeneration. Recently Horie and colleagues isolated and identified the oxidized galectin-1 as a novel factor promoting nerve regeneration, which secreted from a kidney derived cell. However, we do not understand the histological distribution of reduced or oxidized galectin-1 and those involvement in nerve regeneration very well. Therefore, we have been tried to make monoclonal antibodies against reduced and/or oxidized human galectin-1 using recombinant human galectin-1. As a result, we obtained several monoclonal antibodies. In this study, we present characteristics of one of the monoclonal antibodies, 3D11. 3D11 reacted with both reduced and oxidized galectin-1 on ELISA and recognized several different cells on immunohistochemistry of different type of tissues from adult rat. The neurons in primary culture from rat E17 cerebral cortex were also immunostained with 3D11.

P02-F-45

脱髄モデルマウスへの神経幹細胞の移植

○松本 路生¹, 鹿川 哲史¹, Francois Lachapelle², Anne Baron-Van Evercooren², 池中 一裕¹

¹生理研・神経情報, ²INSERM U546, France

多発性硬化症 (MS) をはじめとする脱髄性疾患の治療法として、神経幹細胞の移植が注目されている。我々はこれまでにPLP遺伝子を過剰発現させた自発性の脱髄モデルマウスPLP-4eTgマウスを作製した。MSを始めとする脱髄性疾患の治療に役立てる目的で、PLP-4eTgマウスに神経幹細胞を移植し、ミエリンが再生されるかどうかを検討した。移植には全ての体細胞でGFPを発現させたグリーンマウスより調整した細胞を用いた。正常なPLP遺伝子を持つグリーンマウスの胎生14日胚前脳のganglionic eminenceよりneurosphereを作製し、それらを脱髄の進行した8か月齢のPLP-4eTgマウスのcorpus callosumに移植した。移植後20日目に、移植した細胞の一部が、ミエリンを形成していると思われる成熟したオリゴデンドロサイトに分化している事が分かった。

P02-F-46

Two novel sulfated oligosaccharides derived from P0 of bovine peripheral nerve myelin

○Jianhong Yan, Kunio Kitamura, Masahiko Nomura
Dept. Physiol., Saitama Med. Sch.

Sulfated N-linked oligosaccharides of glycoproteins have been found in the glycoproteins in the PNS myelin, such as P0 and PASII/PMP22. These glycoproteins are involved in the interaction of cell membranes and play some important roles in the formation and maintenance of myelin sheaths. These functions of the glycoproteins are due in part to the oligosaccharides in their molecules. Recently, the structures of the major oligosaccharide of P0 and PASII/PMP22 were reported, showing that each of the proteins contains a sulfated glucuronic acid (SO4-3GlcA), which is known as HNK-1 carbohydrate epitope. Here we report the structures of two novel multi-sulfated oligosaccharides containing HNK-1 carbohydrate epitopes obtained from glycoproteins in bovine PNS myelin, MS1: SO4-3GlcA β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-6(SO4-3GlcA β 1-3Gal β 1-4[SO4-6]GlcNAc β 1-2Man α 1-3) Man β 1-4GlcNAc β 1-4 (Fuc α 1-6)GlcNAc. MS2: Man α 1-3Man α 1-6(SO4-3GlcA β 1-3[SO4-?]Gal β 1-4[SO4-6]GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4(Fuc α 1-6)GlcNAc. In the meantime, in order to know the parent proteins from which the MS1 or the MS2 originated, we determined the glycopeptides containing either MS1 or MS2 by amino acid analysis and sequence analysis. The results showed that both MS1 and MS2 were from P0 glycoprotein.

P02-F-47

Structural studies on the glycoprotein-derived oligosaccharides in the peripheral nerves increased during maturation or by nerve crush injury

○Kunio Kitamura¹, Yuriko Yoshino², Jianhong Yan¹, Masahiko Nomura¹

¹Dept. Physiol., Saitama Med. Sch., ²Dept. Gynecol., Saitama Med. Sch.

We investigated the changes in oligosaccharide structures of rat sciatic nerve glycoproteins in the two cases, one during growth and the other after nerve crush injury. We previously reported that the high-mannose-type oligosaccharides were abundant in premature (5-day to 14-day) rats and also in the nerves at degeneration/regeneration stages of the nerves after crush injury. We also found some changes in the amount of other oligosaccharides in both cases. Among them, we focused on four oligosaccharides; two of them, OIBA1 and OIBA2, increased during maturation, and the other two, MORO1 and MORO2, increased more evidently than the others after nerve crush injury. We analyzed the structures of the four oligosaccharides. Neuraminidase treatment revealed that those were sialylated oligosaccharides. The molecular weights (Mw) of desialylated OIBA1 and desialylated OIBA2 were the same and so were their retention times on HPLC, indicating that the core structures of OIBA1 and OIBA2 are the same. The same thing happened in the case of MORO1 and MORO2. The Mw of MORO2 was bigger than MORO1 by just the Mw of N-acetylneuraminic acid, indicating that MORO2 was sialylated additionally to MORO1. MORO1 and MORO2 were not sulfated, but OIBA1 and OIBA2 were suggested to be monosulfated by time-of-flight mass spectrometry. Further studies suggested that the structure of the desialylated OIBA1/OIBA2 was the same as that of a monosulfated form of immunoglobulin type-H oligosaccharide. Because we have studied on the oligosaccharides obtained directly from the nerve tissues, we do not know so far what type of glycoproteins from which the oligosaccharides originated. Assignments of the parent glycoproteins of the oligosaccharides are now under investigation.

P02-F-48

Mechanism of myelin sheath formation in *shiverer* mutant mouse

○Hiroaki Asou¹, Jin Nakahara^{1,2}, Chika Seiwa¹

¹Dept. Neurobiol., TMIG, ²Dept. Anat., Keio Univ.

The *shiverer* mutant mouse (*shi/shi*) is an autosomal recessive mutant characterized by incomplete myelin sheath formation in the CNS. To investigate the myelin sheath formation in *shi/shi* resulting from the absence of compact myelin, firstly we developed new methods for generating oligodendrocyte precursor cells (OPCs) from an E17 mouse brain, and examined homozygous *shi/shi* OPCs with respect to myelinogenesis in vitro. Following treatment of *shi/shi* OPCs in vitro with PDGF or/and bFGF, proliferation of *shi/shi* OPCs was enhanced similar to that observed in wild-type OPCs. However, the majority of cells from the *shi/shi* remained as A2B5-immunoreactive early OPCs. To determine which molecular events affect the differentiation of *shi/shi* OPCs, we determined the signaling pathway that could be responsible for activating myelin sheath-specific proteins. Treatment of *shi/shi* OPCs with dbcAMP had significant effect on the differentiation of OPCs that became MAG-expressing oligodendrocytes. To further determine the possible mechanism involved in the activation of JNK was markedly stimulated by treatment with dbcAMP, and the phosphorylation factor ATF-2 was also stimulated by dbcAMP. We demonstrated that the MAG-positive *shi/shi* oligodendrocytes extend processes around axons and finally covered the axon, this was clearly observed by immunocytochemistry of *shi/shi* oligodendrocyte-DRG cocultures. These results suggest that ATF-2 coupled to specific signal transduction cascades plays an important regulatory role in MAG expression at a *shi/shi* oligodendrocyte differentiation.

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第45回日本神経化学会・大会長

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第45回日本神経化学学会大会ランチョンセミナー1

■日 時 平成14年7月17日(水) 12:10~13:10

■場 所 ホテル札幌ガーデンパレス B会場

「抗痴呆薬の創薬について」

座長: 北海道大学大学院医学研究科脳科学専攻

神経機能学講座精神医学分野 教授 小山 司 先生

演者: エーザイ株式会社 筑波研究所

創薬第一研究所 所長 杉本 八郎 先生

共 催: 第45回日本神経化学学会大会/エーザイ株式会社・ファイザー製薬株式会社

第45回日本神経化学学会大会ランチョンセミナー2

■日 時 平成14年7月18日(木) 12:10~13:10

■場 所 ホテル札幌ガーデンパレス B会場

「ユビキチンシステムとパーキンソン病」

座長: 北海道大学 神経内科学 教授 田代 邦雄 先生

演者: 順天堂大学 脳神経内科 講師 服部 信孝 先生

共 催: 第45回 日本神経化学学会大会/日本ベーリンガーインゲルハイム株式会社

日本神経化学会

平成14年度奨励賞選考シンポジウム

Young Scientists Lectureship Award

日本神経化学会では2000年度より優秀な神経化学若手研究者を対象に日本神経化学会奨励賞を贈っております。本年度も書類審査を経て、下記の4名のシンポジスト（受賞候補者）が選出されました。4名のシンポジストに研究成果を発表していただいた後、選考委員会で本年度の受賞者を決定致します。

奨励賞選考シンポジウムは一般の参加者にも公開されますので是非ともご参加ください。尚、本シンポジウムの抄録は当日会場入口で配布致します。

2002年7月17日 9:00-11:00 July 17 (Wed) 9:00-11:00

D会場（札幌ガーデンパレス2階） Room D

司会：池中 一裕（国立生理学研究所）

Chairperson：Kazuhiro Ikenaka (Natl. Inst. Physiol. Sci.)

「家族性アルツハイマー病に関連するプレセニリン1変異体は小胞体ストレストランスデューサー群の活性化を阻害する」

Disturbed activation of endoplasmic reticulum stress transducers by familial Alzheimer's disease-linked presenilin1 mutations.

片山 泰一（大阪大学大学院医学研究科）

Taiichi Katayama (Dept. Anat. Neurosci., Osaka Univ. Sch. Med.)

「薬物依存におけるdiazepam binding inhibitor (DBI) 発現とL型電位依存性カルシウムチャネルの機能的関連性」

Functional relationship between diazepam binding inhibitor (DBI) expression and L-type voltage-dependent calcium channels in drug dependence.

桂 昌司（川崎医科大学薬理学教室）

Masashi Katsura (Dept. Pharmacol., Kawasaki Med. Sch.)

「神経系細胞のダイナミズムを制御するリゾフォスファチジン酸シグナル」

Lysophosphatidic acid signaling in the regulation of neural cell dynamics

福島 伸之（北海道大学大学院医学研究科）

Nobuyuki Fukushima (Dept. Biochem. Grad. Sch. Med., Hokkaido Univ.)

「脳のナトリウムレベルセンサー」

Na⁺ channel involved in CNS sodium-level sensing

渡辺 英治（基礎生物学研究所）

Eiji Watanabe (Natl. Inst. Basic for Biol.)

（発表は原則として50音順ですが他のセッションとの関係で順番が変わることもあります。予めご了承ください。）

Author Index

A

- ABE, Kazuho **S8-2**
 ABE, Teruo **S5-1**
 ADACHI, Naoki
 P01-E-51 P02-F-15(MS3-2)
 ADACHI, Tatsumi **P02-E-10** P02-F-7
 AGO, Yukio P01-E-50
 AHMED, Md. Mahiuddin
 P01-F-1 P02-E-50
 AIHARA, Koutoku P02-F-25
 AIMI, Yoshinari P01-E-34(MS5-2)
 AIZAWA, Shinichi P02-F-31
 AKAO, Yukihiko P01-F-17(MS4-3)
 AKAZAWA, Chihiro P02-E-8
 AKIMOTO, Tatsuyuki **P01-E-61**
 ALOKESH, Duttaroy P01-E-4(MS6-3)
 ANDO, Koichi P02-F-31
 ANDO, Kosuke **P01-E-45**
 AMARA, Susan G. **ISN-5**
 AOKI, Shunsuke P01-E-28
 AONO, Sachiko **S10-4**
 AOO, Naoya P01-F-22
 ARAKAWA, Naohisa P02-F-3
 ARAKI, Kazuaki P02-E-55
 ARAKI, Norie P02-E-21
 ARIGA, Hiroyoshi P02-E-17
 ASADA, Akiko
 P01-F-36 P02-E-38 **P02-E-39**
 ASAI, Kiyofumi P01-E-18 P01-E-29
 ASANUMA, Masato
 P01-E-17 P01-E-19 P01-F-27
 ASOU, Hiroaki P02-E-10 **P02-F-48**

B

- BABA, Akemichi
 P01-E-49 P01-E-50 P02-F-3
 BABA, Hiroko **S8-6** P02-E-3
 BABA, Katsuhiro P01-E-8 P02-E-28
 BABA, Kousuke **P02-E-32**
 BALCAR, Vladimir J. P01-E-8
 BANDO, Yoshio P01-F-23 **P01-F-26**
 BANNAI, Hiroko P01-E-32
 BELLIER, Jean-Pierre **P01-E-34(MS5-2)**
 BERNSTEIN, Aran P02-E-44
 BREDT, David S. **PL-3**

C

- CHANG, Ta-Yuan P01-F-2(MS2-4)
 CHEN, Xiao-Liang P01-E-26
 CHIBA, Kenzo P01-F-13 P02-F-4
 CHIKUMA, Toshiyuki
 P01-E-47 P01-F-1 P02-E-50
 CHISAKA, Osamu P02-E-9
 CHOEI, Hiroshi P01-E-59

D

- DATE, Hidetoshi P01-F-24 P01-F-25
 DEZAWA, Mari **S1-4**
 DOHI, Toshihiro P01-E-12
 DUNKLEY, Peter R. **PL-4**

E

- EGUCHI, Naomi P01-E-64 **P02-E-19**
 ENDO, Syogo P01-E-16
 ENDO, Toru P01-F-21
 ENOMOTO, Mariko P02-E-39
 ETO, Yoshikatsu P02-E-21
 EVERCOOREN, Anne Baron-Van
 P02-F-45

F

- FENG, Zijing P01-F-38
 FRANK, Faraci M. P01-E-4(MS6-3)
 FUJII, Takeshi **S11-4**
 FUJIMAKI, Kayo P02-F-44
 FUJIMORI, Sayumi
 P01-E-3 P01-E-9 P01-E-10
 FUJIMOTO, Ken-ichi S1-3
 FUJIMOTO, Sadaki **P01-E-39** P01-E-55
 FUJIMOTO, Takahiro P02-E-60
 FUJINAMI, Yoshiaki **P02-E-23**
 FUJISHITA, Kayoko P02-E-18
 FUJITA, Kimikazu **P01-F-41**
 FUJITA, Naoko **P01-F-27**
 FUJITA, Shinobu C. P01-F-7
 FUJITA, Takuya
 P01-E-1(MS6-2) P01-E-30 P01-E-31
 FUJITA, Yoshihito P01-E-18
 FUJITA, Yuichi P01-F-36
 FUJIWARA, Kentaro P01-E-43 P01-E-46
 FUJIWARA, Michihiro P01-F-22
 FUJIYOSHI, Yoshinori S3-1
 FUKATA, Yuko **P02-F-32(MS1-2)**
 FUKAYA, Masahiro P01-E-14
 FUKUI, Miyuki P01-E-1(MS6-2)
 FUKUNAGA, Kohji
 P01-F-39(MS4-8) P02-E-37
 FUKUNAGA, Noriko P02-F-6
 FUKUSHIMA, Munehisa S8-5
 FUKUSHIMA, Nobuyuki
 P02-E-47(MS1-4)

- FUNAE, Yoshihiko P01-E-48
 FURIHATA, Chie P01-F-43
 FURUICHI, Teichi P01-E-20
 FURUKAWA, Hisako P01-F-22
 FURUKAWA, Shoei P02-F-16(MS3-3)
 P02-F-21 P02-F-22 P02-F-26
 FURUKAWA, Yoshiko **P02-F-21**
 FURUKAWA, Yuriko P02-F-37
 FURUYA, Shigeki **P01-E-16** P02-E-20

G

- GOMI, Fujiya P01-F-30(MS2-1)

GONG, Jian-Sheng

- P01-F-2(MS2-4) P01-F-6 **P01-F-7**
 GOSHIMA, Yoshio **P02-E-46(MS1-5)**
 GULDING, Martyn M. P02-E-7

H

- HACHIYA, Naomi S. P02-E-56
 HADANO, Shinji P01-F-18(MS4-6)
 HAGA, Tatsuya P01-E-11
 HAKOSHIMA, Toshio **S3-4**
 HAMA, Tokiko **P01-E-15**
 HAMADA, Kozo **P01-E-21**
 HAMANOUE, Makoto **P01-F-37**
 HAMAUE, Naoya P01-F-21
 HAMAZUMI, Yuko P02-E-38
 HAN, Chunxi **P02-F-37**
 HARADA, Chikako S8-4
 HARADA, Takayuki S8-4
 HARAOKAWA, Shinpei P02-E-43
 HASEGAWA, Akiko **P02-F-5**
 HASEGAWA, Ayumi P01-E-53
 HASEGAWA, Yoshikazu P02-F-38
 HASHIGUCHI, Mitsuko **P01-F-3**
 HASHIGUCHI, Toshio P01-F-3
 HASHII, Minako P01-E-26
 HASHIKAWA, Tsutomu S7-5
 HASHIMOTO, Eri P02-E-25
 HASHIMOTO, Hitoshi
 P01-E-49 P02-F-3
 HASHIMOTO, Manabu **P02-F-26**
 HASHIMOTO, Norikazu P02-F-35
 HASHIMOTO, Shigeo P01-F-12
 HATANAKA, Hiroshi
 P01-E-51 P02-F-15(MS3-2)

- HATTORI, Mitsuharu P01-E-32
 HAYAISHI, Osamu P01-E-64 P01-E-65
 HAYAKAWA, Yoshika **P02-F-8**
 HAYASHI, Hideki P01-F-7
 HAYASHI, Mitsunori P02-E-34
 HAYASHI, Yoshiyuki P01-F-12
 HEMMINGS, Braian A. **ISN-4**
 HIGASHIDA, Haruhiro **P01-E-26**
 HIGASHI, Youichirou
 P01-E-17 P01-E-19
 HIGUCHI, Haruhisa **P02-F-17**
 HIGUCHI, Hiroshi P01-E-53 P02-E-55
 HINOI, Eiichi
 P01-E-3 P01-E-9 P01-E-10
 HIRABAYASHI, Tetsuya **P01-F-40**
 HIRABAYASHI, Yoshio
 P01-E-16 P02-E-20
 HIRAFUJI, Masahiko P01-F-21
 HIRAI, Satoshi S4-3
 HIRAI, Takao **P02-E-24**
 HIRAKAWA, Hisanori P02-F-41
 HIRAMATSU, Midori **P01-F-44**
 HIRANO, Toshio P02-F-8
 HIROI, Toyoko **P01-E-48**
 HIROKAWA, Nobutaka **PL-1 S3-5**
 HIROSE, Eiji P02-E-49
 HIROSE, Megumi **P02-F-3**

HISAMURA, Ryuuji **P02-E-6**
 HISANAGA, Shin-ichi **P01-F-3**
 P01-F-36 P02-E-38 P02-E-39
 HISANO, Tadashi **P01-E-34(MS5-2)**
 HISAOKA, Tomoko **P01-E-24**
 HOJO, Hiroshi **P02-E-50**
 HOMMA, Keiko **P02-F-39**
 HONDA, Akiko **P02-E-31**
 HONDA, Shizuyo **P02-E-8**
 HONDA, Yukiko **P01-E-15**
 HONMA, Ken-ichi **IL-1**
 HONMA, Naoyuki **P02-E-39**
 HORI, Kei **S5-3**
 HORI, Koichiro **P02-F-44**
 HORI, Takuya **P02-F-26**
 HORIE, Hidenori **P02-F-44**
 HORIE, Masato **P02-F-25**
 HORIE, Syunji **P01-E-41(MS5-4)**
 HORIO, Yoshiyuki **P01-F-42**
 HORIUCHI, Hideki **P01-E-36** **P01-E-40**
 HOSAKA, Sanae **P02-E-34**
 HOSHI, Naoto **P01-E-26**
 HOSODA, Ritsuko **P01-E-58**
 HOSOI, Toru **S11-1** **P01-E-52**
P02-E-62
 HUANG, Zhi-Li **P01-E-64** **P01-E-65**
 HUKUHARA, Nobuyoshi **P01-F-25**

I

ICHIKAWA, Hisashi **P02-E-36**
 ICHIKAWA, Masumi **P01-E-11**
 ICHIRO, Matsuoka **P02-E-51** **P02-F-18**
 IGARASHI, Michihiro **P02-E-65**
 IGARASHI, Shuichi
 P01-F-24 P01-F-25 P01-F-28(MS4-4)
 IIJIMA, Takatoshi **P02-E-44**
 IKEDA, Joh-E
P01-F-18(MS4-6) **P01-F-19(MS4-5)**
 IKEGAYA, Yuji **S4-2**
 IKEGUCHI, Kunihiko **S1-3**
 IKENAKA, Kazuhiro **S6-4** **P02-E-3**
 P02-E-7 P02-E-9 P02-E-11
 P02-F-5 P02-F-10 P02-F-45
 IMAI, Takao **P02-E-44**
 IMAI, Yoshinori **P02-E-48**
 IMAIZUMI, Kazunori
 S9-4 **S9-5** **P02-E-54**
 INAGAKI, Fuyuhiko **S3-3**
 INAGAKI, Masaaki **P01-E-18**
 INAGAKI, Naoyuki **P02-F-40**
 INAMURA, Naoko **P02-E-67**
 INAZU, Masato **P02-E-16**
 INOUE, Atsuko **S11-2**
 INOUE, Kazuhide
 P02-E-8 P02-E-18 **P02-E-22**
 INOUE, Maki **P01-E-10**
 INOUE, Makoto **P01-E-22**
 INOUE, Shunsuke Ben **S9-1**
 INOUE, Takafumi **P01-E-32**
 INUI, Makoto **S7-3** **P01-E-7**
 IP, Nancy Y. **ISN-3**
 IRIE, Atsushi **S10-5**

ISHIBASHI, Daisuke **P01-F-22**
 ISHIBASHI, Tomoko **P02-E-3**
 ISHIDA, Norio **S9-1**
 ISHIDA, Yasushi **P01-E-57**
 ISHIDA, Yusuke **P01-E-23**
 ISHIGURO, Masataro
 P02-E-26(MS6-4) **P02-E-27**
 ISHII, Naoe **P02-E-53**
 ISHII, Yoshiyuki **P02-F-36**
 ISHIKAWA, Yasuyuki **P02-E-57**
 ISHIZAKI, Yasuki **P02-E-12**
 ISHIZUKA, Yuta **P01-E-57**
 ISOJIMA, Yasushi **P01-E-66**
 ITAKURA, Makoto **S7-2** **P02-E-66**
 ITHO, Hiroaki **P01-E-18**
 ITO, Hisanori **P02-F-22**
 ITO, Jinichi **P02-E-5** **P02-F-24**
 ITO, Shigeo **P01-E-6** **P01-E-56**
 ITO, Shingo **P01-E-55**
 ITOH, Jugoh **S11-5**
 ITOH, Tatsuki **P01-F-12**
 IWAI, Miwako **P01-E-32**
 IWAMOTO, Takashi **P01-E-7**
 IWANISHI, Hiroki **P02-F-12**
 IWASAKI, Yasuno **P02-E-11** **P02-F-5**
 IWATA, Ken **P01-E-11**

J

JEON, Yong-Jin **S6-2**
 JOHKOHI, Kuninobu **P02-E-8**
 JUGEN, Wess **P01-E-4(MS6-3)**
 JUNG, Mi-Young **S6-2**

K

KADOYA, Toshihiko **P02-F-44**
 KAGAWA, Tetsushi
 S6-4 **P02-E-7** **P02-F-10** **P02-F-45**
 KAI, Takahiko **P01-E-56**
 KAIBUCHI, Kozo
 P01-F-33 **P02-F-32(MS1-2)** **P02-F-40**
 KAJII, Yasushi **OS-5**
 KAJIZONO, Makoto **P01-F-5**
 KAKIMURA, Jun-ichi
 S8-3 **P01-F-15** **P02-E-1**
 KAKIZAKI, Hidehiro **P02-F-20**
 KAMATA, Reiko **P02-E-56**
 KAMEDA, Yukihiko **P01-F-9**
 KAMETANI, Fuyuki
P01-F-10 **P02-E-33**
 KANAHORI, Yoshiko **P01-E-28**
 KANAMATSU, Tomoyuki **P01-E-63**
 KANAZAWA, Hiroko **P02-E-48**
 KANAZAWA, Masanori **P02-E-53**
 KANBA, Shigenobu **OS-4**
 KANDA, Yasunari **P01-E-27**
 KANEKO, Kiyotoshi **P02-E-56**
 KANEKO, Masayuki
P02-E-26(MS6-4) **P02-E-27**
 KANEKO, Yoko S.
 P01-E-43 **P01-E-44** **P01-E-46**
 KANNAN, Hiroshi **P01-E-57**

KANNO, Manabu
 P01-E-38 **P01-E-42(MS5-3)**
 KANO, Ichiro **P02-E-53**
 KARASAWA, Junnichi **P02-E-33**
 KASAHARA, Jiro **P02-E-37**
 KASAHARA, Kohji **S10-3**
 KASHIMA, Haruo **P02-E-6**
 KATAOKA, Masakazu **P02-E-64**
 KATAYAMA, Taiichi **S9-4** **P01-F-23**
 P01-F-26 **P02-E-31** **P02-E-32** **P02-E-54**
 KATAYAMA, Takahiro **P02-E-15**
 KATHRYN, Lamping G **P01-E-4(MS6-3)**
 KATO, Hirotsugu **P02-E-40**
 KATO, Kanefusa **P02-F-14(MS3-1)**
 KATO, Ryuichi **P02-F-42**
 KATO, Satoru
 P01-E-62 **P01-F-13** **P02-F-39**
 KATO, Takeshi
 P01-E-47 **P01-F-1** **P02-E-50**
 KATO, Toshiaki **P01-F-41**
 KATO-FUKUI, Yuko **P02-E-64**
 KATO-NEGISHI, Midori **P01-E-58**
 KATOH-SEMBA, Ritsuko
P02-F-14(MS3-1)
 KATSUYA, Hirotsugu **P01-E-18** **P01-E-29**
 KAWAGISHI, Toru **P01-E-52**
 KAWAGUCHI, Ayano **P02-F-1**
 KAWAGUCHI, Chihiro **P01-E-49**
 KAWAHARA, Kohichi **P01-F-5**
 KAWAHARA, Masahiro **P01-E-58**
 KAWAHARA, Shigetaka **P02-E-44**
 KAWAI, Yoko **P01-E-29**
 KAWAKAMI, Makiko **P02-E-6**
 KAWAMOTO, Susumu **P01-E-14**
 KAWAMURA, Ken-ichi **P02-E-14**
 KAWAMURA, Yuuki **P01-E-25** **P01-F-8**
 KAWANO, Hiromi **P02-E-17**
 KAWANO, Takayuki **P01-F-39(MS4-8)**
 KAWASAKI, Hiroaki **P01-F-23**
 KAWASHIMA, Koichiro **S11-4**
 KAWASHIMA, Seiichi **P01-F-32(MS2-2)**
 KAWAZOE, Yoko **P02-E-21**
 KAYA, Naoki **P02-E-4**
 KIKKAWA, Yoshiaki **P01-F-32(MS2-2)**
 KIKUCHI, Seiji **P01-F-9**
 KIKUCHI, Tateki **P02-F-30**
 KIM, Jiwon **S6-2**
 KIM, Seong-Soo **S6-2**
 KIM, Seung-Up **S6-2**
 KIM, So-Yeon **S6-2**
 KIMURA, Hiroshi **P01-E-34(MS5-2)**
 KIMURA, Yuki **P02-E-44**
 KINAMERI, Emi **P02-F-19**
 KIRINO, Yutaka **IL-2** **P02-E-44**
 KIRYU-SEO, Sumiko **P02-F-42**
 KITAJIMA, Shigetaka **P02-E-14**
 KITAMURA, Chikara **P02-F-28(MS1-8)**
 KITAMURA, Kunio **P02-F-46** **P02-F-47**
 KITAMURA, Toshio **P01-E-24**
 KITAMURA, Yoshihisa
 S8-3 **P01-F-15** **P02-E-1**
 KITANAKA, Junichi **P01-F-45**
 KITANAKA, Nobue **P01-F-45**

KITANO, Masahiro P02-E-23
 KITAYAMA, Shigeo **P01-E-12** P01-E-35
 KITAYAMA, Tomoya **P01-E-2(MS6-1)**
 KITO, Shozo **P01-E-37** P02-E-29
 KIYAMA, Hiroshi **S1-2** S8-5 P01-F-29
 P01-F-46 P02-F-40 P02-F-42
 KIYOSUE, Kazuyuki P02-F-15(MS3-2)
 KOBASHI, Hiroaki P02-F-41
 KOBAYASHI, Kazuto P01-F-33
 KOBAYASHI, Kenta **P01-F-33**
 KOBAYASHI, Mariko P01-F-7
 KOBAYASHI, Miwako P02-E-17
 KOHSAKA, Shinichi
 P02-E-2 P02-E-8 P02-E-22 P02-E-48
 KOIKE, Nobuya P01-F-43
 KOIKE, Tatsuro P02-E-4
 KOIZUMI, Schuichi
P02-E-18 P02-E-22
 KOJIMA, Takuro P02-F-9
 KOKAME, Koichi P01-F-11
 KOMANO, Hiroto P01-F-8 P01-F-11
 KOMATSU, Katsuko
 P01-F-14 P02-F-27(MS1-7)
 KOMIYA, Yoshiaki
 P02-E-12 P02-E-43 P02-E-65
 KONNO, Daijiro S5-3
 KONNO, Masato P01-E-59
 KORIYAMA, Yoshiki **P01-F-13**
 KOSHIBA, Mamiko **P02-F-30**
 KOYAMA, Tukasa **OS-6** P01-E-61
 KOYAMA, Yutaka P01-E-50
 KOYANAGI, Tomohiko P02-F-20
 KUBO, Tateki **P02-E-30** P02-E-31
 KUBO, Toshikazu P02-F-41
 KUBOTA, Chikara P02-F-31
 KUBOYAMA, Tomoharu
P02-F-27(MS1-7)
 KUDO, Yoshihisa P01-E-45 P02-E-66
 KUMAGAI, Kei P01-E-12
 KUMAKURA, Konosuke P02-E-34
 KUME, Hideaki P01-F-10
 KUME, Shoen P02-F-11
 KUNIMOTO, Manabu P02-E-10 P02-F-7
 KUNITA, Ryota P01-F-18(MS4-6)
 KUO, Che-Hui **P02-F-33**
 KURACHI, Masashi **P02-E-43**
 KURAMOTO, Nobuyuki P02-E-28
 KURIHARA, Tadashi P02-E-2
 KURIYAMA, Kanna **P02-F-13**
 KURODA, Shun'ichi P02-F-25
 KURODA, Yasukazu OS-5
 KURODA, Yoichiro P01-E-58 P02-F-36
 KUROKI, Yoshio P02-E-25
 KUSAKA, Eiko **P02-E-57**
 KUSUMI, Ichiro P01-E-61
 KUWAHARA, Reiko P02-E-64
 KUWAHATA, Yuko P01-E-64
 KUWAJIMA, Takaaki **P01-F-34**
 KYUWA, Shigeru P02-F-36

L

LACHAPPELLE, Francois P02-F-45

LAUNEY, Thomas P01-E-16
 LEE, Suni P02-E-61
 LEE, Young-Don S6-2
 LEMMON, Vance P02-E-61
 LETERRIER, Jean-Francois P02-E-43
 LIU, Zhandong **P02-E-58**

M

MACHINO, Rintaro **P02-F-20**
 MAEDA, Nobuaki **S10-2**
 MAEKAWA, Keiko P02-E-15
 MAENO, Hiroshi OS-1
 MAKIFUCHI, Takao P01-F-25
 MAKITA, Zenji P01-F-9
 MALINOW, Robert **ISN-1**
 MANABE, Takayuki S9-4
 MANO, Yukio P01-E-42(MS5-3)
 MARUOKA, Hisato S5-3
 MARUYAMA, Kei P01-E-25
 MARUYAMA, Kouji P01-E-6
 MARUYAMA, Wakako
P01-F-16(MS4-2) P01-F-17(MS4-3)
 MASUNO, Masaki P02-F-28(MS1-8)
 MATSUBARA, Kazuo **P01-F-47**
 MATSUDA, Toshio P01-E-49 **P01-E-50**
 MATSUKAWA, Toru P02-F-39
 MATSUKI, Norio S4-2
 MATSUMIYA, Teruhiko P02-E-16
 MATSUMOTO, Ken-ichi P02-E-17
 MATSUMOTO, Machiko **P01-E-38**
 P01-E-42(MS5-3) P01-E-60(MS4-7)
 MATSUMOTO, Michio **P02-F-45**
 MATSUMOTO, Tomoya
 P01-E-51 P02-F-15(MS3-2)
 MATSUO, Akinori P01-E-34(MS5-2)
 MATSUO, Yuri **P02-E-40**
 MATSUOKA, Ichiro
 P02-E-17 P02-F-19 P02-F-20
 MATSUOKA, Masato P01-E-11
 MATSUZAKI, Hideo **P01-F-46**
 MATSUZAKI, Shinsuke S9-4
 MATSUZAKI-IYOKU, Yumi P02-F-1
 MAWATARI, Kazuhiro P01-E-62
 MEKADA, Eisuke P02-E-3
 MICHIKAWA, Makoto P01-F-2(MS2-4)
 P01-F-6 P01-F-7 P02-E-21
 MICHIKAWA, Takayuki
 P01-E-20 P01-E-32
 MIKI, Hiroaki P02-E-45(MS1-6)
 MIKI, Naomasa P02-E-36 P02-E-59
 P02-E-60 P02-F-33
 MIKOSHIBA, Katsuhiko
 P01-E-20 P01-E-21 P01-E-32
 P02-E-68(MS1-1) **P02-F-11**
 MIMA, Kazuko **P02-E-41**
 MIMATA, Hiromitu P02-E-42
 MINAMI, Masabumi **P02-E-15**
 MINAMI, Masaru **P01-F-21**
 MINNASCH, Petra P01-E-34(MS5-2)
 MISAWA, Miwa S8-2
 MISHIMA, Kenichi P01-F-22
 MISHINA, Masayoshi **ISN-2** P01-E-14

MITOMA, Jyunya P01-E-16 P02-E-20
 MITSUMOTO, Yasuhide
 P01-F-20(MS4-1)
 MIURA, Naoyuki P02-F-13
 MIURA, Yutaka P01-E-29
 MIYACHI, Eiichi P01-F-41
 MIYAGI, Yohei P01-E-14
 MIYAJIMA, Atsushi P01-E-5
 MIYAMOTO, Eishichi
 P01-F-39(MS4-8) P02-E-37 P02-E-42
 MIYAMOTO, Katsuichi P02-E-21
 MIYAMOTO, Natsuki P01-F-19(MS4-5)
 MIYAMOTO, Shigeaki
P02-E-45(MS1-6)
 MIYATA, Shingo P02-E-35
 MIYATA, Takaki P02-F-1
 MIYATA, Toshiyuki P01-F-11
 MIYAUCHI, Hiroshi P01-E-20
 MIYAZAKI, Ikuko
 P01-E-17 **P01-E-19** P01-F-27
 MIYAZAWA, Chihiro P02-E-14
 MIYAZAWA, Shoko P02-E-63
 MIZOGUCHI, Akira P02-E-60
 MIZOKOSHI, Akito P02-E-22
 MIZUKAMI, Hiroaki S1-3
 MIZUNO, Katsushige P01-E-27
 MOCHIZUKI, Takatoshi P01-E-65
 MOHRI, Ikuko P02-E-19
 MOHRI, Tetsuro P01-F-13 P02-F-4
 MORI, Atsushi **P01-F-20(MS4-1)**
 MORI, Hiroshi P01-F-10
 MORI, Keiji
 P01-E-43 P01-E-44 **P01-E-46**
 MORI, Kohei P01-E-30
 MORI, Norio P01-F-46
 MORI, Nozomu P02-E-49 P02-F-18
 MORI, Yasutake **P02-E-35**
 MORIIZUMI, Tetsuji P01-F-20(MS4-1)
 MORIKAWA, Yoshihiro
 P01-E-5 P01-E-24 P02-F-12
 MORIMATSU, Mitsunori P01-F-25
 MORINO, Tadao P01-E-36 P01-E-40
 MORIOKA, Norimitsu **S11-2**
 MORITA, Katsuya P01-E-12
 MOTEGI, Yoshiko P02-E-64
 MURAKAMI, Koichi S8-5
 MURAKAMI, Shingo P01-E-23
 MURAKAMI, Yutaka P02-F-21
 MURAMATSU, Shin-ichi S1-3
 MURAMOTO, Kazuyo P01-E-58
 MURAOKA, Shin-ichiro OS-5
 MURATA, Yuhji **S11-5**
 MURAYAMA, Ayako **P02-F-1**
 MURAYAMA, Toshihiko
 P01-E-41(MS5-4)
 MUROYAMA, Yuko P02-F-10

N

NABESHIMA, Yo-ichi P02-E-9
 NABESHIMA, Yoko P02-E-9
 NAGANO, Tadasato S7-4

NAGANO, Takashi P02-F-31
 NAGAO, Motoshi S6-1
 NAGASAWA, Kazuki P01-E-39 **P01-E-55**
 NAGATA, Yutaka P01-F-41
 NAGATOMI, Ryoichi **S11-3**
 NAGATSU, Ikuko P01-E-44 P01-E-46
 NAGATSU, Toshiharu P01-E-43
 NAGAYASU, Yuko P02-E-5 P02-F-24
 NAKAFUKU, Masato **S6-1**
 NAKAGAWA, Hiroyuki P02-E-45(MS1-6)
 NAKAGOMI, Saya **P01-F-29** P02-F-40
 NAKAHARA, Jin P02-F-48
 NAKAHARA, Yoko P02-E-64
 NAKAHIRA, Eiko **P02-E-7**
 NAKAI, Masami P01-F-20(MS4-1)
 NAKAJIMA, Kazunori P02-F-29(MS1-3)
 NAKAJIMA, Kazuyuki **P02-E-2** P02-E-14
 NAKAJIMA, Ryoko **P01-E-47**
 NAKAMURA, Junji P02-E-15
 NAKAMURA, Shun P02-F-30
 NAKAMURA, Takeshi P01-E-32 P02-F-18
 NAKAMURA, Yasuko P02-E-8
 NAKAMURA, Yoichi **P02-E-13**
 NAKANO, Imaharu S1-3
 NAKASHIMA, Akira **P01-E-43** P01-E-44 P01-E-46
 NAKASHIMA, Kinichi S6-3
 NAKATANI, Toshiyuki **P02-E-17** P02-E-51
 NAKATA, Yoshihiro S11-2
 NAKAYAMA, Hitoshi P01-F-5
 NAKAYAMA, Kenji P01-E-58
 NAKAYAMA, Tomohiro P01-E-32
 NAKAZIMA, Aki P02-F-22
 NAMBA, Hiroki **S2-3**
 NAMBA, Hisaaki S7-4
 NAMIKAWA, Kazuhiko S1-2 **S8-5** P01-F-46
 NAMIKI, Yoshiko P02-E-33
 NAOI, Makoto P01-F-16(MS4-2) **P01-F-17(MS4-3)**
 NARISAWA-SAITO, Mako S7-4
 NARUSE, Satoshi P01-F-28(MS4-4)
 NAWA, Hiroyuki S7-4 P02-E-55 P02-E-67
 NEGISHI, Takayuki P02-F-36
 NIINUMA, Yoshifumi P02-E-26(MS6-4) **P02-E-27**
 NISHIDA, Keigo P02-F-8
 NISHIDA, Kentaro P01-E-39
 NISHIKAWA, Toru **OS-5** P01-E-33(MS5-1)
 NISHIMOTO, Takeharu **IL-3**
 NISHINOKUBI, Izumi S9-1
 NISHIO, Chika P01-E-51
 NISHIO, Masahiro **P01-E-27**
 NISHIYAMA, Norito P01-F-31
 NITTA, Atsumi P02-F-16(MS3-3) P02-F-21

NODA, Mami S8-4 P01-E-26 **P01-E-28**
 NOMURA, Kazushige P02-E-65
 NOMURA, Masahiko P02-F-44 P02-F-46 P02-F-47
 NOMURA, Tadashi **S4-5**
 NOMURA, Takeo P02-E-42
 NOMURA, Yasuyuki S11-1 P01-E-52
 P01-F-43 P02-E-26(MS6-4)
 P02-E-27 P02-E-62
 NOMURA, Yoshio P02-E-42
 NORITA, Masao P01-E-11
 NORO, Miyuki P02-F-28(MS1-8)
 NOZUMI, Motohiro P02-E-45(MS1-6)
 NUKADA, Toshihide P02-E-33
 NUMAKAWA, Tadahiro P01-E-51 **P02-F-15(MS3-2)**
 NUMAKAWA, Yumiko P01-E-51 P02-F-15(MS3-2)

O

ODA, Ryo P02-F-41
 ODA, Takamasa P01-E-7
 ODAGAKI, Yuji **P01-E-13**
 OGATA, Akihiko P01-F-21
 OGATA, Tadanori P01-E-36 **P01-E-40**
 OGAWA, Masaharu P01-E-4(MS6-3) P02-E-68(MS1-1)
 OGAWA, Norio P01-E-17 P01-E-19 P01-F-27
 OGAWA, Yasuhiro **P02-E-11**
 OGITA, Kiyokazu P01-F-31 P02-E-23
 OGURA, Akihiko **P02-F-35**
 OGURA, Toshihiko S3-1
 OHASHI, Satoshi P01-E-38
 OHASHI, Toya P02-E-21
 OHKAWA, Hiroko **P02-E-25**
 OHKUBO, Tadatashi P02-F-4
 OHNAMI, Soichiro S4-3
 OHNISHI, Hiroshi S7-2
 OHNUKI, Reiko P01-F-26
 OHSAKO, Shunji **P02-E-52**
 OHSAWA, Keiko **P02-E-48**
 OHSHIMA, Toshio **P02-E-68(MS1-1)**
 OHSUMI, Yoshinori **IL-3**
 OHTA, Toshio P01-E-6 **P01-E-56**
 OHTAKI, Ko-ichi P01-F-47
 OKA, Michiko P01-E-1(MS6-2) P01-E-30
 OKABE, Shigeo **S5-4**
 OKADA, Kazushi P01-E-48
 OKADA, Masato **S7-1** P02-E-57 P02-F-23
 OKADA, Masayoshi **P01-E-53**
 OKADA, Takashi S1-3
 OKADA, Tetsuya P01-E-65
 OKAJIMA, Seiichi **P02-F-41**
 OKAMOTO, Hitoshi **S6-5**
 OKAMOTO, Kazuya P01-E-63
 OKANO, Akira P02-E-63
 OKANO, Hideyuki **S1-5** P02-E-44
 P02-F-1 P02-F-8 P02-F-9
 OKANO-UCHIDA, Takayuki **P02-E-12**

OKAZAKI, Mitsuhiro P02-E-1
 OKI, TORU P02-F-38
 OKUDA, Hiroaki **P01-F-31**
 OKUDA, Kenji P01-E-14
 OKUMA, Yasunobu **S11-1** P01-E-52 P01-F-43 P02-E-62
 OKUNO, Toshiaki P01-E-14
 ONO, Kenji **P02-F-43**
 ONO, Rei P02-E-43
 ONO, Tomio **P01-F-32(S1S2-2)**
 ONODERA, Osamu P01-F-24 P01-F-25
 ONOZAKI, Kikuo P02-F-43
 ONUKI, Reiko **P01-F-23**
 OOHASHI, Toshitaka **S10-1**
 OOHIRA, Atsuhiko S10-4
 OONO, Kayoko **P02-E-54**
 OSAKA, Hitoshi S8-4 P01-E-28
 OSHIRO, Satoru **P02-E-14**
 OSUGA, Hitoshi P01-F-18(MS4-6)
 OSUMI, Noriko S4-5 P01-F-33
 OTA, Akira P01-E-43 P01-E-44 P01-E-46
 OTOMO, Asako P01-F-18(MS4-6)
 OTSUKI, Taisuke P01-E-63
 OYAKE, Mutsuo P01-F-25
 OZAKI, Miwako **S7-5**
 OZAWA, Hiroki P01-E-59
 OZAWA, Keiya **S1-3**

P

PARK, Masami P01-E-34(MS5-2)

Q

QU, Wei-Min P01-E-64 P01-E-65

R

RASBAND, Matthew P02-E-11

S

SADATO, Norihiro **S2-1**
 SAI, Xiaorei P01-F-8 **P01-F-11**
 SAITO, Hideya P01-E-60(MS4-7)
 SAITO, Naoaki P01-E-22
 SAITO, Osamu P01-F-47
 SAITO, Taro P01-F-3 **P01-F-36**
 P02-E-38 P02-E-39
 SAITO, Toshikazu **OS-2** P01-E-59 P02-E-25
 SAITO, Yumiko **P01-E-25**
 SAKAGUCHI, Takuya **S4-1**
 SAKAI, Rie P01-E-59 P02-E-25
 SAKAKIBARA, Shin-ichi P02-F-9
 SAKAKI, Yoshiyuki P01-F-43
 SAKAMOTO, Jun P01-F-42
 SAKAMOTO, Tsuyoshi P02-E-21
 SAKASEGAWA, Yuji P02-E-56
 SAKAUE, Masaki P01-E-50
 SAKURAI, Shinichiro P01-E-33(MS5-1)
 SANADA, Noriko **P02-E-28**
 SANAI, Yutaka S10-3

SANEYOSHI, Takeo P02-F-11
 SANO, Yasuteru P01-F-24 **P01-F-25**
 SANTO-YAMADA, Yuko OS-1
 SASAKAWA, Nobuyuki **P02-E-34**
 SASAKI, Nobuyuki P01-F-9
 SASAKI, Yasuharu P02-E-40
 SASAKI, Yo **P02-E-8** P02-E-48
 SASAKI, Yuki P01-E-61
 SASAKI, Yukio P02-E-46(MS1-5)
 SATO, Chikara **S3-1**
 SATO, Kohji P01-F-46 P02-F-13
 SATO, Makoto **P02-F-31**
 SATO, Masahiko S3-1
 SATOH, Masamichi P02-E-15
 SATOU, Takao P01-F-12
 SAWADA, Makoto P02-E-6 P02-F-43
 SAWAMURA, Naoya
 P01-F-2(MS2-4) P01-F-7
 SAYA, Kikuchi **P02-F-18**
 SEIWA, Chika P02-F-48
 SEKIGAWA, Akio **P02-E-38**
 SEKIGUCHI, Mariko P02-E-66
 SEKINO, Yuko S5-5
 SENBA, Emiko
 P01-E-5 P01-E-24 P02-F-12
 SHAMOTO-NAGAI, Masayo
 P01-F-16(MS4-2)
 SHEN, Jin-Song P02-E-21
 SHIBASAKI, Futoshi P02-E-42
 SHIBATA, Shinsuke **P02-F-9**
 SHIBATA, Takashi P02-F-20
 SHIBATA, Yasuhiro P01-E-23
 SHIBAYAMA, Kenzo P01-F-41
 SHIGEMOTO-MOGAMI, Yukari
 P02-E-22
 SHIMADA, Aki P01-E-28
 SHIMADA, Shoichi P01-E-23 P01-E-54
 SHIMAZAKI, Takuya P02-F-1 P02-F-8
 SHIMAZU, Dai P01-E-33(MS5-1)
 SHIMIZU, Hiroki P02-F-38
 SHIMIZU, Katsuji P02-F-16(MS3-3)
 SHIMIZU, Keiko P01-F-47
 SHIMIZU, Motohiro **P02-E-20**
 SHIMIZU, Takao P01-F-40
 SHIMIZU, Takeshi **P02-F-10**
 SHIMODA, Masami S9-1
 SHIMOHAMA, Shun
 S8-1 P01-E-39 P01-F-15
 SHIMOHATA, Mitsuteru
 P01-F-28(MS4-4)
 SHIMOHATA, Takayoshi
 P01-F-28(MS4-4)
 SHIMOSATO, Kazuaki **P01-E-35**
 SHINGO, Akiko S. P01-E-37 **P02-E-29**
 SHINGOU, Tetsurou **S1-1**
 SHINODA, Yoko P01-E-16 P02-E-20
 SHINOMIYA, Kenji
 P01-E-1(MS6-2) **P01-E-31**
 SHINTANI, Norihito **P01-E-49** P02-F-3
 SHIONO, Hiroshi P01-F-47
 SHIRAIISHI, Hirohisa **P01-F-8** P01-F-11
 SHIRAKAWA, Masahiro **S3-2**
 SHIRAO, Tomoaki **S5-5** P02-E-49

SHIROISHI, Toshihiko **S9-3**
 SHITASHIGE, Miki P02-E-42
 SHOUGUCHI-MIYATA, Junko
 P01-F-19(MS4-5)
 SHUO, Takuya S10-4
 SIOMI, Haruhiko **S9-1**
 SIOMI, Mikiko Chihara S9-1
 SOBUE, Kazuya **P01-E-18** P01-E-29
 SOBUE, Kenji **S5-3** P01-E-7
 SOHMA, Hitoshi P02-E-25
 SOMEYA, Akiyoshi **P01-E-41(MS5-4)**
 SONODA, Tomoko P01-E-14
 SORA, Ichiro P02-E-33
 SUGIMURA, Haruhiko P02-F-13
 SUGITANI, Kayo **P02-F-39**
 SUGIURA, Hiroko
 P01-E-11 P02-E-59 P02-E-60
 SUHARA, Tetsuya **S2-2**
 SUH-KIM, Haeyoung **S6-2**
 SUYAMA, Eigo P01-F-23
 SUZUKI, Katsuzi P01-E-61
 SUZUKI, Kinuko P02-E-19
 SUZUKI, Kyoko P02-E-59
 SUZUKI, Naoko P01-F-32(MS2-2)
 SUZUKI, Rika P02-E-64
 SUZUKI, Ryo P01-F-8 P01-F-11
 SUZUKI, Takahiro P01-E-43
 SUZUKI, Tatsuo **P02-E-63**
 SUZUKI, Yasuhiro P01-F-29 **P02-F-40**
 SUZUKI, Yasuo P02-E-20

T

TABATA, Hidenori **P02-F-29(MS1-3)**
 TAGA, Tetsuya **S6-3**
 TAGUCHI, Takahisa
 P01-E-51 P02-F-15(MS3-2)
 TAIRA, Eiichi P02-E-36
 TAIRA, Kazunari P01-F-23 P01-F-26
 TAKADA, Shinji P02-F-10
 TAKAHASHI, Hideto P02-E-49
 TAKAHASHI, Hitoshi P01-F-25
 TAKAHASHI, Kazuaki P01-F-33
 TAKAHASHI, Masami
 S7-2 P01-E-45 P02-E-64 P02-E-66
 TAKAHASHI, Masanori P01-F-33
 TAKAHASHI, Mitsuo P01-F-22
 TAKAHASHI, Tetsuya P01-F-24
 TAKAI, Satomi **P02-F-23**
 TAKAMATSU, Ken P01-E-66 P01-F-37
 TAKAMATSU, Yoshiki P02-E-52
 TAKAMOTO, Akiko P01-E-8
 TAKANAGA, Hiromi
 P02-E-10 **P02-F-7**
 TAKANO, Katsura P02-E-13
 TAKANORI, Noguchi P01-E-4(MS6-3)
 TAKARADA, Takeshi **P01-E-8**
 TAKATA, Kazuyuki P01-F-15 **P02-E-1**
 TAKATUKI, Kanako P02-E-44
 TAKAZAKI, Risa **P02-F-2(MS3-4)**
 TAKEBAYASHI, Hirohide
 P02-E-9 P02-E-11
 TAKEDA, Hiroshi **P02-E-16**

TAKEDA, Yasuo **P02-E-61**
 TAKEHASHI, Masanori
 P01-F-4(MS2-3)
 TAKEI, Nobuyuki **S7-4** P02-E-67
 TAKEMIYA, Takako P02-E-59
 TAKEMORI, Akihiro P01-E-10
 TAKEMURA, Motohiko P01-F-45
 TAKENAWA, Tadaomi
 P02-E-45(MS1-6)
 TAKESHITA, Sen P02-E-39
 TAKEUCHI, Masayoshi **P01-F-9**
 TAKII, Takemasa P02-F-43
 TAKIZAWA, Noboru P01-E-62
 TAKIZAWA, Takumi S6-3
 TAKUYA, Mori P02-E-51
 TAMAI, Ikumi P01-E-55
 TAMARU, Teruya **P01-E-66**
 TAMURA, Shinobu **P01-E-5**
 TAMURA, Takayuki **P01-F-14**
 TANAKA, Fumiko **P02-F-38**
 TANAKA, Hidekazu
 P02-E-59 **P02-E-60**
 TANAKA, Hiroyuki P02-E-31 **P02-F-34**
 TANAKA, Jun P01-E-52
 TANAKA, Kazuhiro P01-E-49
 TANAKA, Kazunori **P01-F-19(MS4-5)**
 TANAKA, Ken-ichi P01-F-27
 TANAKA, Kenji P02-E-6 P02-F-43
 TANAKA, Masahiko **S4-4** S10-2
 TANAKA, Masamitsu P02-F-13
 TANAKA, Masashi P01-F-16(MS4-2)
 TANAKA, Masayuki **P01-E-62**
 TANAKA, Minoru P01-E-5
 TANAKA, Satoshi S5-5
 TANAKA, Seigo **P01-F-4(MS2-3)**
 TANAKA, Shuuitsu **P02-E-4**
 TANIGUCHI, Go P01-E-33(MS5-1)
 TANIGUCHI, Takashi
 S8-3 P01-F-15 P02-E-1
 TANIKE, Masako P02-E-19
 TANIURA, Hideo
 P01-F-34 P02-F-2(MS3-4) **P02-F-6**
 TANIZAWA, Katsuyuki P02-F-25
 TASHIRO, Kunio P01-F-21
 TASHIRO, Tomoko P01-F-43
 P02-E-43 P02-F-28(MS1-8)
 TATEISHI, Yoko **P01-E-32**
 TATENO, Masaru P01-E-59
 TERADO, Mutsuko P01-F-21
 TERAOKA, Keiji S1-3
 TERASHIMA, Toshio P02-F-31
 TERZIS, Julia P02-F-41
 TETSUKA, Mitsue P01-E-25
 TIAN, Qing Bao P02-E-63
 TODA, Fumiaki **P02-E-51**
 TOGASHI, Hiroko P01-E-38
 P01-E-42(MS5-3) **P01-E-60(MS4-7)**
 TOHDA, Chihito
 P01-F-14 P02-F-27(MS1-7)
 TOHYAMA, Masaya S9-4 P01-F-23
 P01-F-26 P02-E-30 P02-E-31
 P02-E-32 P02-E-35 P02-E-54
 P02-F-17 P02-F-34

TOHYAMA, Yoko P02-E-2
TOKITA, Yoshihito S10-4
TOMINAGA-YOSHINO, Keiko

P02-F-35
TOOYAMA, Ikuo P01-E-34(MS5-2)
TORIHATA, Keita P02-E-40
TORIMITSU, Keiichi P02-F-37
TRIMER, James P02-E-11
TSUCHIDA, Hideto P01-E-33(MS5-1)
TSUCHIDA, Ken P01-E-47
TSUDA, Makoto P02-E-22
TSUGU, Toshimitsu P01-E-43
TSUJI, Akira P01-E-55
TSUJIMOTO, Yoshihide
PL-2 P01-F-17(MS4-3)
TSUJIMURA, Jun P02-E-66
TSUJI, Shoji
P01-F-24 P01-F-25 P01-F-28(MS4-4)
TSUKADA, Yasuzo P01-E-63

U

UCHIDA, Keiko P01-E-20 P01-E-32
UCHIDA, Yoko P01-F-30(MS2-1)
UEDA, Hiroshi P01-E-22
UEDA, Kunihiro P01-F-4(MS2-3)
UEDA, Takashi P01-E-23 P01-E-54
UEHARA, Takashi P01-F-43
UEKI, Takatoshi P02-F-13
UENO, Ken-ichi
P01-E-38 P01-E-60(MS4-7)
UENO, Sachiko P02-E-5
UENO, Satoshi P02-E-51
UENO, Yutaka S3-1
UESAKA, Naofumi S4-3
UETSUKI, Taichi
P02-F-2(MS3-4) P02-F-6
UEZU, Akiyoshi P02-E-37
UGAWA, Shinya P01-E-23 P01-E-54
UKAI, Wataru P01-E-59 P02-E-25
UMEDA, Masaaki P01-E-63
UMINO, Asami OS-5 P01-E-33(MS5-1)
URADE, Yoshihiro
P01-E-64 P01-E-65 P02-E-19
USHIO, Yukitaka P01-F-39(MS4-8)
USUDA, Nobuteru P02-E-63
USUI, Shinichi S5-3

W

WADA, Etsuko OS-1
WADA, Keiji OS-1 S8-4 P01-E-28
WADA, Miyuki P01-E-1(MS6-2)
WADA, Sachiyo P02-E-62
WADA, Tamaki P02-F-10
WAKAYAMA, Yuta P02-F-28(MS1-8)
WANG, Dexin P01-F-38 P02-E-58
WANG, Jiawei P01-F-38
WANG, Lijun S1-3
WANG, Yu-Lai S8-4 P01-E-28
WATABE, Kazuhiko P02-E-21
WATAI, Takayuki P01-F-9
WATANABE, Hidehiro P01-E-63

WATANABE, Kazutada P02-E-61
WATANABE, Masahiko
P01-E-14 P01-E-16
WATANABE, Satoru P01-E-35
WATANABE, Yasuyoshi OS-3 S2-5
WATANABE, Yoshifumi P01-E-7
WAWANABE, Yasuhiro P01-E-27
WEILLIE, Zhang P01-E-4(MS6-3)

Y

YAGI, Hideshi P02-F-31
YAGINUMA, Hiroyuki P01-F-33
YAMADA, Kazuyuki OS-1 P01-E-14
YAMADA, Masahisa P01-E-4(MS6-3)
YAMADA, Masashi P02-F-15(MS3-2)
YAMADA, Shingo P01-E-59
YAMADA, Yasue P01-E-7
YAMADA, Yoshihisa P02-F-16(MS3-3)
YAMAGATA, Kanato
P01-E-11 P02-E-59 P02-E-60
YAMAGISHI, Satoru P01-E-51
YAMAGISHI, Sho-ichi P01-F-9
YAMAGUCHI, Mitsune P02-E-50
YAMAGUCHI, Tsuyoshi P02-E-55
YAMAKAWA, Eiko P02-E-39
YAMAKUNI, Hisashi P02-E-15
YAMAMORI, Saori S7-2
YAMAMOTO, Akira
P01-E-1(MS6-2) P01-E-31
YAMAMOTO, Daisuke S9-2
YAMAMOTO, Haruyasu
P01-E-36 P01-E-40
YAMAMOTO, Hideko
P01-F-35 P02-E-33
YAMAMOTO, Hideyuki P02-E-42
YAMAMOTO, Masaru
P01-F-1 P02-E-50
YAMAMOTO, Megumi P01-E-59
YAMAMOTO, Naoki P01-E-18
P01-E-29 P01-E-33(MS5-1)
YAMAMOTO, Nobuhiko S4-3
YAMAMOTO, Toshifumi
P01-F-35 P02-E-33
YAMAMOTO, Yasuhiro P01-F-31
YAMAMURA, Naoshi P01-F-43
YAMAMURA, Takashi P02-E-21
YAMANAKA, Akira P01-E-30
YAMANAKA, Satoru
P01-E-1(MS6-2) P01-E-30 P01-E-31
YAMASHITA, Atsue P02-E-50
YAMASHITA, Tetsuji P01-E-14
YAMASHITA, Toshihide P02-E-30
P02-E-31 P02-F-17 P02-F-34
YAMATODANI, Atsushi P01-E-65
YAMAUCHI, Masamitsu P01-F-41
YAMAUCHI, Shinobu S10-4
YAMAUCHI, Takashi
P01-E-11 P02-E-41
YAMAUCHI, Toshio P01-E-13
YAMAZAKI, Hiroyuki S5-5 P02-E-49
YAMAZAKI, Matsumi
P01-F-13 P02-F-4

YANAGISAWA, Katsuhiko
P01-F-2(MS2-4) P01-F-6 P01-F-7
P01-F-8 P01-F-11 P02-E-21
YANAI, Kazuhiko S2-4
YAN, Jianhong P02-F-46 P02-F-47
YASUDA, Shin P02-E-59 P02-E-60
YASUHARA, Osamu P01-E-34(MS5-2)
YASUI, Saori P02-E-27
YASUMOTO, Fumie P02-F-36
YAWO, Hiromu S5-2
YI, Hong P01-F-17(MS4-3)
YOHDA, Masfumi P02-F-30
YOKOMAKU, Daisaku
P01-E-51 P02-F-15(MS3-2)
YOKOYAMA, Minesuke P02-E-64
YOKOYAMA, Shigeru P01-E-26
YOKOYAMA, Shinji P02-E-5 P02-F-24
YONEDA, Takashi P01-E-48
YONEDA, Takunari P02-E-54
YONEDA, Yukio P01-E-2(MS6-1)
P01-E-3 P01-E-8 P01-E-9 P01-E-10
P01-F-31 P02-E-13 P02-E-23
P02-E-24 P02-E-28
YONEKAWA, Hiromichi
P01-F-32(MS2-2)
YONEYAMA, Masanori
P01-E-2(MS6-1)
YOON, Yong-Soo S6-2
YOSHIDA, Akira P01-E-22
YOSHIDA, Eri P01-F-18(MS4-6)
YOSHIDA, Kazuyuki
P01-E-16 P02-E-20
YOSHIDA, Shigetaka P01-F-26
YOSHIDA, Shosei P02-E-9
YOSHIFUJI, Shigeyuki P02-F-4
YOSHIHARA, Ken P02-F-43
YOSHIKAWA, Kazuaki
P01-F-34 P02-F-2(MS3-4) P02-F-6
YOSHIKAWA, Yasuhiro P02-F-36
YOSHIMOTO, Nobuko P01-F-41
YOSHIMURA, Kazunori P02-F-44
YOSHINO, Yuriko P02-F-47
YOSHIOKA, Michiyo P01-F-27
YOSHIOKA, Mitsuhiro P01-E-38
P01-E-42(MS5-3) P01-E-60(MS4-7)
YUE, Li P01-E-25
YUYAMA, Kohei P01-F-35

Z

ZENG, Ling-Hui P02-F-33
ZHANG, Chun P02-E-14
ZHANG, Jian P02-E-58
ZHANG, Jia-Sheng P01-E-26
ZOU, Kun P01-F-6 P01-F-7

Keyword Index

A

ACETYLCHOLINE [ACH]
S11-4 S11-5 P01-E-34(MS5-2)

ACETYLCHOLINESTERASE [ACHE]
S2-3

ACETYLTTRANSFERASE
P01-E-34(MS5-2)

ACID
P01-E-54

ACTIN
S5-5 P02-E-45(MS1-6) P02-E-48
P02-E-49

ACTION POTENTIAL
S4-3 S10-1

ACTIVATION
P02-E-38

ACTIVITY
S7-5 P01-F-32(MS2-2) P02-F-35

ADDICTION
P01-E-35

ADENOSINE
P02-E-1 P02-E-8 P02-F-14(MS3-1)

ADENYLATE CYCLASE
S3-2 P02-E-29

ADHESION
S3-4 P02-E-59 P02-E-60 P02-E-61

ADP-RIBOSYL CYCLASE
OS-2 P01-E-26

ADRENERGIC
P01-E-27

AFFINITY
P02-E-33

AGGRESSION
P01-E-50

AGING [AGEING]
S2-4 P01-F-42 P01-F-43 P01-F-44

AGONIST
P01-F-27

ALCOHOL
OS-2

ALLELE
P02-E-64

ALPHA
P02-E-35

ALUMINUM
P01-E-39 P01-E-55

ALZHEIMER
S2-3 S8-2 S8-3 S9-4 OS-2
P01-F-1 P01-F-2(MS2-4) P01-F-3
P01-F-4(MS2-3) P01-F-6 P01-F-7
P01-F-8 P01-F-9 P01-F-10 P01-F-11
P01-F-12 P01-F-13 P01-F-14
P01-F-15 P01-F-17(MS4-3)
P01-F-30(MS2-1) P01-F-32(MS2-2)
P02-E-63

AMINO ACID
P01-E-1(MS6-2) P01-E-8 P01-E-16
P01-E-23 P02-E-20

AMPA
S7-4 P02-E-66

AMPHETAMINE
P01-E-12

ANTAGONIST
P01-F-1 P02-F-36

ANTIBODY
P02-E-11 P02-F-1 P02-F-44

ANTICONVULSANT
P01-E-61

ANTIDEPRESSANT
P01-E-12 P02-E-16

ANTIPSYCHOTIC
P01-E-13

ANXIETY
OS-1 P01-E-38 P01-E-42(MS5-3)

ANXIOLYTIC
P01-E-42(MS5-3)

APOPTOSIS
P01-E-59 P01-F-4(MS2-3) P01-F-13
P01-F-17(MS4-3) P01-F-19(MS4-5)
P01-F-22 P01-F-23 P01-F-26
P01-F-29 P01-F-30(MS2-1)
P01-F-31 P01-F-32(MS2-2)
P01-F-33 P01-F-34 P01-F-35
P01-F-36 P01-F-37 P01-F-38
P01-F-46 P02-E-13 P02-E-19
P02-E-42 P02-E-54 P02-E-57
P02-F-35

APP
P01-F-8

ARACHIDONIC
P01-E-41(MS5-4) P02-E-58

ASTROCYTE [ASTROGLIA]
P01-E-17 P01-E-18 P01-E-19
P01-E-29 P01-F-4(MS2-3) P02-E-10
P02-E-11 P02-E-14 P02-E-15
P02-F-5 P02-F-7 P02-F-13 P02-F-24

ASTROCYTE[ASTROGLIA]
S1-5 P02-E-5

ATROPHY
P02-F-27(MS1-7)

AXON
S4-2 S4-3 S8-6 S10-5 P02-E-3
P02-E-30 P02-F-27(MS1-7)
P02-F-32(MS1-2)

AXONAL TRANSPORT [AXOPLASMIC
TRANSPORT]
P01-F-18(MS4-6) P02-E-43
P02-E-46(MS1-5)

AXOTOMY
P02-E-8 P02-F-16(MS3-3) P02-F-44

B

BASAL FOREBRAIN
P01-E-64

BEHAVIOR
S9-1 S9-2 OS-4 P01-E-49
P01-E-60(MS4-7)
P01-F-20(MS4-1) P01-F-22

BETA
P01-F-4(MS2-3) P01-F-30(MS2-1)

BINDING
P01-E-15 P02-F-9

BLOOD
P01-E-4(MS6-3)

BLOOD FLOW
S2-5

BLOOD-BRAIN
P01-E-55

BRADYKININ
P02-E-40

BRAINSTEM
P01-E-52

C

CA1
P01-F-39(MS4-8)

CA3
P02-E-1

CALCIUM [CA]
S11-4 P01-E-6 P01-E-20 P01-E-23
P01-E-32 P01-E-45 P01-E-53
P01-E-56 P01-E-58 P01-E-61
P01-F-32(MS2-2) P01-F-40 P02-E-4
P02-E-25 P02-E-36 P02-E-42
P02-E-48 P02-F-11
P02-F-15(MS3-2) P02-F-36

CALMODULIN
P01-E-45 P01-E-61 P02-E-5
P02-E-42 P02-F-33

CAM
S3-4 P02-E-35 P02-E-41

CANNABINOIDS
P01-E-41(MS5-4)

CAPSAICIN
P01-E-41(MS5-4)

CASPASE
P01-F-35 P01-F-37 P01-F-38
P02-E-42

CATECHOLAMINE
P01-E-44 P01-E-56

CAUDATE
P02-F-12

CDNA
P01-F-43

CEREBELLUM
S4-4 S7-5 S10-2 P01-E-14 P01-F-24
P01-F-25 P02-E-12 P02-E-28
P02-E-44 P02-F-28(MS1-8)

CHANNEL
S3-1 S5-2 S7-3 S8-6
P01-E-1(MS6-2) P01-E-7 P01-E-18
P01-E-20 P01-E-21 P01-E-29
P01-E-53 P01-E-54 P01-E-56

CHAPERONE
S8-3 P01-F-15

CHEMOKINE
S11-3 P02-F-26

CHOLINERGIC
S11-4 P02-F-30

CHROMAFFIN
P01-E-56 P02-E-34

CIRCADIAN
P01-E-66

COCAINE
P01-E-12

COGNITIVE
P01-E-60(MS4-7) P02-E-58

COLLATERAL
P02-F-31

CORD
P02-F-21

CORTEX
S2-1 S4-3 S4-5 P01-E-13 P01-E-57
P01-E-58 P01-F-44 P02-E-7
P02-E-39 P02-E-46(MS1-5)
P02-F-29(MS1-3) P02-F-31

CULTURE
S4-3 S4-5 S8-2 P01-E-2(MS6-1)
P02-E-10 P02-E-21 P02-F-7
P02-F-37

CURRENT
P01-E-28

CYCLIC ADP-RIBOSE
P01-E-26

CYCLIC AMP
P01-E-30 P01-F-13 P01-F-28(MS4-4)
P02-E-36 P02-F-17

CYTOPLASM
P02-F-34

CYTOSKELETON
S3-4 S5-1 P01-F-33 P02-E-30
P02-E-43 P02-E-47(MS1-4)
P02-E-48 P02-E-49 P02-F-28(MS1-8)

D

D1 [D-1]
P02-F-36

DEAFFERENTATION
P02-F-5

DEATH
P01-F-31 P01-F-39(MS4-8)
P02-E-54

DEGENERATION
S8-1 P01-F-17(MS4-3)
P01-F-19(MS4-5) P01-F-24
P01-F-25 P01-F-41 P02-F-35
P02-F-47

DEMENTIA
S9-5 P01-E-63 P01-F-44

DEMYELINATION
S8-6 P02-E-19 P02-F-45

DENDRITE
S10-2 P02-E-35 P02-E-44
P02-E-46(MS1-5) P02-E-63
P02-F-27(MS1-7)

DENTATE
P01-F-31 P02-E-15

DEPRESSION
OS-2

DESENSITIZATION
P01-E-11 P01-E-22

DEVELOPMENT
S4-1 S4-5 S5-5 S6-1 S6-2 S6-4
S8-1 S9-2 S9-3 OS-3 OS-4
P01-E-5 P01-E-24 P01-E-62
P02-E-7 P02-E-9 P02-E-10
P02-E-11 P02-E-17 P02-E-51
P02-F-1 P02-F-5 P02-F-8 P02-F-9
P02-F-10 P02-F-11 P02-F-12
P02-F-19 P02-F-29(MS1-3) P02-F-31

DEVELOPMENTAL DISORDER
OS-3

DEXAMETHASONE
P02-E-62

DIABETES
P01-F-9 P02-F-20

DIFFERENTIATION
S6-1 S6-3 S6-4 S6-5 P02-E-12
P02-E-17 P02-E-51 P02-F-2(MS3-4)
P02-F-6 P02-F-7 P02-F-10 P02-F-13
P02-F-18 P02-F-22 P02-F-32(MS1-2)
P02-F-34 P02-F-48

DNA
P01-F-31 P01-F-44 P02-E-49
P02-F-33

DOPAMINE
S1-3 S2-2 P01-E-11 P01-E-17
P01-E-19 P01-E-35 P01-E-38
P01-E-42(MS5-3) P01-E-43
P01-E-46 P01-E-48 P01-E-50
P01-F-21 P01-F-27

DOPAMINERGIC
P02-F-36

DOPAMINERGIC NEURON
P01-F-47

DORSAL
P02-F-10

DORSAL ROOT
P01-E-34(MS5-2)

DORSAL ROOT GANGLION [DRG]
S11-2 P01-E-54 P02-E-21 P02-F-38
P02-F-42

DROSOPHILA
S9-1 S9-2 P02-E-52

DYE
P02-F-1

E

EEG [ELECTROENCEPHALOGRAM]
P01-E-64

ELECTNIC
S3-1

EMBRYO
S9-3 P01-E-5 P01-E-24

EMOTION
OS-1

ENTERIC
P01-E-34(MS5-2)

ENVIRONMENTAL
OS-4

ENZYME
P01-E-47

EPILEPSY
P01-E-62

ER
P01-F-26

ER [ENDOPLASMIC RETICULUM]
S9-4 P01-F-23 P02-E-26(MS6-4)
P02-E-27 P02-E-54

ESTROGEN [ESTRADIOL]
P01-E-37 P01-E-51 P01-F-41
P02-E-29

ETHANOL
P02-E-25

EVOLUTION
OS-4

EXERCISE
S11-3

EXOCYTOSIS
S5-2 S7-2 P01-F-18(MS4-6) P02-E-34
P02-E-64 P02-E-65 P02-E-66

F

FATIGUE
S2-5

FATTY ACID
P02-E-58

FEEDBACK
P01-E-43

FEEDING
S11-1

FILOPODIA
P02-E-45(MS1-6)

FLUORESCENCE
P02-F-1

FOREBRAIN
P02-F-1

FORSKOLIN
P02-E-36 P02-F-40

FRONTAL
P01-E-57

G

G PROTEINS
S3-2 OS-2 P01-E-13 P01-F-8
P01-F-18(MS4-6)

GABA

S7-5
GABA [GAMMA-AMINOBUTYRIC]
P01-E-3
GABAERGIC
P01-E-38
GAMMA
P01-F-8
GANGLIOSIDE
S10-3
GENE
S1-3 S6-2 S9-3 OS-1 OS-4
P01-E-23 P01-F-18(MS4-6) P01-F-43
P02-E-41 P02-E-55 P02-E-64
P02-F-3
GENE EXPRESSION
OS-5
GFAP
P02-E-10 P02-F-5 P02-F-7
GFP
S5-4 P01-E-22 P01-E-32 P01-F-42
P02-E-45(MS1-6) P02-F-43
GLIA
S4-4 S6-3 S6-4 S8-1 S8-2 S8-4
S8-5 S11-1 P01-E-16 P01-E-17
P01-E-19 P01-E-30 P02-E-3
P02-E-6 P02-E-12 P02-E-14
P02-E-16 P02-E-18 P02-E-19
P02-E-20 P02-E-62 P02-F-8
P02-F-10 P02-F-13 P02-F-19
GLUCOCORTICOID
S11-3 P02-E-62
GLUCOSE
P01-E-63
GLUTAMATE
S8-2 P01-E-10 P01-E-14
P01-E-33(MS5-1) P01-E-38 P01-E-39
P01-E-51 P01-E-63 P01-F-44
P02-E-23 P02-E-37
P02-F-15(MS3-2)
GLUTAMATE RECEPTOR
P01-E-26
GLUTAMATE TOXICITY
P01-F-47
GLYCINE
P01-E-33(MS5-1)
GLYCOLIPID
P02-E-32
GLYCOPROTEIN
P02-E-32 P02-E-61 P02-F-46
P02-F-47
GOLGI
P02-E-32
GROWTH
P02-F-6
GROWTH CONE
S4-3 P02-E-38 P02-E-45(MS1-6)
P02-E-46(MS1-5) P02-F-40
GROWTH FACTOR
S3-3 S7-4 S8-5 P01-F-46 P02-F-24
P02-F-25

GRP [GLUCOSE-REGULATED
PROTEIN
P02-E-33
GUIDANCE
P02-E-30
H
HALOPERIDOL
P01-E-59
HINDBRAIN
S6-5
HIPPOCAMPUS
S4-1 S4-2 S5-2 S5-4 P01-E-13
P01-E-37 P01-F-13 P01-F-31
P01-F-41 P01-F-43 P02-E-1
P02-E-15 P02-E-17 P02-E-18
P02-E-23 P02-E-29 P02-E-37
P02-E-60 P02-E-66 P02-E-67
P02-F-9 P02-F-14(MS3-1)
P02-F-37
HISTAMINE
S2-4 P01-E-65 P01-F-45
HPLC
P02-E-53
HSP [HEAT SHOCK PROTEIN]
P01-E-37
HYPERACTIVITY
P01-E-49
HYPERALGESIA
P01-E-36 P01-E-40
HYPOTHALAMUS
S11-1 P01-E-52
HYPOTHERMIA
P01-E-49
HYPOXIA
S9-4
I
IMAGING
S2-1 S2-2 S2-3 S2-4 P02-E-56
IMMUNOREACTIVITY
S11-5 P01-E-52
INJURY
S1-5 S8-5 P02-F-21 P02-F-26
P02-F-42
INOSITOL
P01-E-20 P01-E-21 P01-E-32
P02-F-11
INTERLEUKIN
S11-1 S11-2 P01-E-57 P02-E-62
ION
P01-E-21
ISCHEMIA
P01-F-39(MS4-8) P01-F-40
P01-F-41
ISOLATION
P01-E-50

K

KAINIC ACID
P02-E-1 P02-E-17 P02-E-23
P02-E-67
KINASE
S7-2 S7-4 S7-5 P01-F-2(MS2-4)
P01-F-29 P02-E-22 P02-E-37
P02-E-39 P02-E-46(MS1-5)
P02-F-25
KINESIN
S3-5

L

LATERALIZATION
P02-F-30
LEARNING
OS-3 P01-F-12 P01-F-14
LEUCINE-ZIPPER MOTIF
S5-3
LEUKEMIA
P02-E-15
LIGHT
P02-F-30
LINEAGE
P02-F-8
LIPID
P01-F-7 P01-F-40 P02-E-31
P02-E-47(MS1-4) P02-E-56
LIPOPROTEIN
P01-F-7 P02-F-24
LIVER
P02-E-33
LOCALIZATION
P02-E-44 P02-E-56
LOCOMOTION
P01-F-45

M

MAGNETIC
P01-E-42(MS5-3) P02-E-24
MEDIAL
P01-E-57
MEMBRANE
P02-E-3 P02-E-31 P02-E-56 P02-E-63
MEMORY
P01-E-60(MS4-7) P01-F-12 P01-F-14
METABOLISM
P01-E-63
METHAMPHETAMINE
OS-5 P01-E-27 P01-E-35
P01-F-45 P01-F-46
MICRODIALYSIS
P01-F-47
MICROGLIA
S8-3 P01-F-5 P01-F-15 P01-F-40
P02-E-1 P02-E-2 P02-E-4 P02-E-6

P02-E-8 P02-E-13 P02-E-14
P02-E-22 P02-E-48 P02-F-43
MICROTUBULE
S3-5 P02-F-28(MS1-8)
P02-F-32(MS1-2)
MIDBRAIN
P02-F-36
MIGRATION
S6-4 P01-F-40 P02-E-7
P02-E-47(MS1-4) P02-E-68(MS1-1)
P02-F-29(MS1-3)
MITOCHONDRIA
P01-E-6 P01-F-16(MS4-2)
P01-F-17(MS4-3) P02-E-23
MONOAMINE
P01-E-48 P01-F-20(MS4-1)
P02-E-16
MONOAMINE OXIDASE
P01-F-21
MONOCLONAL
P02-E-11
MOSSY FIBER
P02-F-37
MOTILITY
P02-E-48
MOTONEURON [MOTOR NEURON]
S6-5 P01-F-18(MS4-6) P02-E-9
MOTOR
S3-5 P01-F-33 P02-F-12
MOVEMENT [MOTION, MOTOR
ACTIVITY]
P01-F-20(MS4-1) P01-F-21
MPTP
P01-F-20(MS4-1)
MRNA
S9-3 P01-F-43 P02-E-35 P02-E-36
P02-E-63 P02-F-3
MUSCARINIC
S11-4 P01-E-4(MS6-3)
MUTANT
S9-2 P01-E-43 P01-E-64 P02-E-19
P02-E-21 P02-E-64 P02-F-8
P02-F-9
MYELIN
S8-6 S10-1 P02-E-3 P02-E-7
P02-E-21 P02-F-45 P02-F-46
P02-F-47 P02-F-48

N

NAD
P01-E-26
NEURITE
S10-3 P02-E-47(MS1-4)
P02-F-27(MS1-7) P02-F-28(MS1-8)
P02-F-34 P02-F-35 P02-F-38
NEUROBLASTOMA
P02-E-41 P02-E-45(MS1-6)
P02-E-56 P02-F-4

NEUROENDOCRINE
P01-E-48
NEUROFILAMENT
P02-E-43
NEUROMODULATION
P01-E-41(MS5-4)
NEURON
S1-5 S5-5 S6-2 S6-3 S8-1 S8-4
P01-E-5 P01-E-24 P01-E-39
P01-F-33 P01-F-34 P01-F-38
P02-E-8 P02-E-12 P02-E-14
P02-E-51 P02-F-2(MS3-4)
P02-F-3 P02-F-6 P02-F-13
P02-F-33
NEUROPEPTIDE
OS-1 P01-E-49 P01-E-53 P02-E-50
P02-F-3
NEUROTOXICITY
P01-E-31 P01-E-59 P01-F-3
P01-F-6 P01-F-9 P01-F-46
NEUROTRANSMITTER
S7-2 P01-E-28 P01-E-31 P01-E-45
P01-E-46 P02-E-40 P02-F-3
NEUROTROPHIC
S1-3 S7-4 S8-3 S8-4 P01-E-15
P01-F-15 P02-E-20 P02-E-51
P02-F-4 P02-F-14(MS3-1)
P02-F-16(MS3-3) P02-F-17
P02-F-19 P02-F-23 P02-F-25
P02-F-26
NF-KAPPAB
P01-F-4(MS2-3) P01-F-37 P02-E-25
NGF [NERVE GROWTH FACTOR]
P02-E-31 P02-F-21 P02-F-22
P02-F-38
NICOTINE
S11-5 P01-E-37 P01-E-60(MS4-7)
NICOTINIC
S11-4
NITRIC OXIDE [NO]
P01-E-57 P02-F-4
NMDA [N-METHYL-D-ASPARTATE]
S7-3 S7-5 P01-E-2(MS6-1) P01-E-7
P01-E-9 P01-E-33(MS5-1) P01-F-1
P01-F-32(MS2-2)
NMR
S3-2 S3-3 P01-E-63
NODE
S8-6 S10-1
NOREPINEPHRINE
[NORADRENALINE]
P01-E-12 P01-E-44 P01-E-50
NOS [NO SYNTHASE]
P02-F-4
NUCLEUS
S9-5 P01-F-42 P02-E-41

O

OBESITY
P01-E-25
OLFACTORY
P01-E-46
OLIGODENDROCYTE
[OLIGODENDROGLIA]
S6-4 P01-F-37 P02-E-7 P02-E-9
P02-E-10 P02-F-10 P02-F-45
OOCYTE
P01-E-7
OPIOID
P01-E-22 P01-E-56
OPTIC
S1-4
ORGAN
S3-1
OXIDATION
P01-F-6

P

PAIN
S2-4 P01-E-36 P01-E-40 P01-E-54
P02-E-22
PARAQUAT
P01-F-47
PARKINSON
S1-3 S1-4 S2-3 P01-E-17
P01-E-19 P01-E-43 P01-F-16(MS4-2)
P01-F-17(MS4-3) P01-F-20(MS4-1)
P01-F-21 P01-F-22 P01-F-27
PARKINSON DISEASE
P01-F-47
PEPTIDE
S3-2 P01-E-15 P01-E-25
PET [POSITRON EMISSION
TOMOGRAPHY]
S2-1 S2-2 S2-3 S2-4 OS-3
PHENCYCLIDINE
OS-5 P01-E-33(MS5-1)
PHENOTYPE
P02-E-17
PHEOCHROMOCYTOMA [PC12, PC-12]
P01-E-6 P01-E-28 P01-F-29
P02-F-4 P02-F-38
PHOSPHATASE
S4-4 S7-2 S10-2 P01-F-2(MS2-4)
P02-E-42
PHOSPHATIDYLINOSITOL
P01-E-51
PHOSPHOINOSITIDE
P01-F-45
PHOSPHOLIPASE
P01-E-39 P01-E-41(MS5-4)
P02-E-5
PHOSPHOLIPID
P01-F-2(MS2-4)

PHOSPHORYLATION

S7-1 S7-3 S10-4 P01-F-2(MS2-4)
P01-F-3 P02-E-38 P02-E-40
P02-E-43 P02-E-57 P02-E-64
P02-E-65 P02-E-68(MS1-1) P02-F-7

PLATELET

P01-E-61

POLYAMINE

P02-E-13

POSTNATAL DEVELOPMENT

OS-5

POSTSYNAPTIC

P02-E-63

POSTSYNAPTIC DENSITY

S5-3

POTENTIATION

P02-E-60

PRESENILIN

S9-4 P01-F-10 P01-F-11

PRESYNAPTIC

S5-2 P01-E-45

PROGESTERONE

P01-E-51

PROLIFERATION

P01-E-2(MS6-1) P02-E-12

PROSTAGLANDIN

S11-2 P01-E-64 P01-E-65
P02-E-19 P02-E-40

PROTEASE

P01-F-1 P01-F-36 P02-E-50
P02-F-5 P02-F-42

PROTEASOME

P02-E-26(MS6-4) P02-E-27

PROTEIN

P01-F-11 P01-F-16(MS4-2)
P01-F-28(MS4-4) P02-F-33

PROTEIN KINASE

S7-1 S8-2 S10-3 P01-E-7 P01-E-18
P01-E-22 P01-E-28 P01-E-29
P01-E-37 P01-E-61 P01-F-3
P01-F-13 P01-F-35 P01-F-36
P01-F-39(MS4-8) P02-E-2 P02-E-29
P02-E-34 P02-E-38 P02-E-41
P02-E-49 P02-E-59 P02-E-65
P02-F-17

PROTEIN SYNTHESIS

S9-3 P02-E-67

PROTEOGLYCAN

S10-1 S10-2 S10-3 S10-4 S10-5

PROTEOLIPID

P02-F-45

PROTEOLYSIS

P01-F-36

PURIFY

P01-E-34(MS5-2)

PURINERGIC

P02-E-18 P02-E-22

PURKINJE CELL

S4-4 S10-2 P01-E-14 P01-E-16
P02-E-44

PUTAMEN

P02-F-12

PYRAMIDAL

P02-F-31

R

REACTIVE OXYGEN SPECIES

P01-F-35

RECEPTOR

S3-2 S7-3 S11-3 OS-1 P01-E-3
P01-E-4(MS6-3) P01-E-5 P01-E-7
P01-E-9 P01-E-10 P01-E-11
P01-E-14 P01-E-15 P01-E-20
P01-E-21 P01-E-23 P01-E-24
P01-E-27 P01-E-32 P01-E-40
P01-E-52 P01-E-65 P02-E-31
P02-E-33 P02-E-62 P02-E-66
P02-F-11 P02-F-17

REGENERATION

S1-2 S1-4 S8-1 S8-5 P02-F-19
P02-F-39 P02-F-40 P02-F-41
P02-F-44 P02-F-47

RELEASE

P01-E-20 P01-E-45

RETINA

S8-4

RETINAL GANGLION CELL

S10-5 P02-F-39

RHO

S3-4

RHYTHM

P01-E-66

RNA

S9-1 S9-4 S9-5 P01-E-15 P02-E-44
P02-E-52 P02-E-53 P02-F-9
P02-F-40

S

SCHIZOPHRENIA

S2-2 OS-5 P01-E-33(MS5-1)

SCHWANN CELL

S8-5 P02-E-21 P02-F-19

SCIATIC

S1-4 P02-E-50 P02-F-16(MS3-3)
P02-F-47

SECRETION

P01-F-11

SEIZURE

P02-E-15 P02-E-59

SENSITIZATION

P01-F-45

SENSORY NEURONS

S6-5 P01-E-54

SEROTONIN

P01-E-13 P01-E-36 P01-E-40
P01-E-42(MS5-3) P01-E-49
P01-E-50 P02-E-5

SEX

S9-2

SIGMA

P02-E-33

SIGNAL TRANSDUCTION

S6-1 P01-E-26

SLEEP

P01-E-64 P01-E-65

SLICE

S4-1 S4-4 P02-F-37

SODIUM

S3-1

SODIUM [NA]

P01-E-6

SPINAL

S1-5 P01-F-33 P02-E-9 P02-F-21
P02-F-26

SPROUTING

S4-1 P02-F-37

STAIN

P02-E-11

STEM CELL

S6-1

STRESS

S8-3 P01-E-38 P01-F-15 P01-F-23
P01-F-26 P02-E-14
P02-E-26(MS6-4) P02-E-27 P02-E-54

STRUCTURE

P01-E-21

SUBCELLULAR

P01-F-24

SUBSTANCE P

S11-2 P01-E-47

SUBSTANTIA NIGRA

P01-F-21 P01-F-22 P02-F-23

SYMPATHETIC

P02-E-51

SYNAPSE

S4-1 S5-1 S5-2 S5-4 S5-5 S7-4
P01-E-14 P01-E-51 P01-F-14
P02-E-18 P02-E-60 P02-E-61
P02-E-66 P02-F-15(MS3-2)
P02-F-35

SYNAPSIN

P01-E-58 P02-E-40

SYNAPTIC TARGETING

S5-3

SYNAPTogenesis

P01-E-58 P02-E-38 P02-F-28(MS1-8)

T

TASTE

P01-E-23

THALAMUS

P02-F-30

THYROXINE

P01-E-58

TOLERANCE

P01-E-22

TRACT

P02-F-31

TRANSCRIPTION

P01-E-2(MS6-1) P01-E-66

P01-F-19(MS4-5) P01-F-28(MS4-4)

P01-F-29 P01-F-30(MS2-1)

P01-F-39(MS4-8) P02-E-9 P02-E-20

P02-E-23 P02-E-24 P02-E-28

P02-E-36 P02-E-49 P02-E-55

P02-F-12 P02-F-22 P02-F-33

TRANSDUCTION

S3-3 P02-F-8

TRANSMISSION

S5-1

TRANSPLANT

S1-4 P02-F-43 P02-F-45

TRANSPORT

S3-5 P01-E-1(MS6-2) P01-E-8

P01-E-16 P01-E-30 P01-E-31

P01-E-55 P01-F-8 P01-F-10

P02-E-16

TRIGEMINAL

S6-5

TROPHIC

P02-F-15(MS3-2)

TUBULIN

S5-1 P02-F-32(MS1-2)

TYROSINE

S7-1 S7-2

TYROSINE HYDROXYLASE

P01-E-43 P01-E-44 P01-E-46

P01-F-27

U

UBIQUITIN

S8-4 P01-E-28 P01-F-11 P01-F-12

UBIQUITINATION

P01-F-12 P02-E-26(MS6-4)

P02-E-27

UPTAKE

P01-E-12 P01-E-17 P01-E-19

V

VAGUS

S11-1

VESICLE

S3-5

VIRUS

P01-F-22 P02-F-23

VISUAL

P02-F-30

W

WATER

P01-E-18 P01-E-29

Z

ZINC

S7-3

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TEL:03-3475-5618 FAX:03-3475-5619 E-mail:naka-atsu@muj.biglobe.ne.jp

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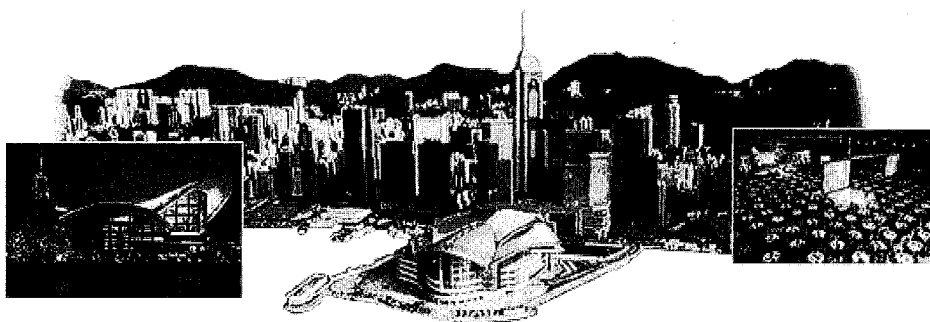
編集後記

日本神経化学会誌「神経化学」第41巻第3号をお届け致します。本号は第45回日本神経化学会（札幌）大会の抄録号です。今回はプログラム号並びに抄録号におけるプログラムにおいて、タイトル、発表者及び所属を含め、日時、会場等について和文とともに英文を付けましたので、いずれの冊子体もやや厚くなりましたがご了承下さい。これは国際化を目指す本学会の方針を受けて、海外からの参加者の便を考えて行いました。ご協力頂きました発表者の皆様に感謝致します。

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Enquiry:

Dr Alfreda Stadlin
 Dept of Anatomy
 Chinese University of Hong Kong
 Shatin, NT
 Hong Kong
 Email: astadlin@cuhk.edu.hk
 Fax: 852-26035031
 Phone: 852-26096783
 ISN website: <http://www.neurochem.org/>



Cell Signaling

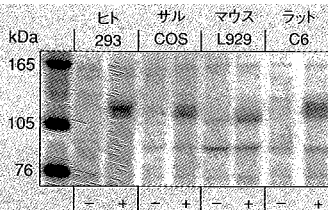
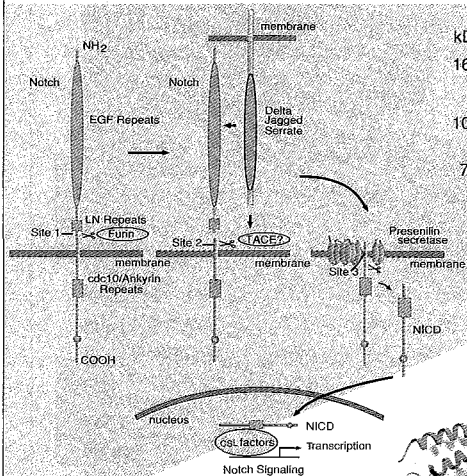
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細胞内タンパクのシグナル伝達系関連の研究に

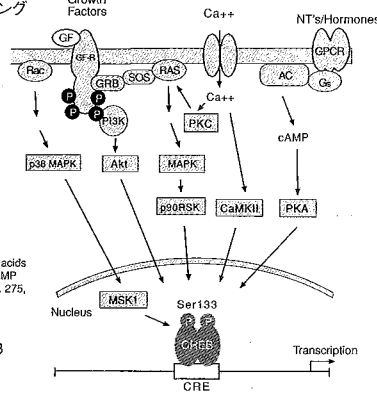
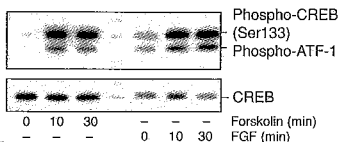
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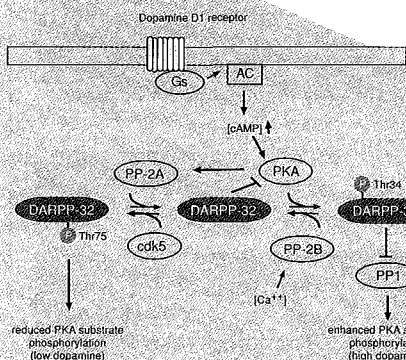


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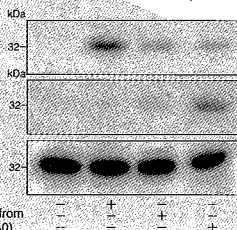
<NOTCH>**<CREB>**

虚血性状態のラット海馬の染色像 (白)

(Provided by Dr. Bingren Hu, Miami University School of Medicine, Florida.)



Dopamine levels modulate PKA and PP1 activities by dual phosphorylation of DARPP-32. (Adapted from Bibb, J.A. et al. 1999, Nature 402: 669; and Nishi, A. et al. 2000, Proc. Natl. Acad. Sci. USA 97: 12940.)

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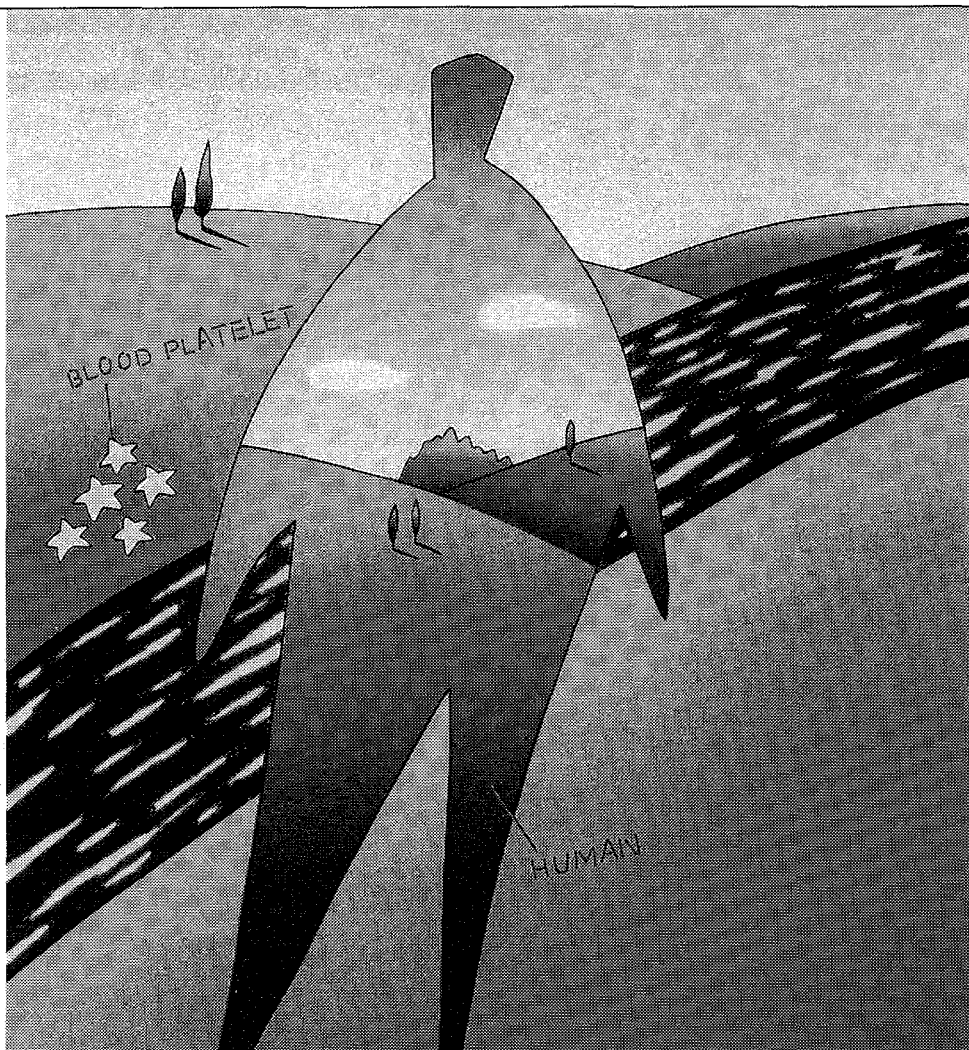
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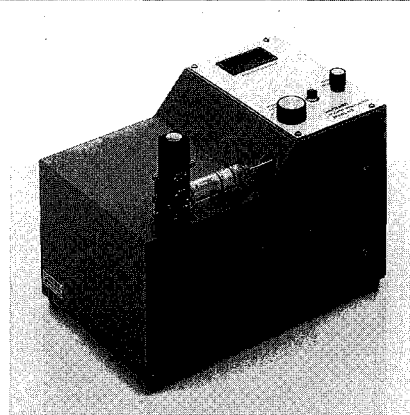
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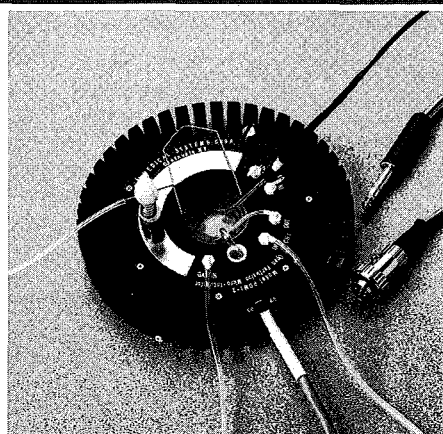


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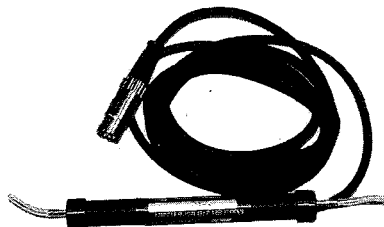
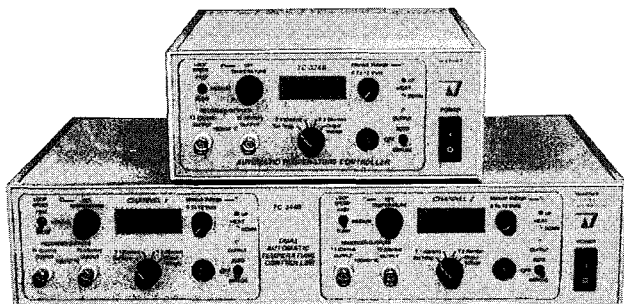
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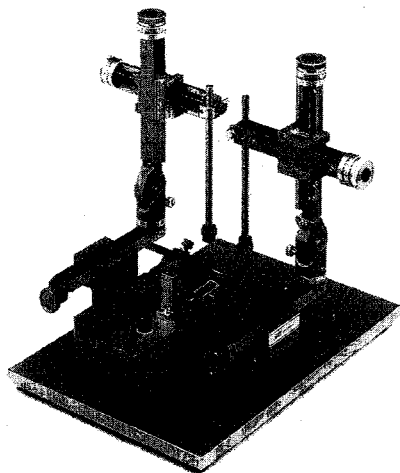
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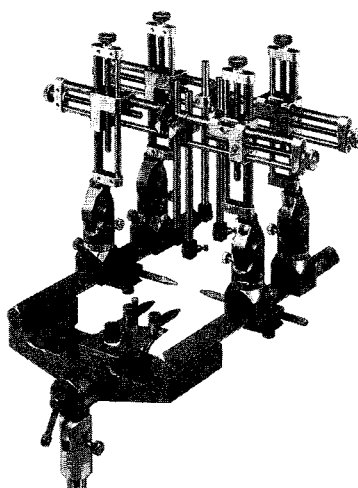


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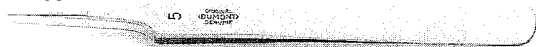
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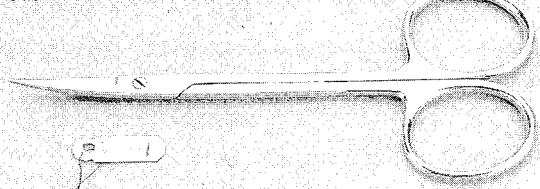
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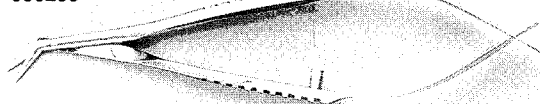
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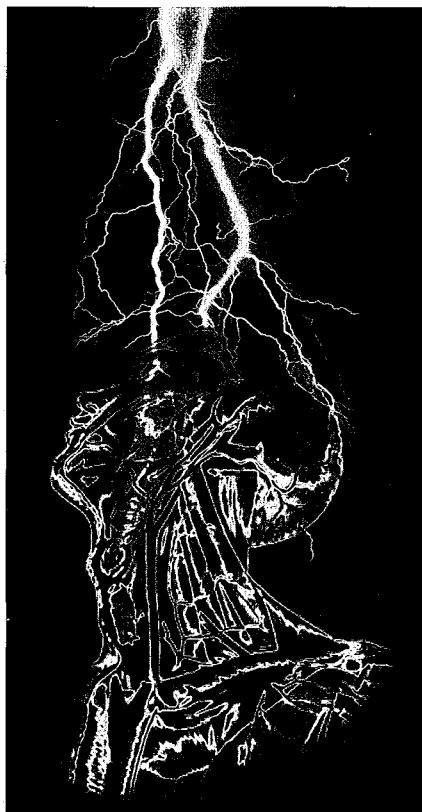
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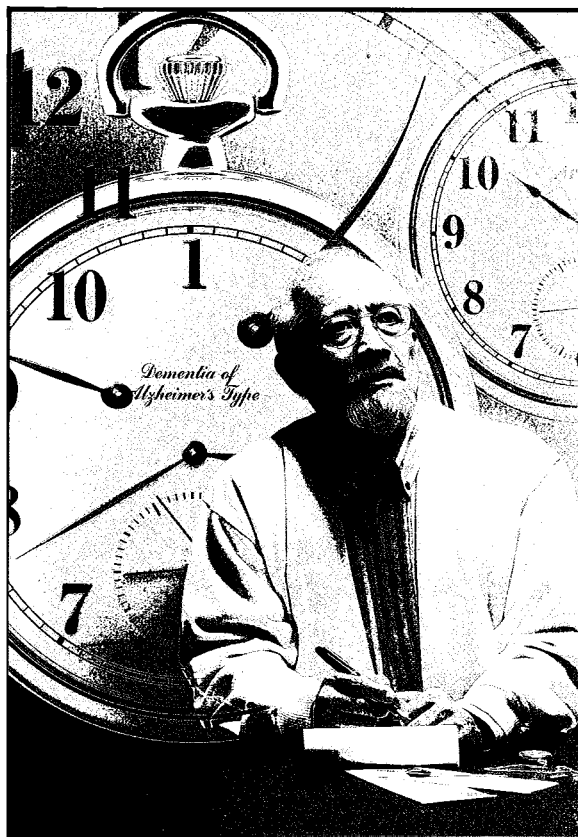
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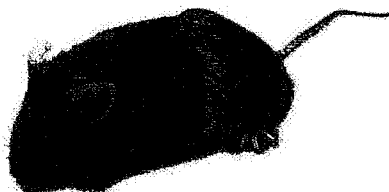
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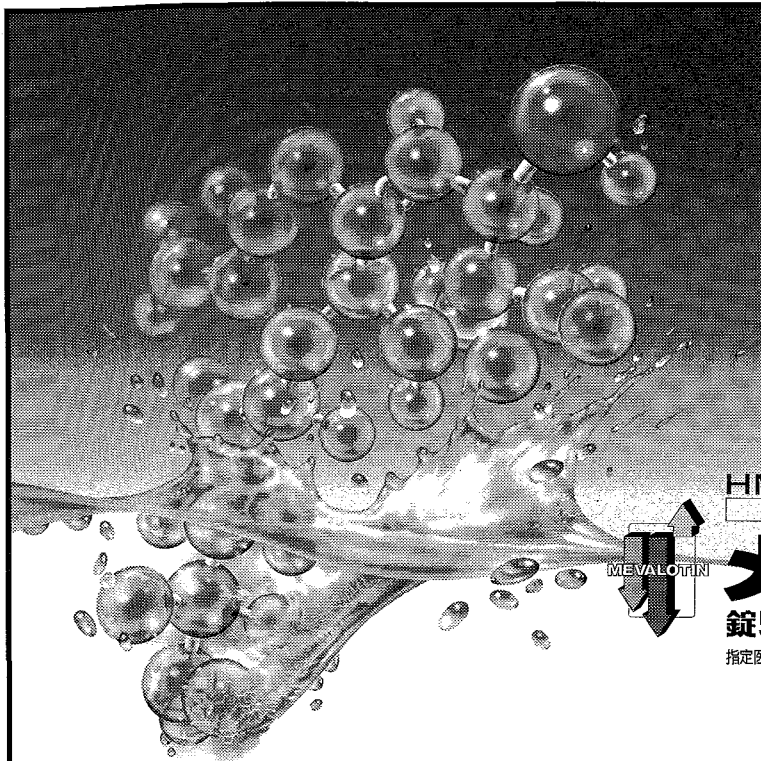
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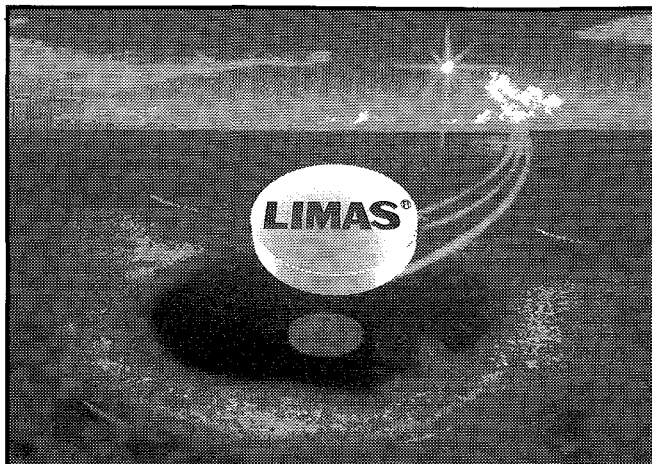


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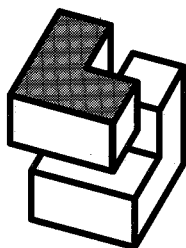
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- (1) 血清リチウム濃度が1.5mEq/Lを越えたときは臨床症状の観察を十分に行い、必要に応じて減量又は休薬等の処置を行うこと。
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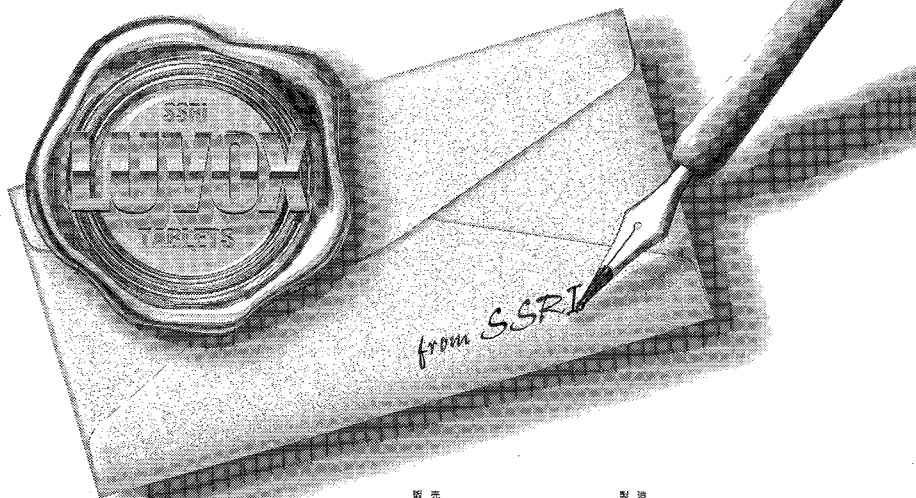
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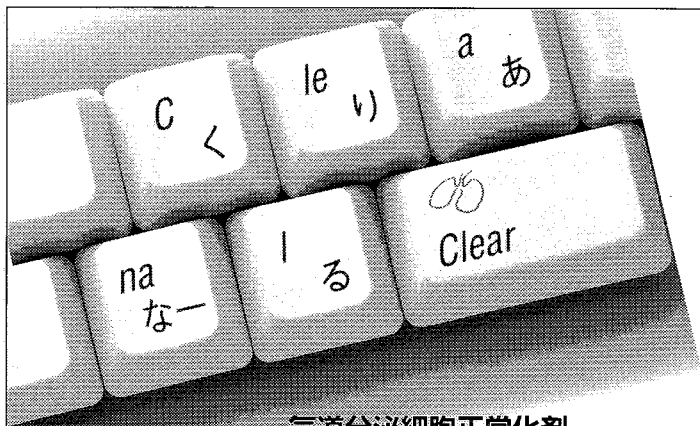


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岐路に立つ日本の科学教育

—21世紀の数学・理科教育はいかにあるべきか—



理数系学会教育問題連絡会 編／代表世話人 浪川幸彦／四六判／170頁／定価（1,600円＋税）

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| <p>① 総合的な学習の時間と科学教育
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日本教育方法学会代表理事 柴田 義松</p> <p>③ 産業人として理科教育に望むもの
元 日本化学会会長、化学教育協議会議長 館 糾</p> <p>④ 科学記者からみた日本社会と自然科学教育
日本経済新聞社科学技術部長 高木 勲生</p> <p>⑤ 「ゆとり」の中でいかに科学教育の水準を保つか？
文部科学省大臣官房審議官 寺脇 研</p> <p>⑥ “理科離れ・数学離れ”が何故起きるのか
日本総合学習学会会長 上野 健爾</p> <p>⑦ 「ゆとり教育」より「考える教育」を
日本物理学会理事（物理教育委員長） 波田野 彰</p> <p>⑧ 文部省の「科学」と「技術」についての考え方に対する疑問
日本化学会化学教育協議会 副議長 細矢 治夫</p> | <p>⑨ 教育にもっと投資を
日本学術会議会員／日本動物学会会長 星 元紀</p> <p>⑩ 学力低下の歯止め策—教える中身か、考え方か—
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日本学術会議物理学研究連絡委員会
物理教育委員会幹事 兵頭 俊夫</p> <p>⑫ 教科書作りと学習指導要領を切り離すべきだ
東京大学大学院総合文化研究科 松田 良一</p> <p>⑬ 日本の科学リテラシー
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3. 理数系学会教育問題連絡会活動概略
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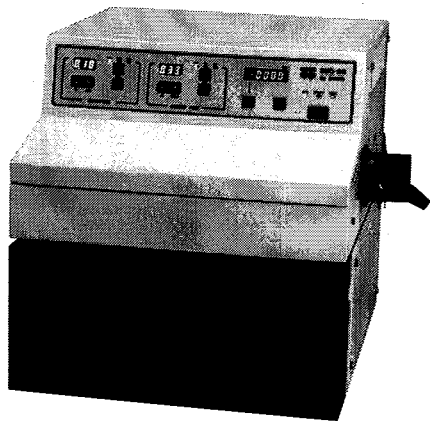
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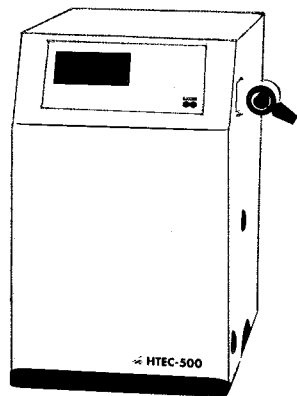
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