ノックアウトマウスと非侵襲的イメージング法を用いた創薬科学の新展開と応用

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（基盤研究(B)(2)）
研究成果報告書

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はじめに

本報告書は、平成１２年度から１４年度まで基盤研究(B)(2)の助成を受けて、ポジトロン標識合成法の開発と新しいＰＥＴ検査を用いた神経薬理学研究への応用展開研究をまとめたものである。最近の画像医学の進歩はめざましく基本的には認知、感情などすべてのヒトの精神活動はイメージとして定量的に捕らえられる時代になってきている。我々は本研究助成によりポジトロンエミッションントモグラフィー（ＰＥＴ）を用いてヒト脳の神経伝達を研究し薬理学に応用することを行った。特にスーパーコンピューターにて画像再構成させる新しい 3D-PET を用いて研究を行いその有用性を実証することができた。3 次元データ収集 PET による受容体測定法はヒトの薬理学特に臨床薬理学に将来性のある方法であることを強調したい。

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研究成果：

1. 新しい ^{11}C-ヨウ化メチル合成法（Gas-Phase 法、メチルトリフレート合成法の自動化）：

現在、脳の神経伝達機能診断薬剤として臨床的に使用される標識リガンドは ^{11}C-標識薬剤にほぼ限られている。我々は本研究助成により ^{11}C-標識化合物の自動合成装置開発に携わり、作られた標識薬剤を臨床研究に応用した。一般的に ^{11}C-ヨウ化メチル合成は THF に溶解した Lithium aluminum hydride を還元剤として用いるが、最近はガス相法（図１）にて ^{11}C-ヨウ化メチルを作る方法が、簡便で高比放射能の放射性リガンドを作成できるために注目されている。また [^{11}C]methyl triflate（図２）は反応収率が高く優れた方法である。本研究助成によりガス相法による ^{11}C-ヨウ化メチル合成法を確立し、さらに新規の[^{11}C]methyl triflate により ^{11}C-標識リガンドを作成した。さらにループ法という簡便な方法を開発して標識合成法を自動化した薬剤管理委員会（倫理委員会）にデータを提出し、審査を経て平成１３年度から新標識薬剤合成法により東北大学の ^{11}C-リガンド臨床研究を開始した（^{11}C - ドキセピン、メチオニン、ラクロプライド）。現在このような新しく開発されたシステムを用いて ^{11}C - ドネペジルを新規 ^{11}C - 標識化合物として開発している。

図 1. ガス相法のヨウ化メチル合成法

$$^{11}CO_2 \xrightarrow[\text{Nickel, } H_2]{} {}^{11}CH_4 \xrightarrow[I_2]{} {}^{11}CH_3I$$

図２．[^{11}C]メチルトリフレート合成法の確立と自動化

$$^{11}CH_3I \xrightarrow[\text{AgOTf-Graphic GC column}]{} {}^{11}CH_3OSO_2CF_3$$

新しい ^{11}C-メチルトリフレート法により、^{11}C-ドネペジル（アセチルコリンエステラーゼ阻害薬）を新規に合成することに成功した。さらにループ法という簡便な方法とセッパックによる HPLC 分離時の塩類を除去する方法で、より安全な ^{11}C 標識薬剤の臨床応用に成功した。^{11}C-ドネペジルを用いてインビトロ・インビボでの結合を評価した。図３に示すようにアセチルコリンエステラーゼへの結合を ^{11}C-ドネペジルにて見ることができた。次に脳スライスを用いてインビボ・スライス PET 法による評価を行った。すなわち、生きている脳スライスと ^{11}C-ドネペジル標識薬剤をインキュベートして ^{11}C-ドネペジルの取りこみをイメージングプレートにて測定して、生きている状態でアセチルコリンエステラーゼが測定できることを証明した。さらに臨床応用に関する当該倫理委員会へ提出するデータ（無菌テスト、被曝量、安全性データなど）を得て臨床応用の準備を終了した。現在、倫理委員会に審査資料を提出して審査中で、今年 7 月以降にヒトに投与を開始する予定である。

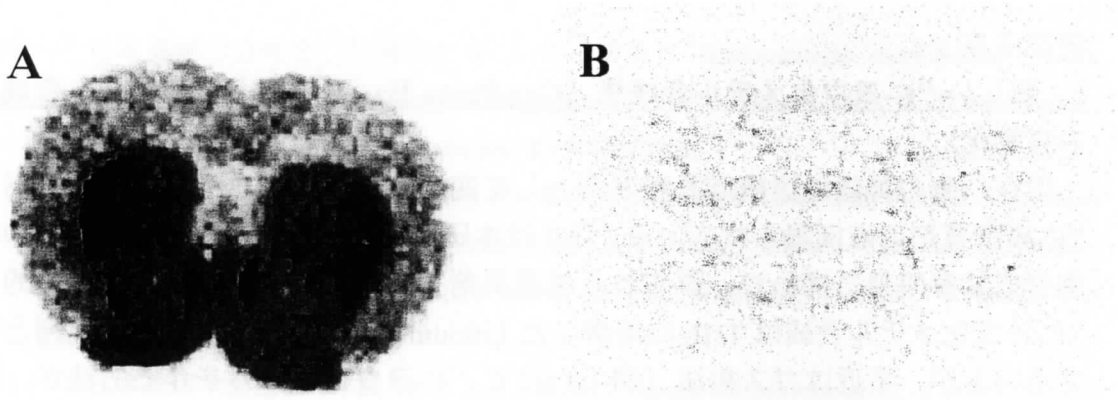


図３:インビトロ脳スライスにおける^{11}C-ドネペジルの結合。A: Total binding, B: Non-specific binding。高い結合が線条体に認められるが、多量のドネペジルでこの特異結合が消失する。

2. 新しい ^{18}F 標識法の開発（^{18}F-標識ヨウ化フルオロベンジル）：

^{11}C-ヨウ化メチルと同様に汎用性のあるポジトロン標識前駆体である ^{18}F-標識ヨウ化フルオロベンジル反応を確立した。^{18}O-濃縮水をターゲットとする ^{18}O(p,n) ^{18}F 核反応で ^{18}F-フッ素イオンを得たあと ^{18}F-標識ヨウ化フルオロベンジルを合成し、標識合成の迅速で効率的な ^{18}F-標識フルオロベンジルイミダゾイルプロピルエーテル（^{18}F-FUB193、^{18}F-fluoropropoxyfan）の反応条件を確立した（図４）。９９％以上の放射化学的純度を持った ^{18}F-fluoropropoxyfan を照射終了後２時間以内に６－１０％の収率で合成することができた。作成した ^{18}F-fluoropropoxyfan をインビトロにおける結合実験を行い、特異的にＨ３受容体に結合していることが分かった。Ｈ３受容体は、ドパミン神経などの徐神経によって起きる supersensitivity に関連して増加することが我々の研究から明らかになっている。このような新しい標識薬剤の開発によりドパミン神経の supersensitivity をイメージングできる可能性が拓けた。

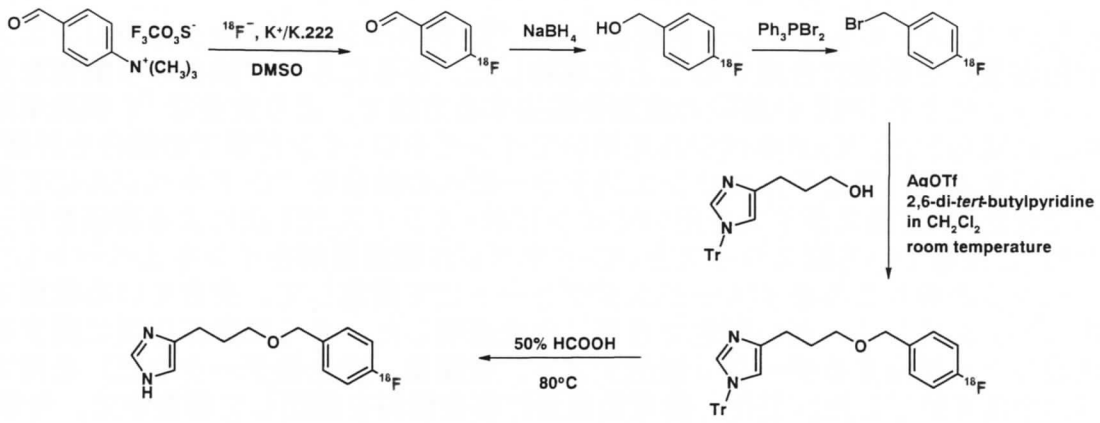


図４．^{18}F-標識ヨウ化フルオロベンジルを用いた 18F-標識 H3 受容体アンタゴニストの開発。

また図５に示すように ^{18}F-標識アセチルコリンエステラーゼ阻害薬の TAK-147 の新規合成に成功し、PET イメージングが可能であるかについて研究している。

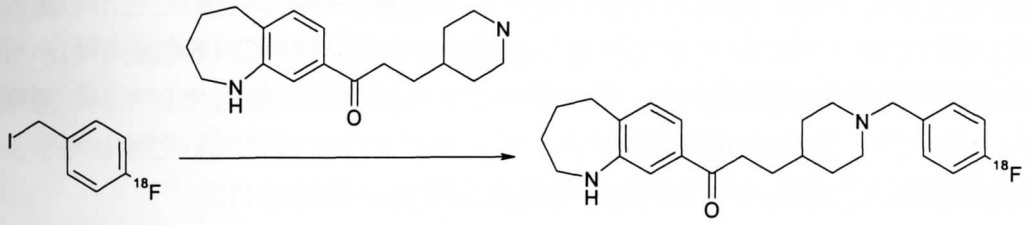


図５．^{18}F-標識アセチルコリンエステラーゼ阻害薬の TAK-147 の新規合成

３．遺伝子改変マウス（ノックアウトマウス）を用いた研究。

ヒスタミン関連ノックアウトマウス作成：近年様々な遺伝子改変動物が薬理学に応用され、多くの新しい知見が得られるようになっている。遺伝子ノックアウトマウスはその特異性が高くいままで薬理学的方法によって明らかにされてきた遺伝子産物の生体における機能をより明確にすることができる。痛み反応や攻撃性、ストレス反応や薬物依存など幾つかの神経系機能に関してより純系のマウスを作成する必要性がある。５世代以上 C57B 系マウスに交配し、遺伝的に均一な C57B マウスのヒスタミン H１,H2 受容体ダブルノックアウトマウスを作成した。

ヒスタミン関連ノックアウトマウスを用いた痛みの受容研究：ノックアウトマウス PET 研究と併行して、痛み受容メカニズムについてノックアウトマウスを含むマウスで詳細に研究を行った。作成した H1,H2 受容体ダブルノックアウトマウス、H1 受容体遺伝子ノックアウトマウス、H2 受容体ノックアウトマウスを用いてアロディニアなどの神経障害後の痛み受容においても H1、H2 受容体とも協調的に作用して痛みの受容促進に作用していることがわかった。

モルヒネ鎮痛とヒスタミン（図６）：痛み反応におけるヒスタミンＨ１受容体の役割とモルヒネの鎮痛効果におけるヒスタミンの役割を明らかにした。ヒスタミンは H1 受容体を介して痛みの受容を促進している。モルヒネは肥満細胞あるいはヒスタミン神経からヒスタミンを遊離させて鎮痛効果を減弱させていることがわかった。臨床的にモルヒネと抗ヒスタミン薬の併用が強い鎮痛効果を発揮することがノックアウトマウスを用いて証明された。さらにモルヒネによる鎮痛作用における H1 受容体の役割について H1 受容体遺伝子ノックアウトマウスを用いて明らかにできた。

痛みの受容メカニズムにおけるオレキシンの役割：オレキシンはヒスタミン神経を興奮させてヒスタミン神経系と協調して覚醒を引き起こす。しかし痛みの受容については異なる作用を持っていることを明らかにした。オレキシンは

覚醒反応を強力に惹起するが、脳内や脊椎内に投与するとモルヒネ様の鎮痛作用を起こした。中枢ヒスタミンは H1、H2 受容体を介してオレキシンと同様に覚醒反応を起こすが、痛みの受容については促進させた。このことから痛みの受容についてオレキシンとヒスタミンは逆の作用を持っていることがわかった。各種ヒスタミン関連遺伝子ノックアウトマウスにおける脳内オレキシン濃度を測定したところ、H1 受容体遺伝子ノックアウトマウス脳においてオレキシン濃度の低下があり、オレキシンと H1 受容体の関連が示唆された。

Ｈ２受容体ノックアウトマウスについても一般的な行動薬理学的評価を行い、その基本的な性質はＨ１受容体ノックアウトマウスと同様であることを明らかにした。痛み反応や攻撃性、ストレス反応や薬物依存など幾つかの神経系機能に関してより純系のマウスを作成する必要性がある。H1、H2 受容体ノックアウトマウスを C57B 系マウスに交配し、さらに両方のマウスをかけ合わせてヒスタミンＨ１,H2 受容体ダブルノックアウトマウスを作成した。現在、ダブルノックアウトマウスを含めて、ストレスの受容について実験が進行中である。

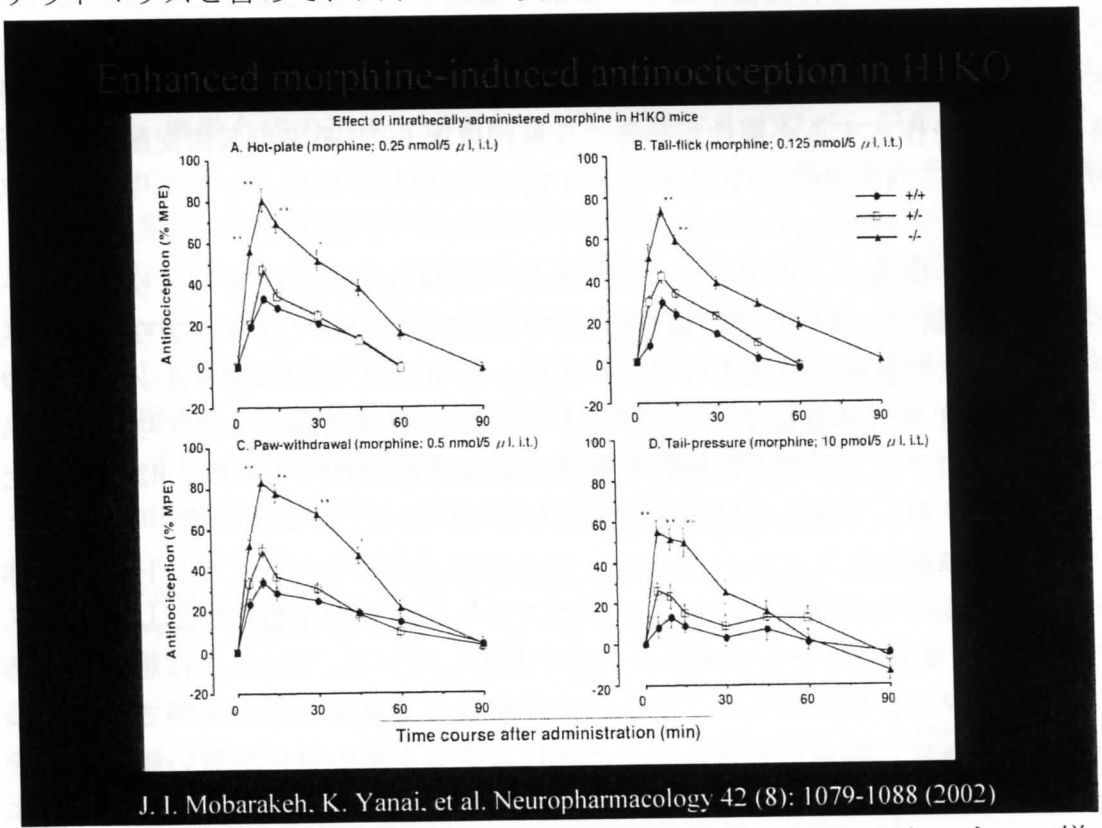


図６．モルヒネによる鎮痛効果は H1 受容体遺伝子ノックアウトマウスで増大する。

４．３次元ＰＥＴカメラを用いたヒト脳の神経伝達量測定法の開発と疾患への応用。

ヒト脳内神経受容体の非侵襲的定量化法の確立を行った。まず測定感度が高いために投与する放射能が少なくてすみ、放射線被曝が軽減される３次元データ収集モードを用いた画像再構成法の高速化を行った。通常の汎用コンピューターでは画像解析に時間がかかるためにスーパーコンピューターを用いて画像再構成をおこなう方法を開発した。３次元データ収集モードで通常数日かかっていたデータ解析がスーパーコンピューターによりほとんどリアルタイムでおこなえるようになった。ＰＥＴによる受容体測定の時に今までは動脈血採血をおこなって定量してきたが、無採血非侵襲的定量法を考案して速度定数や結合パラメーターの算出をおこなった。疾患への応用では、アルツハイマー病、うつ病、統合失調症への応用をおこなった。3 次元 PET カメラの臨床応用としてアルツハイマー型痴呆患者においてヒスタミンＨ１受容体量を測定し、アルツハイマー病の認知機能低下に伴ってＨ１受容体が減少することが分かった（図７）。同様に軽症うつ病患者からインフォームドコンセントを取り PET を用いて H1 受容体を測定した。予測したように H1 受容体の減少が観察された（図８）。本研究によりＨ１受容体が AD 患者や正常老人、うつ病患者の認知機能低下に関係していることが示唆された。アルツハイマー病やうつ病における神経伝達の異常は、多くの神経伝達物質に異常が生じることが知られている。本 PET 研究からヒスタミン神経系の機能低下がうつ病やアルツハイマー病の中枢機能の低下に関係している可能が考えられた。現在、精神分裂病において H1 受容体を測定している。

図７．アルツハイマー病の H1 受容体は正常老人と比較して有意に減少する。結合のパラメーターである binding potential (Bmax/KD) を若年正常者 (YOUNG)、老年正常者 (OLD)、アルツハイマー病(AD)で比較した。またアルツハイマー病における H1 受容体結合能と脳血流量を認知スケールの MMSE（mini-mental score examination）でプロットした（下図）。脳血流の変化より H1 受容体結合量の減少が大きいことがわかる。H1 受容体の減少が認知機能低下に関係していることを示している。

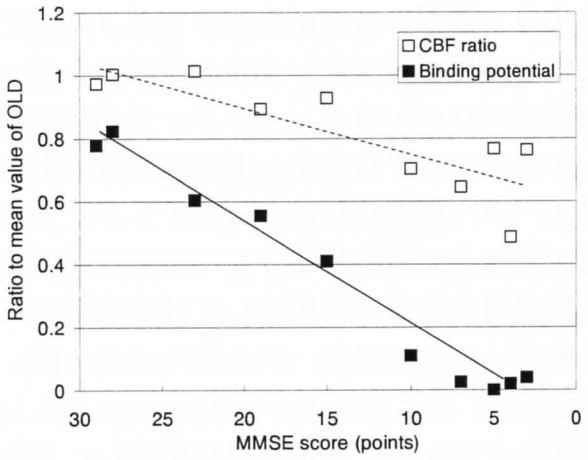
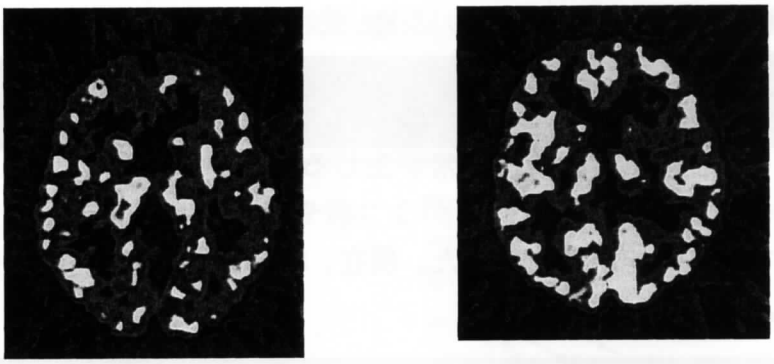
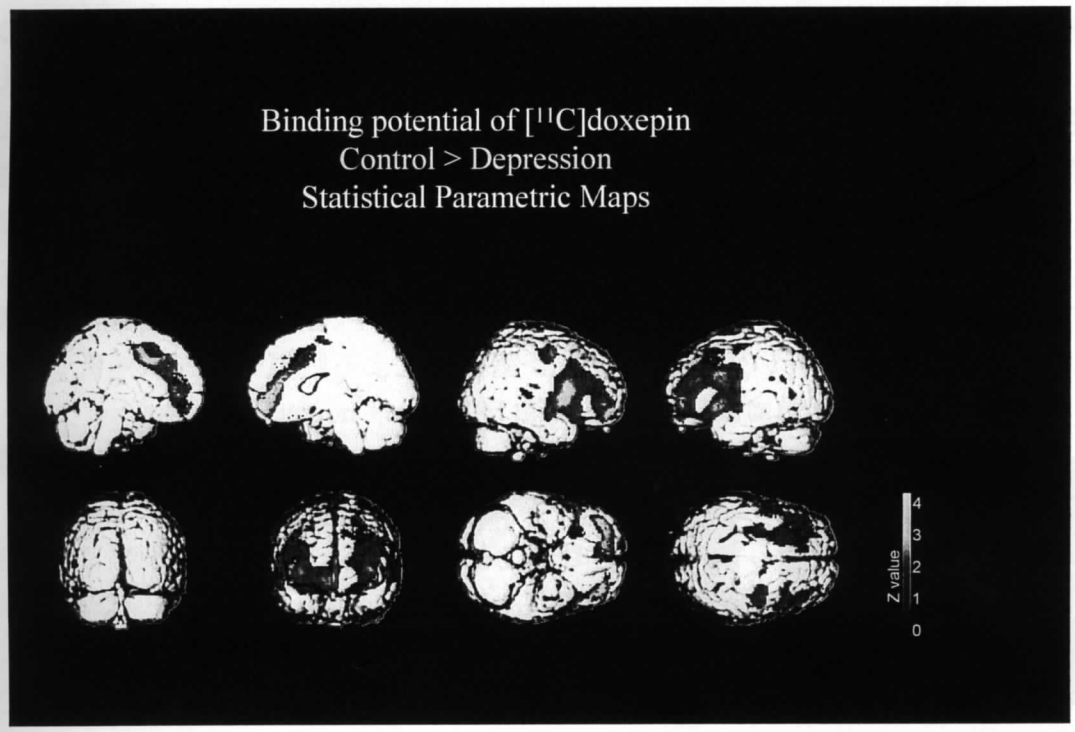


図８．うつ病におけるヒスタミン H1 受容体量結合量の変化



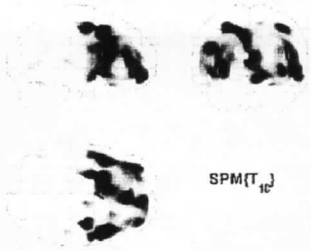
59 歳　男性（うつ病）　　５４歳　男性（正常者）

^{11}C-ドキセピンの結合を年齢と性があった正常被験者と比較した。上図は 1 例の提示であるが、明らかに H1 受容体の結合量の減少がわかる。10 例のうつ病患者と性と年齢があった健常者群について SPM を用いて比較した。うつ病患者の前頭葉皮質、前帯状回、前頭前野などにおいて H_1 受容体結合能の低下を認めた。低下が確認された脳部位は、うつ病に関係するといわれている前頭葉を中心に広く観察された（下図）。うつ病の症状の SDS スコアと逆相関する部位も主に前頭葉・帯状回を中心に認められた。本研究によりＨ１受容体がうつ病患者の認知機能低下に関係していることが示唆された。アルツハイマー病やうつ病における神経伝達の異常は、多くの神経伝達物質に異常が生じることが知られている。本 PET 研究からヒスタミン神経系の機能低下がうつ病やアルツハイマー病の中枢機能の低下に関係している可能が考えられた。



B

対照群でうつ病群よりも
H1受容体結合能が有意に高い領域



Area (Brodman area)	Side	Z-score	Talairach coordinates		
			x	y	z
Middle Fronrtal Gyrus (10)	L	3.69	−34	50	−6
Inferior Frontal Gyrus (44)	R	3.64	48	14	16
Orbitofrontal cortex (11)	R	3.49	8	58	−14
Inferior Frontal Gyrus (44)	L	3.45	−44	10	26
Anterior Cingulate gyrus (24,32)	L	3.44	−10	10	40
Frontal Lobe	L	3.43	38	−10	52
Precentral gyrus (4)	R	3.4	38	−10	52
Anterior Cingulate gyrus (24,32)	R	3.33	2	38	22
Middle Fronrtal Gyrus (10)	R	3.19	38	52	−4
Inferior Frontal Gyrus (47)	L	3.12	−40	14	−6
Medial Fronrtal Gyrus (6)	R	3.74	12	−10	58
Temporal Lobe	L	2.58	−48	−58	−22
Middle Temporal Gyrus (22)	L	2.49	−48	−54	22

5．脳賦活試験法による高次脳機能の研究

①脳賦活法を用いたヒスタミン神経系の研究

様々な神経活動に伴う神経伝達機能を非侵襲的に測定することは極めて重要な研究である。本研究助成により脳賦活法を用いて我々が長く取り組んできた

ヒスタミン神経系の機能研究をヒトボランティアにて行った。脳内ヒスタミンは覚醒や日内リズムに重要な役割を果たすことが知られている。それに関連して、常用量の第１世代 H1 ブロッカーの投与により眠気や認知能力の低下が生じることが知られている。しかしその発生メカニズムについて十分な研究はなされていない。本研究は、まず①[^{11}C]ドキセピンを用いてＨ１受容体占拠率を測定し、眠気発生や認知能力低下にどれだけのＨ１受容体占拠が必要か明らかにすることを試みた。さらに②$H_2{}^{15}O$ と 3D-PET を用いて視覚認知課題遂行時の脳機能イメージングを行い、抗ヒスタミン薬投与による眠気や認知機能低下の発生メカニズムを調べた。

２０－２７歳の正常若年男子ボランティア３９名に協力していただいた。視覚認知課題はタキストスコープを用いて、数字とひらがなを１，３，５，７，１０，２０msec 間呈示して、弁別させる Odd-Ball 課題を与え反応時間と正答率を測定した。d-ｸﾛﾙﾌｪﾆﾗﾐﾝ 0,1,2ｍｇを静注して認知機能をタキストスコープ検査により、主観的な眠気は Stanford Sleepiness Scale (SSS)を用いてインタビューにより調べた。またＨ１受容体占拠率は、d-ｸﾛﾙﾌｪﾆﾗﾐﾝ１，２，５mg 投与後に[^{11}C]ドキセピンを静注して２Ｄモードにて脳の放射能を計測して受容体のほとんどない小脳を基準に求めた。さらに d-ｸﾛﾙﾌｪﾆﾗﾐﾝ２mg 投与前後に脳血流量を３次元データ収集モードによる $H_2{}^{15}O$ 静注法により９回測定し、脳機能賦活部位をＳＰＭ９６にて解析した。

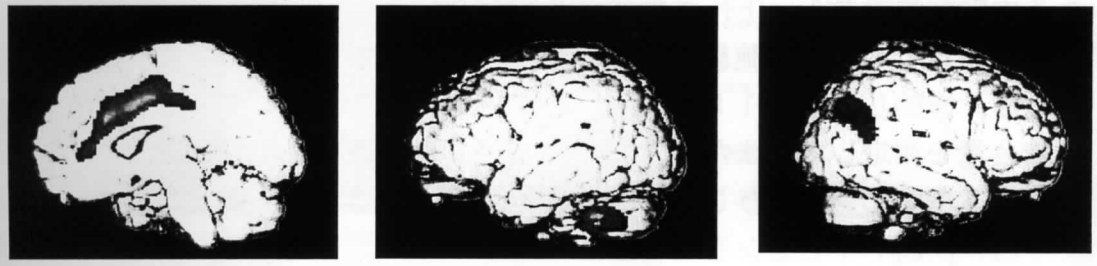
主観的な眠気のスケールである SSS ではプラセボ、d-ｸﾛﾙﾌｪﾆﾗﾐﾝ 1mg、2mg 投与によりほとんど有意な変化は認められなかった。タキストスコープによる視覚認知課題では、プラセボ群では全く変化がなかったが、1mg 投与群において反応時間の延長と正答率の低下が、有意差はみられなかったもののある程度認められ、特に刺激条件が厳しいほど（5,7msec の呈示時間）その傾向は強かった。d-ｸﾛﾙﾌｪﾆﾗﾐﾝ 2mg 投与時の認知課題試験で提示時間が 5ms のときにプラシーボとの間で反応潜時の遅延と正答率の低下が有意に認められた。2mg での H1 受容体占拠率は 77%であり、1mg 投与時の H1 受容体占拠率は 56％であった。以上のことからヒト脳内 H1 受容体遮断に伴い認知能力の低下をもたらすと考えられ、有意な認知能力の低下には 70％以上の H1 受容体が遮断される必要があると結論づけられる。$H_2{}^{15}O$ のよる脳機能の計測では、d-ｸﾛﾙﾌｪﾆﾗﾐﾝ静注により減少しプラセボ（生食）では変化しなかった脳の部位は、right midbrain, right medial thalamus, left middle temporal gyrus, left inferior frontal gyrus などであった。抗ヒスタミン薬はこれらの部位におけるＨ１受容体を介する神経伝達を阻害することにより、眠気や認知能力の低下が起こる可能性を指摘したい。

さらに、d-クロルフェニラミン 6mg 内服による Choice reaction time (CRT)課題施行時の脳活動をプラセボと比較した。CRT 施行時に右帯状回（BA24）、右頭

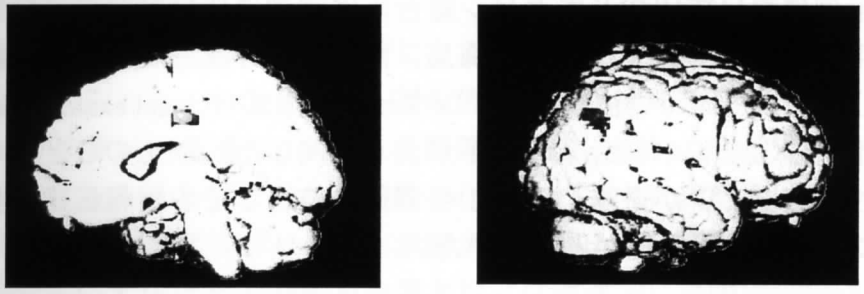
頂葉（BA40）、左小脳が賦活される。BA40 は左右の弁別に関連する脳部位であり、クロルフェニラミン投与による CRT 課題の低下に関連して活動が低下した。また BA24 は注意に関連する領域で、認知機能低下に伴って課題遂行に注意を必要とするために BA24 の活動は増加した。認知機能低下に伴ってタスクに依存した脳活動の変化が証明された（図９）

図９　クロルフェニラミン（6mg）内服による脳活動の変化

Choice Reaction Time (CRT)課題遂行中の脳血流量増加部位



クロルフェニラミン投与により脳活動が変化した部位



活動増加部位（帯状回）　　活動低下部位（BA40）

ヒトの神経伝達を観察する一般的方法は、ＰＥＴやＳＰＥＣＴを用いて標識した特異的リガンドの脳内動態を調べることによりなされる。また一方 $H_2{}^{15}O$ による脳機能イメージングを用いてもある程度特異的神経伝達を観察できると思われる。今回、抗ヒスタミン薬のＨ１受容体占拠率と認知機能との関係を[^{11}C]ドキセピンとタキストスコープを用いて明らかにした。さらに脳賦活試験遂行時（視覚弁別課題）の脳機能イメージングを $H_2{}^{15}O$ 静注法により行い、d-クロルフェニラミンを投与することにより認知能力低下や眠気に関与する脳の特異的作用点を描出することに成功した。本研究助成によりヒトの神経伝達機能を研究する新しい方法を開発できた。

②リガンド賦活法による神経伝達物質遊離測定。

最近のＰＥＴ研究の進歩には、このような３次元データ収集ＰＥＴを用いた $H_2{}^{15}O$ による脳賦活試験とリガンド賦活法によるヒト脳における神経伝達物質遊離測定の開発が挙げられる。我々は、[^{11}C]ドキセピンを用いたヒスタミンH1受容体測定法を用いて、２回ＰＥＴ検査をすることによりヒトの脳内においてヒスタミン遊離が測定できるかを試みた。

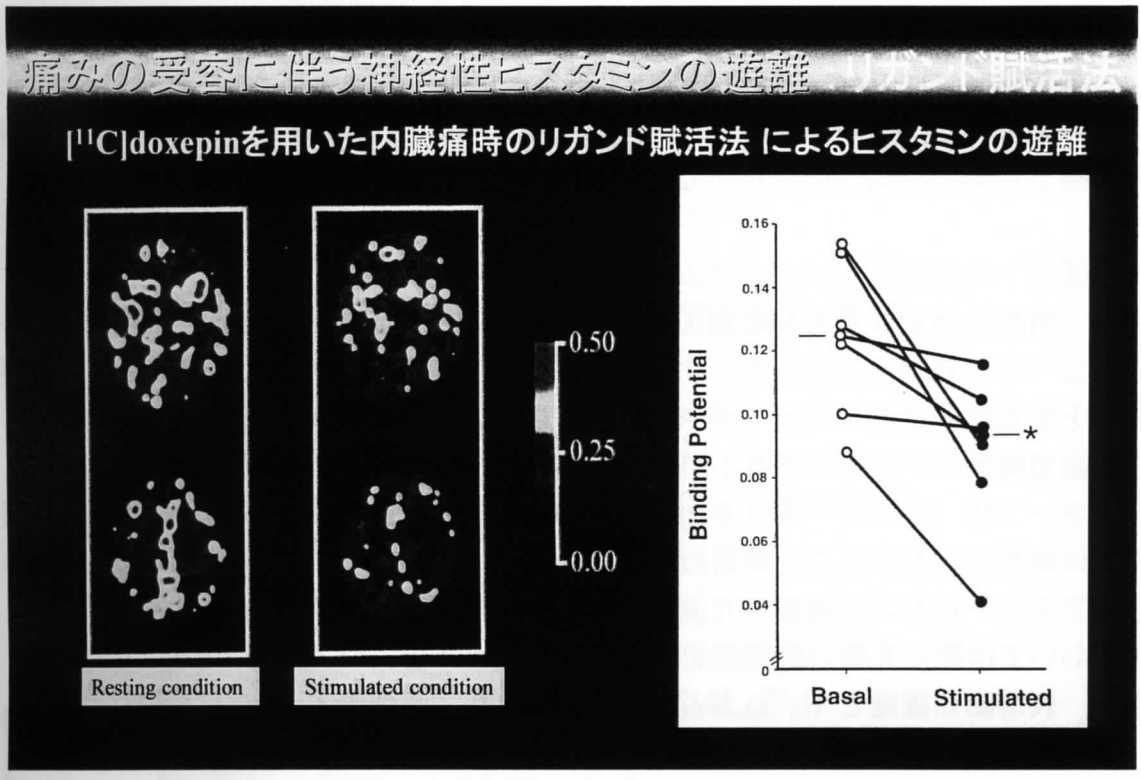
[^{11}C]ドキセピンは通常の Lithium aluminum hydride を用いる方法により[^{11}C]ヨウ化メチルを作成し、N-メチル化反応により標識した。被験者は２０－２７歳の男性被験者を広告にて募集し書面にて承諾書を得た。タスクは腸管の拡張による内臓刺激である。下行結腸にバルーンを留置して、６０mmHg にて１０秒間膨らませて２０秒間弛緩させ６０分間に渡り計１２０回膨らませた。バルーン刺激を開始してから１５分後に[^{11}C]ドキセピンを投与して９０分間Ｈ１受容体結合量を測定した。また別な日にバルーンは留置したが、刺激しないで同様に[^{11}C]ドキセピンを投与して９０分間Ｈ１受容体結合量を測定した。計７人に１４回、[^{11}C]ドキセピンを用いた研究を行った。画像解析法は我々が以前に開発した、Bmax/Kd をピクセルごとに求めたパラメトリック画像を SPM（statistic parametric mapping）にて処理した。

７人全例において[^{11}C]ドキセピン結合能は刺激に伴い結合量が減少した。ＳＰＭを用いた解析では、帯状回、海馬、前頭葉、一次感覚野などが特に結合量が減少した。また同時に被験者にインタビュー形式（visual analog scale）により調べた不快感と全脳の結合量の低下がよく相関した。以上のことから、腸管刺激によりヒスタミンが遊離してＨ１受容体に結合しその情報伝達の脳内処理が不快感に関係する可能性が考えられた。

我々は現在までに、ヒスタミンＨ１受容体遺伝子ノックアウトマウスを用いた解析によりＨ１受容体が欠損していると内臓痛や体性痛ともその感受性が低下することを明らかにしている。基礎研究から得られた痛みの受容にＨ１受容体が関係していることのヒトにおける証明について、今回ＰＥＴを用いて行うことができた。リガンド賦活法により刺激に伴う神経伝達物質遊離を測定できることは受容体測定法のいっそうの発展に繋がると思われる。

図１０．リガンド賦活法によるヒスタミン遊離測定法の開発。

[^{11}C]ドキセピンの PET スキャンを２回行うことによりその結合量の変化からヒスタミンの遊離を測定した。腸管刺激により有意に[^{11}C]ドキセピン結合が減少する。現在、精神的な集中ストレスと運動ストレスにおけるヒスタミンの PET 遊離測定を計画中である。



③ヒトの神経薬理学

我々はさらにアルコール(EtOH)負荷による眠気や認知機能障害について、まずタスク面から検討した。EtOH による運転機能や認知機能の低下に関する報告は多いが、血中エタノール濃度(BAC)との関係を詳細に検討した報告はほとんどない。EtOH はアセトアルデヒドから酢酸へ代謝され、主代謝酵素はアルコール脱水素酵素およびアセトアルデヒド脱水素酵素である。両代謝酵素には遺伝的多型性による酵素活性の個人差が認められており、代謝速度に差がみられる。また食事の有無や薬物の服用等により胃排泄速度が影響を受け、EtOH の消化管からの吸収速度が変化する。したがって代謝酵素活性の差や食事や薬物の影響により、同量の飲酒でも個人間あるいは個人内で BAC に差が現れる可能性がある。

健常成人男性被験者に eye-hand coordination 課題およびタキストスコープによる視覚刺激課題を与えて認知機能を評価し、EtOH 負荷前後で比較した。視覚刺激課題は、odd-ball 課題と二者選択反応時間課題(CRT)および単純反応時間課題(SRT)を採用した。Eye-hand coordination 課題は coordination 達成率(%)を、視覚刺激課題は反応時間と正答率を測定し、ウィスキー(30、60、90、120ml)および水の計 5 種類の用量を single blind 試験により検討した。課題終了後（飲酒後約 1 時間）に血液を採取し、BAC を測定した。

BAC が 50mg/dl 以上で、coordination 達成率は有意に低下し、視覚刺激課題の反応潜時は有意に遅延した。正答率は BAC の増加と有意ではないが低下傾向で

あった。以上の結果より、BACの上昇と共に認知機能が低下したが、その認知機能障害にはBACの閾値が存在することが明らかとなり、おおよそ50mg/dlと考えられた。将来このように中枢神経系作用薬の作用メカニズムをタスクに関連した脳の活動性の変化として捉えていきたい。

④ヒトの表情認知メカニズムの研究。

情動を測定するために顔の認知タスクの開発（図１１）と評価を行い、アルコールの効果を研究した。③で記述したようにアルコールは高度な注意を必要とする課題においてはウイスキー3杯以上あるいはBAC50mg/dl以上において認知機能低下が認められるが、情動が関係するヒトの表情認知課題ではウイスキー１杯（アルコール３０ｍｌ）あるいはBAC１０－２０mg/dl程度にて笑顔の認知が亢進することが初めて明らかになった。情動が関係する場合低用量のアルコールが認知機能を亢進させることが明確になった。BAC50mg/dl以上においては逆に笑顔の認知機能であっても低下する。

表情認知課題と$H_2{}^{15}O$静注法PETにより、感情のイメージングを行い、アレキシサイミア（失感情症）と正常反応者において表情認知の脳内メカニズムを研究した（図１２）。アレキシサイミアとは、心身症に特徴的な認知、感情様式を表す概念で、感情を認識して感情と情動喚起に伴う身体感覚を区別することが困難な性格傾向である。自分の感情を表現することが乏しく、他者の感情について語ることも難しい。様々な顔の表情をイメージをPsyScope (free soft, http://psyscope.psy.cmu.edu/)を用いてモニターに呈示した。マッキントシュにボタンボックスを接続して反応時間と正答率を正確に測定した。今回用いた刺激は、様々な表情を持った顔（幸福、悲しみ、驚き、怒り）とそれぞれの情動レベルをNeutralとの間で４段階変化させた画像である。心理学的アンケートにより選出されたアレキシサイミア群で、その脳内表情認知メカニズムを$H_2{}^{15}O$静注法PETにより調べると、アレキシサイミア群では有意に左半球を用いて顔の表情認知を行っていることが判明した。

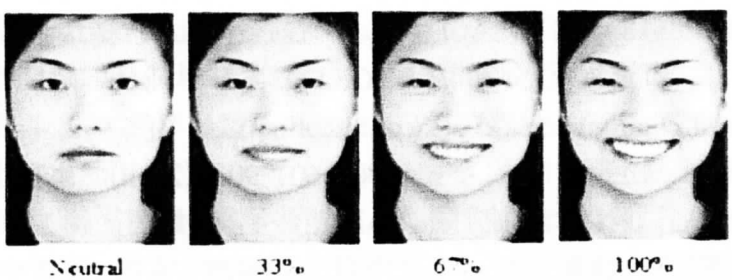
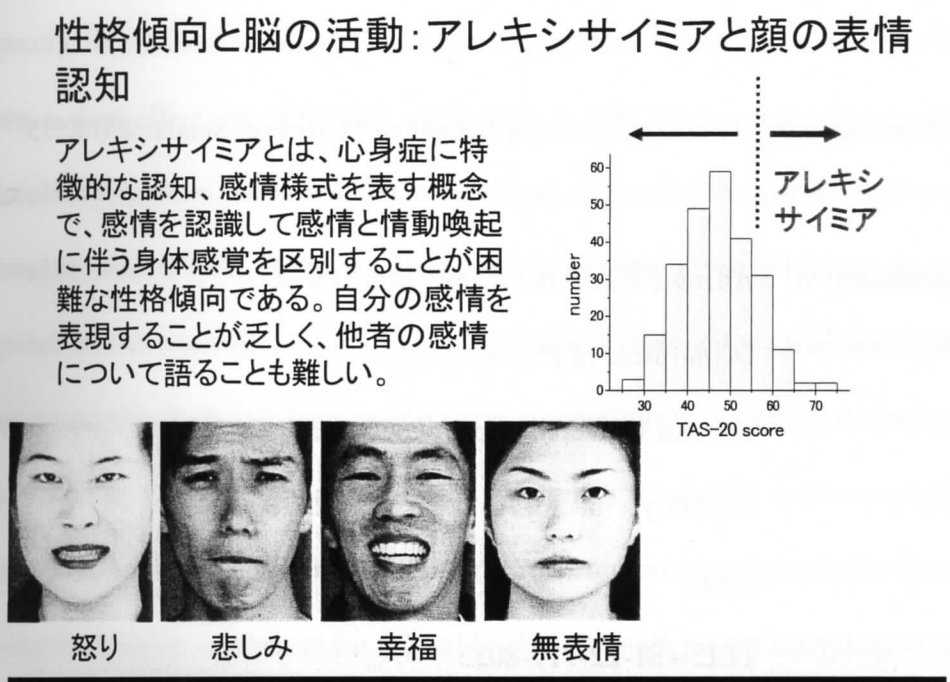
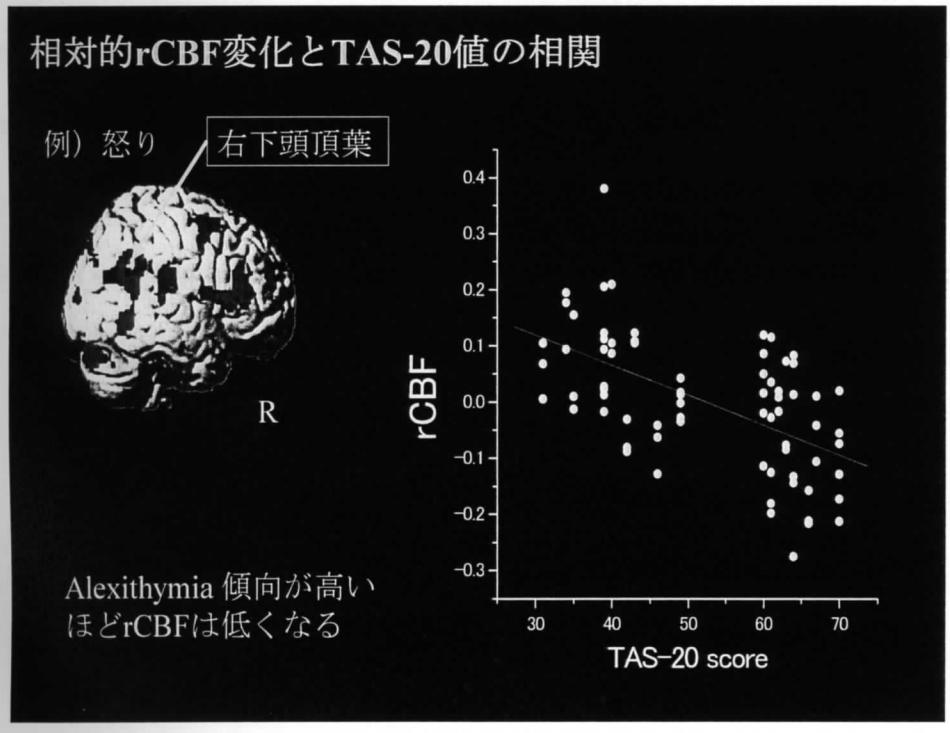


図１１．顔の表情認知機能を調べるタスク。コンピューターにて100％の笑顔と中性の顔を３３％、６７％混合させている（モルフィング）。

図１２．情動のイメージング。

アレキシサイミア（失感情症）を TAS-20 という心理検査にて分類してそのスコアと脳血流量が相関する部位を描出している。アレキシサイミアは心身症の発症と密接な関係がある。





Histamine as a neurotransmitter in the central nervous system

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Abstract

Histamine neurons are exclusively located in the posterior hypothalamus, and send their outputs to almost all regions of the brain. They are involved in many functions such as spontaneous locomotion, arousal, sleep-wake cycles, appetite control, seizures, learning and memory, behavioural sensitisation, stress and emotion. We propose here that histamine neurons have a dual effect on the CNS, with both a stimulatory and suppressive function. As a stimulatory function, neuronal histamine is one of the most important systems to maintain and stimulate wakefulness. Brain histamine also functions as an inhibitory bioprotection system against various noxious and unfavourable stimuli of convulsion, drug sensitisation, denervation supersensitivity, ischemic lesions and stress susceptibility. A significant amount of research has been done to clarify the functions of the histaminergic neuron system using histamine-related gene knockout mice and human PET (positron emission tomography) studies. The activity of histamine neurons is inherent for mental health, although potent activators of histamine neurons have not been clinically available until now. Here, we summarize the bimodal function of histamine neurons.

Key words: Histamine, histamine neurons, pharmacology, brain, knockout mice, PET (positron emission tomography).

Histamine is an active amine with a wide spectrum of biological actions at the central and peripheral level. Since its discovery in 1910, histamine has been a challenge for many scientists; it has been studied extensively in various fields of biology. Histamine is involved in various physiological functions through H1-, H2-, H3-, and H4-receptors [1-3]. In particular, it is involved in the symptoms of allergic rhinitis and urticaria. Orally administered antihistamines are useful for the relief of allergic symptoms. Most of people believed for a long time that histamine was a harmful mediator. However, recent studies reveal that histamine plays an important part for homeostasis in the brain. The histaminergic neuron system was first identified immunohistochemically with the antibody raised against L-histidine decarboxylase (a histamine-forming enzyme, HDC) as a marker by Watanabe *et al.* [4]. The strategy of their study was to purify HDC from HDC-rich placenta and to prepare its antibody (Fig. 1). In fact, histamine neurons were simultaneously visualized using antibodies against histamine itself by Panula *et al.* and Steinbusch *et al.*, independently, in the same year. The fundamental structure of the histaminergic neuron system was elucidated a few years later through intense world-wide collaboration.

The functions of the histaminergic neuron system have been extensively studied using pharmacological histaminergic agents. The general morphology and various functions of histamine neurons have been examined from these pharmacological experiments [5-7]. Based on accumulated data from neuropharmacological and behavioural studies, a role for brain histamine has long been thought to be involved in arousal, the sleep-awake cycle, appetite control, seizures, learning and memory, aggressive behaviour, and emotion [8]. These data were mainly obtained from rodents through classical pharmacological experiments and confirmed recently by the study of knockout mice. Because there are considerable differences among species in histamine biology, it can not be totally accepted to deduce the functions in humans

from those of rodents. For this purpose, autopsied human brains and cerebrospinal fluid (CSF) have been utilized for a long time in human brain chemistry. Alternative approaches to human brain chemistry are non-invasive brain imaging modalities [9]. Imaging techniques enable us to assess the properties of brain tissues and to obtain information of how the brain works across scales from system level to molecular level. Among several imaging modalities, molecular PET (positron emission tomography) techniques enable us to focus directly on human pharmacology and brain functions in living subjects. Here, the functions of histamine neurons are described followed by brief explanations of the used methods. We propose that the functions of histamine neurons are classified into stimulatory and suppressive roles of the net CNS effects (Table 1). An adequate activity of the histaminergic neuron system is indispensable to maintain a healthy neuronal condition.

Methods used for studies on functions of histamine neurons.

Classical pharmacological tools: Functions of the histaminergic neuron system have been studied pharmacologically in various ways, e.g. by inactivating the histaminergic neuron system with an HDC inhibitor, (S)-α-fluoromethylhistidine (FMH), H1 and H2 antagonists (e.g., pyrilamine (mepyramine) and zolantidine), and an H3 agonist, (R)-α-methylhistamine (MeHA). FMH has been particularly useful in earlier studies [10]. For its activation, L-histidine, a precursor amino acid of histamine, metoprine, an inhibitor of the histamine inactivating enzyme *N*-methyltransferase (HMT), 2-thiazolylethylamine, an H1 agonist, and thioperamide and clobenpropit, H3 antagonists have been administered to animals. The results obtained by these pharmacological studies are summarized in previous reviews [11, 12].

Knockout mice of histamine related genes: An alternative approach to clarify the physiological functions of histamine neurons is an approach which manipulates the histamine-related genes. The use of genetically-altered mice has become routine in many fields

of biomedical research over the past decade. For the investigators that utilize rodents as experimental systems, the technical development of production of mice with specific genetic alteration has provided a unique opportunity for a wide variety of sophisticated investigations. As shown in Table 2, there are 5 different knockout mice available for experiments on the histaminergic neuron system [13-16]. Nowadays, many laboratories are using the knockout mice of histamine-related genes in combination with classical pharmacological approaches in order to clarify the functional roles of the histaminergic neuron system.

Non-invasive PET imaging of histamine receptors in the human brain: The histamine receptors in human brains were firstly visualized non-invasively by our group. We have used PET techniques since 1990 in order to examine the functions of histamine neurons in the living human brain. There had been only a few studies on the pathophysiology of histamine neurons in the human brain until PET techniques were developed. The distribution of H1-receptors in a living human brain was measured with [^{11}C]-mepyramine (pyrilamine) or [^{11}C]-doxepin as a radiotracer by PET [17]. The densities of H1-receptors are most prominent in the frontal, temporal and parietal cortices, the anterior cingulate, thalamus, and hippocampus, less prominent in the striatum and occipital cortex, and least prominent in the pons-medulla oblongata and cerebellum (Fig. 2). The visualized binding was completely blocked by the premedication of *d*-chlorpheniramine before the PET scan. The further validity of the use of [^{11}C]-doxepin as a radiotracer for H1-receptor imaging was later obtained by the *in vitro* doxepin binding experiments of histamine H1-receptor gene knockout (H1KO) mice. Doxepin did not bind specifically to membrane fractions prepared from brains of H1KO mice, though a specific binding was detected with wild-type mice [13].

Bimodal actions of histamine neurons: Stimulatory functions

Sleep-wake cycle: Histamine is believed to be a wake amine and is involved in circadian

rhythm [18]. In pharmacological experiments, FMH shortened the waking time in the dark period and prolonged the slow-wave-sleep (SWS) time in the light period in rats. In accordance with this study, thioperamide (an H3 antagonist) increased the waking time and MeHA (an H3 agonist) decreased the S2-SWS time in cats [19]. These results were confirmed by studies using knockout mice of histamine-related genes. The daytime activity (6 a.m.-6 p.m.) in home cages was more enhanced in H1-receptor gene knockout (H1KO) mice than in the wild-type. The nighttime (6 p.m.-6 a.m.) activity was less among the H1KO mice. Thus the day to night ratio of ambulation was 0.15 in wild-type but was 0.55 in H1R-KO mice [13]. These data show that the H1KO mice move less at night and move more during the day. It is very interesting that the same distorted circadian rhythms were observed in the H2- and H3-receptor gene knockout mice as well as in the histidine decarboxylase (HDC) knockout mice.

Neuronal histamine plays a central role in maintaining wakefulness. In addition, activity in the histaminergic neuron system can be modified by several neurotransmitters and neuromodulators such as orexins, GABA, adenosine and prostaglandin D_2 [20]. In particular, orexins are newly-discovered neuropeptides in the lateral hypothalamus. Orexins deficiency causes the sleep disorder narcolepsy in various models and in human patients, suggesting that the functions of these peptides might be the regulation of sleep. The activation and inactivation of the histaminergic neuron system by these modulators are important in sleep-wake control [21]. The schematic representation on the role of the histaminergic neuron system in cortical activation is illustrated in Fig. 3 [22].

Brain cortical activation: The PET imaging studies of H1-receptors in the living human brain substantiated the histamine-mediated cortical activation theory. It is well known that classical antihistamines can cause considerable sedation in humans. We determined the values for brain H1-receptor occupancies of classical and second-generation antihistamines. The second-generation antihistamines are generally believed to be non-sedative. As shown in Fig. 4,

the H1-receptor occupancies of the second-generation antihistamines were lower than those of the sedating antihistamines. The sedative properties of antihistamines have been extensively studied by using [^{15}O]H_2O-PET and [^{11}C]-doxepin-PET techniques [23, 24]. These results clearly suggest that histamine causes wakefulness through H1-receptors in the human brain.

Spontaneous locomotor activity: Spontaneous locomotor activity in an open field changed after histaminergic agents were administered to rodents. For example, locomotor activity decreased significantly by administration of the HDC-inhibitor FMH and the H3-agonist MeHA. FMH and MeHA decrease the release of histamine from histamine neurons. On the other hand, the locomotor activity increased by administration of the HMT-inhibitor metoprine and the H3-antaginist thioperamide. Metoprine and thioperamide were reported to increase the release of histamine. In accordance with these data, H1-receptor gene knockout mice showed impaired locomotor activity in an open field [13, 25]. The results indicate that histamine is involved in the activation of ambulation through H1-receptors.

Responses to nociceptive stimuli: High doses of histamine impair several nociceptive responses in rodents, while lower doses enhance them. The same biphasic effects were observed with several H1 agonists. Even though H1 antagonists inhibit the analgesia evoked by histamine, some H1-blockers also produce analgesic activity when given alone or with opiates. Antihistamines were empirically administered as mild analgesics in the clinic. Such ambiguities exist in pharmacological experiments. The knockout mice with histamine-related genes are also useful to clarify the roles of histamine on nociception. H1KO mice were less sensitive to thermal, mechanical and chemical nociceptive stimuli than wild type mice [26]. HDC-KO also showed less sensitivity to nociceptive stimuli. These data suggest that histamine enhances the responses to nociceptive stimuli through H1-receptors. Moreover, H1KO mice showed enhanced analgesic responses to morphine [27]. Similar results were obtained with H2KO mice, suggesting that both H1- and H2-receptors are synergistically functioning on the stimulation of

nociceptive transmission.

Cognition: Intracerebroventricular administration of histamine improves learning and memory in rats, whereas administration of sedating H1-blockers cause a dose-dependent impaired effect on avoidance-responses of aversive stimuli and on spatial cognitions of radial maze experiments. Several H3 antagonists shorten the response latencies of cognitive tasks through the activation of histamine neurons. However, these effects on cognition could not be confirmed using knockout mice with histamine-related genes.

Age-related declines in H1-receptor binding were demonstrated in normal human brains by ^{11}C-doxepin-PET studies. In normal aging, the H1-receptor binding decreases in the prefrontal, temporal, cingulated and hippocampal regions, which are closely associated with attention and cognitive functions [28]. In addition, a significant decrease of H1-receptors is demonstrated in the frontal and temporal regions of Alzheimer's disease. The decrease in H1-receptors correlates with the cognitive severity of Alzheimer's disease as assessed by Mini-Mental State Examination scores [29]. A decrease in H1-receptor binding has also been observed in depressive and schizophrenic patients. Decreased H1-receptor-mediated neurotransmission might thus contribute to cognitive dysfunctions of these diseases. Several atypical antipsychotics are reported to release neuronal histamine through the blockage of 5-HT2 receptors [30], and might thereby enhance the cognition of schizophrenics through the activation of histamine neurons. Although there are several opposite findings in rodents, the activation of histamine neurons may improve cognition in humans.

Bimodal actions of histamine neurons: Suppressive functions

Convulsion: The roles of histamine on convulsion were examined in several seizure models of electroconvulsion, pentylenetetrazole (PTZ)-kindling and amygdala kindling. In pharmacological experiments, convulsion duration was prolonged by the deletion of histamine

by FMH administration, and was shortened by the increase of histamine contents by metoprine (HMT-inhibitor) or L-histidine. These data were further supported by experiments using H3 ligands. Selective H1 agonists inhibit the convulsion in several seizure models, suggesting that the inhibitory action of histamine is mediated through H1-receptors. These pharmacological experiments suggest that histamine functions as an endogenous anticonvulsant. This idea was further confirmed by studies using histamine-related gene knockout mice /with histamine-related genes. The H1KO mice showed longer periods of electroconvulsion in maximal electroshock models and they were more susceptible to pentylenetetrazole-induced kindling [31]. Similar results were obtained with HDC-KO mice.

It is well known that brain-penetrating H1-blockers cause convulsions as serious side effects. Our PET studies showed that H1-receptors are increased in the foci of epileptic patients with complex partial seizures. This result might be explained by an up-regulation of H1-receptors, which diminishes the spreading of abnormal firing. In accordance with human data, the binding potential (Bmax/KD) of H1-receptors have increased in the amygdala kindling model of rats [32].

Hyperactivity caused by stimulants and stress: Neuronal histamine increases the locomotion in ordinary conditions as described above. In contrast, neuronal histamine has a calming effect on the hyperactivity of locomotion caused by stress and stimulants such as methamphetamine (MAP), amphetamine and cocaine. When repeatedly treated with stimulants, locomotion gradually increases day by day. The increased locomotor activity known as behavioural sensitisation or reverse tolerance is a result of stimulant abuse and schizophrenia in animal models. Increased locomotor activity caused by single and multiple doses of MAP is attenuated by treatment with L-histidine, while the hyperactivity itself can be significantly enhanced by FMH. The formation of behavioural sensitisation to MAP was also facilitated in HDC-KO mice [33]. All these results indicate that neuronal histamine inhibits the formation of

behavioural sensitisation to stimulants.

Chronic stress as well as stimulants can also induce hyperlocomotion. Food-deprived activity stress is often used as a natural model of stress-caused hyperlocomotion. This model is defined as the condition in which rats are forced to run on a wire wheel with restricted food consumption. Research has shown that food-deprived activity stress gradually increases hyperactivity on the running wheel, and actually results in decreased body weight. Intracerebroventricular injection of histamine and peripheral administration of L-histidine reduces hyperactivity caused by food-deprived activity stress, although it did not affect the spontaneous locomotor activity [34]. These findings suggest that hyperexcitation caused by stimulants and chronic stress can be inhibited by the activation of histamine neurons.

Appetite control: Neuronal histamine is thought to be involved in the regulation of appetite and energy control [35]. Continuous administration of histamine into the hypothalamus suppresses food intake in rats and treatment with metoprine (HMT-inhibitor) increases endogenous histamine and suppresses food intake, as well. In contrast, FMH (HDC-inhibitor) increases feeding-associated behaviour. The suppressive effects of neuronal histamine on appetite have also been confirmed by studies using receptor gene knockout mice. Recently, it has been reported that the appetite-suppression of leptin is mediated through H1-receptors [36]. Hypothalamic histamine probably functions as one of the anorectic neurotransmitters, suggesting that clinically available H1 agonists may be useful for the treatment of abnormal obesity.

Protective roles in ischemia-induced neuronal damages and denervation supersensitivity: Several neurotransmitters are involved in the development of delayed neuronal cell death after short-term brain ischemia. Some neurotransmitters are neuroprotective, whereas others are neurotoxic. The protective roles of histamine on delayed neuronal cell death were demonstrated by examining the effects of FMH on delayed neuronal cell death [37].

Several reports showed that depletion of brain histamine aggravates neuronal death following brain ischemia. Neuronal histamine probably functions as one of the protective transmitters against ischemic insults.

Neuronal histamine is also involved in denervation supersensitivity caused by chemical and physical nerve injury [38, 39]. H3-receptors increase in the rat striatum after 6-hydroxydopamine-induced dopaminergic denervation and in the superior colliculus follwoing unilateral orbital enucleation. These data indicated that H3-receptors were highly up-regulated in the postsynaptic sites of injured neurons in association with denervation supersensitivity. Because the activation of H3-receptors decreases cAMP through inhibitory G-protein, the up-regulated H3-receptors may suppress denervation supersensitivity.

Conclusion

The histaminergic neuron system distributes to almost all regions of the brain. The structures constitute a diffuse system of information transfer with non-directed synapses. These structural characteristics resemble other aminergic neuron systems, suggesting a wide variety of regulatory functions. Integrated studies using pharmacological agents, knockout mice of histamine-related genes and PET reveal that histamine neurons have stimulatory and suppressive functions in normal and pathological conditions. The functions of histamine neurons are characteristic, although the precise action mechanism for the bimodal CNS effects is still unknown. Neuronal histamine is indispensable for maintaining mental health. Unfortunately, there are no clinically available medications to activate the histamine neurons at present. New drugs that activate histamine neurons would be useful for the progress of therapeutic strategies against neurological and psychiatric diseases.

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Legends to Figures

Figure 1. Distribution of histamine neurons in the rat brain. Immunohistochemical localization of histamine neurons was demonstrated using antibodies raised against rat HDC. Histamine neurons are exclusively located at the posterior hypothalamus, and distribute their fibers to almost all brain regions. A: lower magnification. B: higher magnification.

Figure 2. Distribution of H1-receptors in the human brain as measured by [^{11}C]doxepin-PET. The density of H1-receptors is high in the cingulate cortex, prefrontal cortex, fronto-temporal cortex, thalamus, amygdala and hippocampus. H1-receptors are low in the cerebellum.

Figure 3. Hypothetical roles of histamine neurons in sleep-wake mechanism. Histamine neurons are functioning as the most important center of wakefulness. VLPO (ventrolateral preoptic nucleus) and orexin neurons can inhibit and stimulate the activities of histamine neurons, respectively. PGD_2 and adenosine activate the sleep center of VLPO.

Figure 4. Histamine H1-receptor occupancy by H1-antagonists in the human brain. H1-antagonists are well known to cause sedation and sleepiness. We have used PET imaging methods to determine differences in penetration through the blood-brain barrier (BBB) of classical and so-called 2nd generation H1-antagonists. The H1-receptor occupancy is measured in the human cortex after single oral doses. If it does not penetrate BBB at all, the value of occupancy is estimated to zero. H1-receptor occupancies of less than 20%, 20-50% and over 50% are considered to be "non-sedative", "less-sedative" and "sedative", respectively.

Table 1. Functional roles of the histaminergic neuron system: A stimulatory and suppressive function

Functions	Deduced functions of histamine neurons
Stimulatory CNS functions	
Sleep-wake cycle	Maintenance of wakefulness
Locomotion	Increased locomotor activity
Cognition	Augmented learning and memory
Energy metabolism	Induction of brain glycogen hydrolysis
Nociception	Increased pain perception
Suppressive CNS functions	
Feeding	*Inhibition of feeding behaviour*
Convulsion	*Inhibition*
Stress	*Inhibition of stress-induced excitation*
MAP-induced psychosis	*Inhibition of kindling formation*
Neural plasticity	*Inhibition of denervation-induced supersensitivity*

Table 2. Available knockout mice in studies of histamine CNS functions

Histamine H1-Receptor Gene Knockout Mice (H1KO) (ref. 13)
Histamine H2-Receptor Gene Knockout Mice (ref. 14)
Histamine H3-Receptor Gene Knockout Mice (ref. 16)
Histamine H1- and H2-Receptor Gene Double Knockout Mice
Histidine Decarboxylase Gene Knockout Mice (HDC-KO) (ref. 15)

Figure 1.

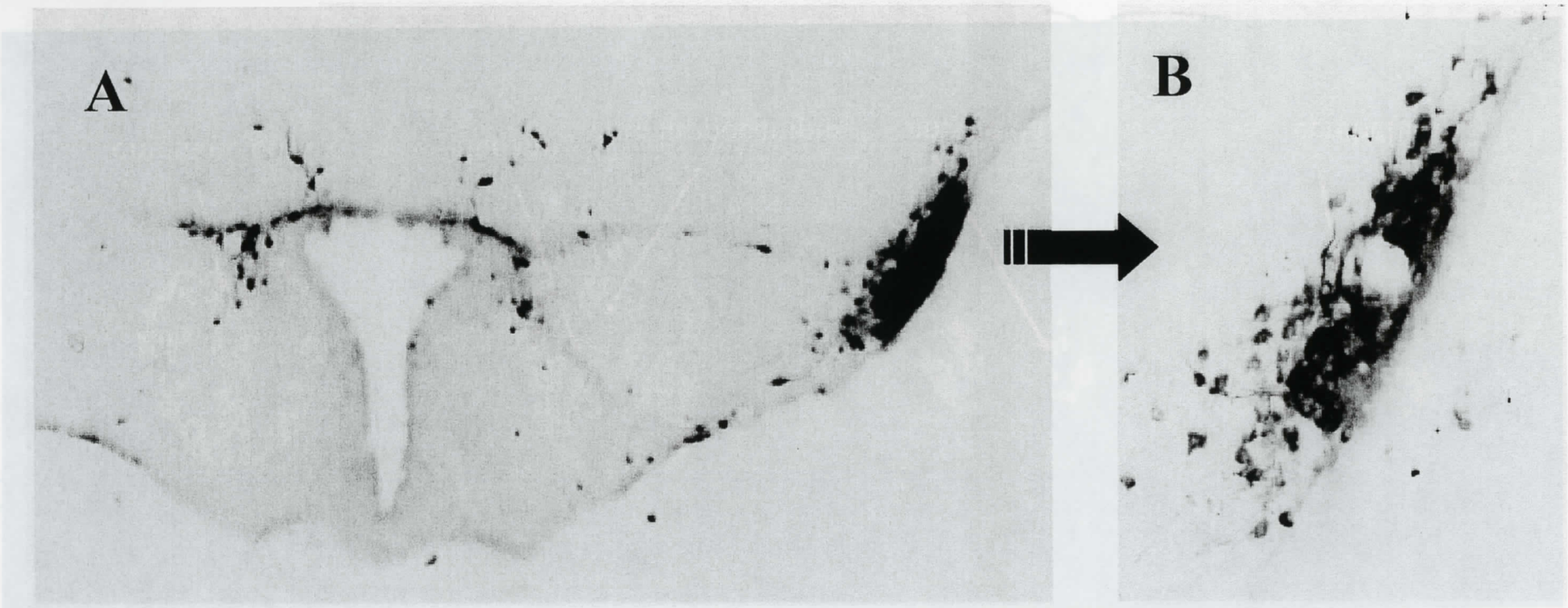


Figure 2.

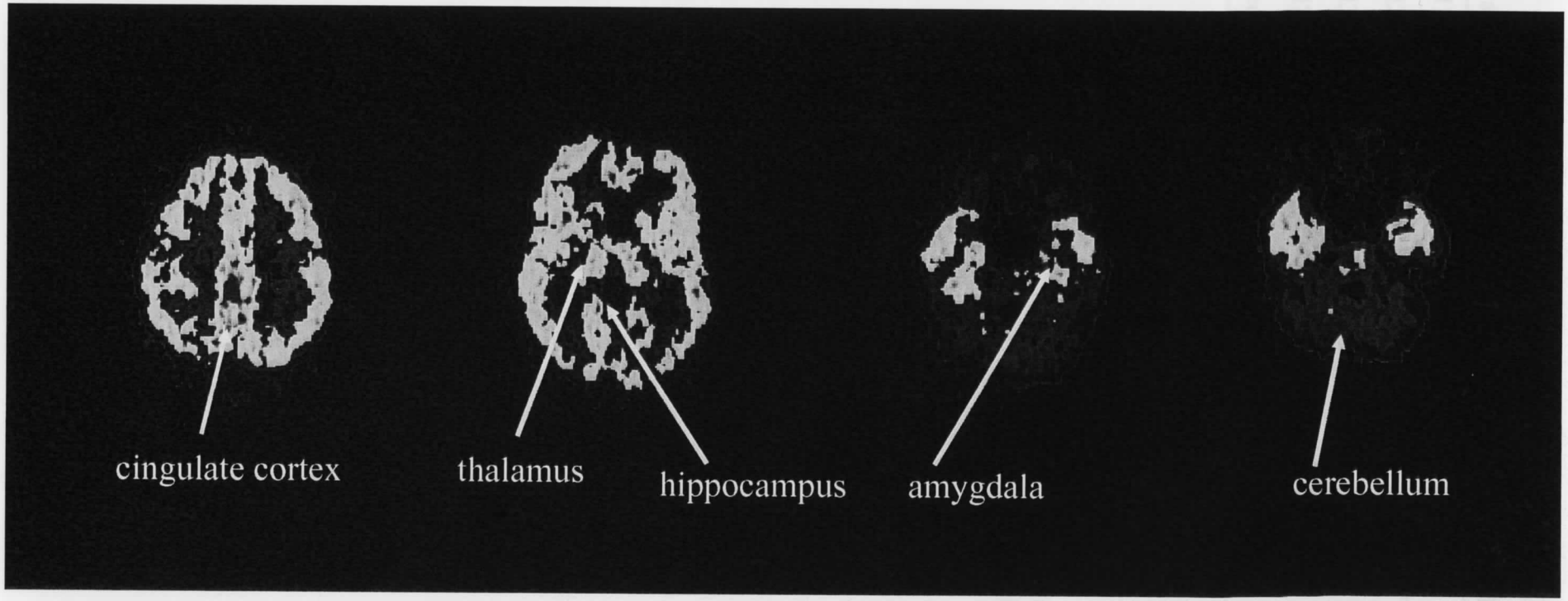

cingulate cortex
thalamus
hippocampus
amygdala
cerebellum

Figure 3.

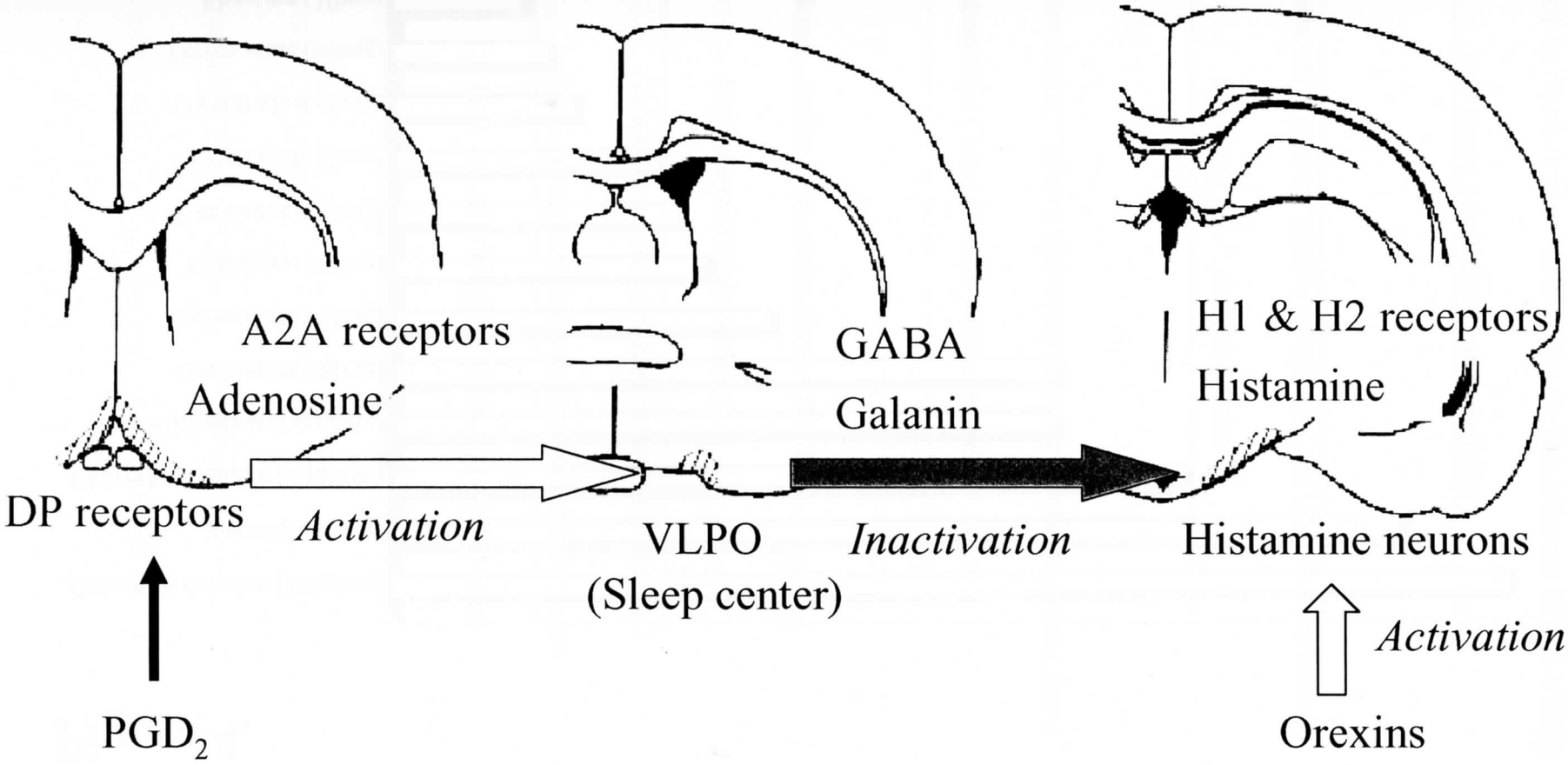
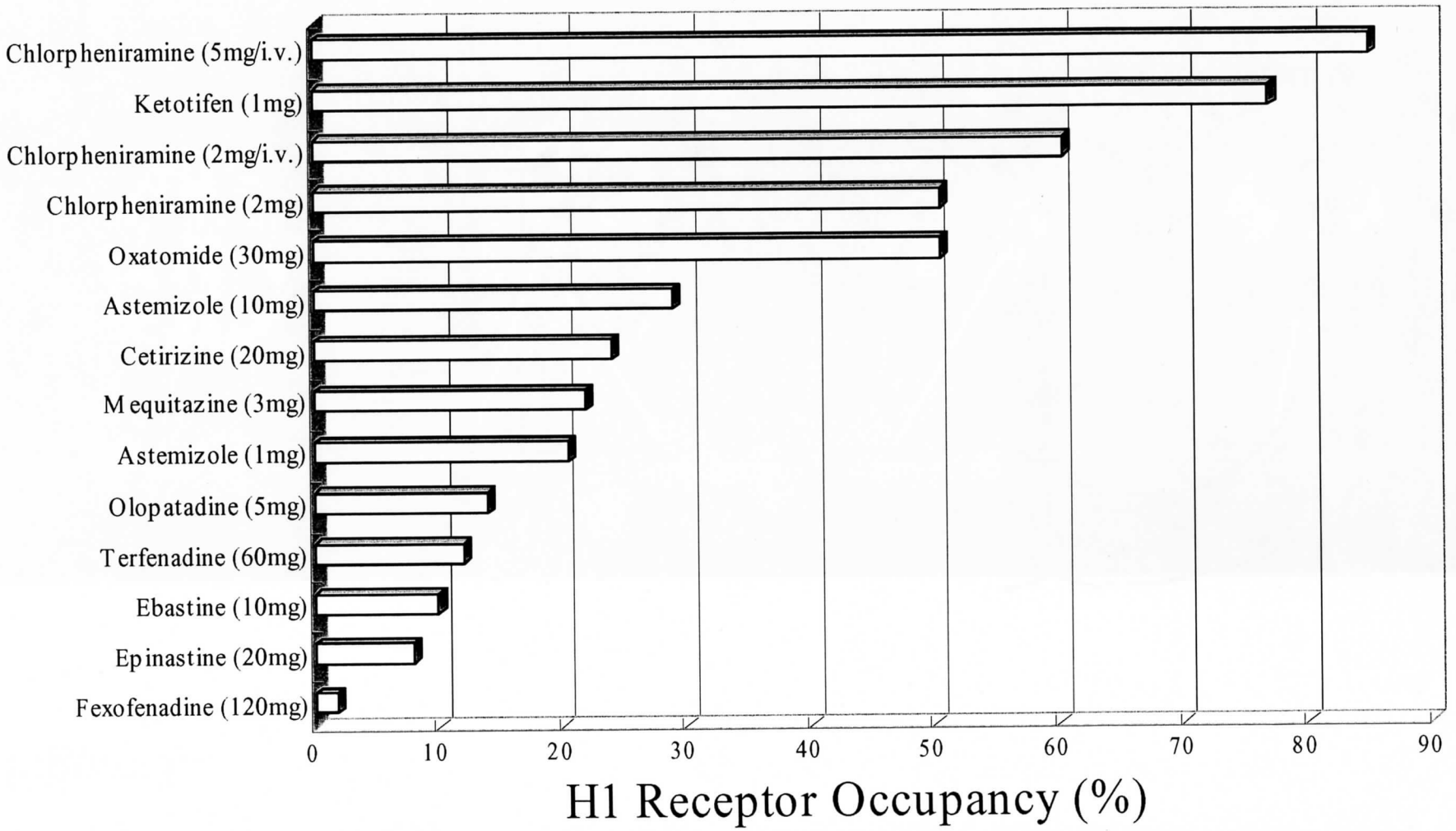

A2A receptors
Adenosine
GABA
Galanin
H1 & H2 receptors
Histamine
DP receptors
Activation
VLPO
(Sleep center)
Inactivation
Histamine neurons
Activation
PGD2
Orexins

Figure 4.


Chlorpheniramine (5mg/i.v.)
Ketotifen (1mg)
Chlorpheniramine (2mg/i.v.)
Chlorpheniramine (2mg)
Oxatomide (30mg)
Astemizole (10mg)
Cetirizine (20mg)
Mequitazine (3mg)
Astemizole (1mg)
Olopatadine (5mg)
Terfenadine (60mg)
Ebastine (10mg)
Epinastine (20mg)
Fexofenadine (120mg)
0
10
20
30
40
50
60
70
80
90
H1 Receptor Occupancy (%)

Imaging of central itch modulation in the human brain using positron emission tomography

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Abstract

The unpleasantness of itching is reduced by cooling. Although previous research suggests the presence of a central itch modulation system, there is little documentation about how the modulation system works in the brain. In the present study, we investigated the modulating system of the itching sensation in the human brain using positron emission tomography (PET) and $H_2{}^{15}O$. The significant increases of regional cerebral blood flow (rCBF) caused by histamine stimuli using iontophoresis were observed in the left anterior cingulate cortex (BA24), the left thalamus, the right anterior parietal cortex (BA40), the right posterior parietal cortex (BA7), the bilateral dorsolateral prefrontal cortex (BA46) and the right premotor cortex (BA6). We did not observed any changes in the secondary somatosensory cortex (S2) in the mild and intense itching stimulus conditions. That was partly consistent with the previous reports that itching did not activate S2 and thalamus. Activation in these areas related to itching and subjective evaluation of the itching sensation were decreased by cold pain stimulus simultaneously given to the opposite side of the itching stimulus, as compared to itching alone. Interestingly, midbrain including periaqueductal gray matter (PAG) was activated only during the simultaneous stimulation of itching and cold pain. PAG is known to be modulating noxious stimulus. Here we hypothesize that the activation of PAG may also

be related to the itch modulation in the CNS. These findings indicate that the modified brain activities in the PAG, the cingulate, the frontal and the parietal cortex might be associated with the itch modulation in the CNS and that the S2 might not be primarily involved in processing the itching perception in the brain since the activity of S2 was not observed in any concentration of itching stimuli.

Keywords: Itch modulation; Periaqueductal gray matter; Secondary somatosensory cortex; Histamine; Positron emission tomography

1. Introduction

Itching can be defined as "an unpleasant sensation associated with the desire to scratch" (Rothman, 1941). Scratching behavior in atopic dermatitis can become just as unbearable and debilitating as chronic pain, leading to depression and suicidal thoughts (Keele et al., 1964). Itching is related to the excitation of unmyelinated C-fibers triggered by histamine release from mast cells (Van et al., 1972; Handwerker et al., 1987). Generally, antihistamines are prescribed for patients with allergic diseases to suppress itching symptoms through the blockade of histamine H1 receptors (H1R) (Assanasen et al., 2002). However, administration of antihistamines, especially of the first generation, interfere with the activities of daily living and with work that requires full alertness, since they elicit sedation and impair various cognitive functions such as psychomotor speed and learning (Mochizuki et al., 2001; Nicholson, 1985; Shamsi and Hindmarch, 2000). The unpleasant sensation caused by itching can also be reduced by cooling. Skin cooling can reduce itch sensation without aggravation while scratching tends to aggravate the symptom and while antihistamines often cause sedation. Therefore, it has been considered that understanding of the itch inhibitory mechanism by cooling is clinically important. Actually many researchers have studied about the itch modulation by cooling. Melton et al. noted that a cold environment appreciably

shortened the duration and intensity of itch sensation (Melton et al., 1950). Cormia et al. found that lowering the skin temperature increased the threshold for histamine-induced itchiness (Cormia et al., 1953). At least, two hypotheses have been proposed for the mechanism of itch inhibition by cooling in the central nervous system (CNS). One hypothesis is that the itch modulation system exists in the spinal cord. Bromm et al. indicated the possibility that an pruriceptive C-fibers and cold-mediating A-delta fibers were interacting somewhere within the spinal cord, that would be analogous to the gate control theory of pain (Bromm et al., 1995). Jinks et al. also reported that decreased activity of the dorsal horn neurons related to histamine was associated with the activation of spinal inhibitory interaction evoked by cooling stimuli to the itchy site (Jinks et al., 1998). The other hypothesis assumes that the itch modulation system lies in the supraspinal regions. For example, Murray referred to Melzack's proposal that the central itch modulation system in order to account for the itch attenuating effect of cold stimulus should be in the supraspinal regions (Murray et al., 1975). However, as far as the authors know, little has been reported focusing on the itch inhibitory mechanism by cooling in the brain. Even it is still unclear whether such mechanism exists in human brains or not.

In pain studies, on the other hand, presence of the central pain modulating system has

been already identified. The periaqueductal gray matter (PAG) is known as one of the central pain modulation systems. The descending pathway from PAG modulates pain by restricting afferent neural signals caused by pain in the spinal cord (Mayer, 1984).

Itch and pain are regarded as closely related sensations. Some indicated that itch was a subliminal form of pain (Graham et al., 1951). Several investigators have studied the neural mechanisms of itch and pain. Both sensations are conveyed to the brain via C-fibers and the spinothalamic tract (Bear et al., 2001; Schmelz et al., 2001). Handwerker et al. investigated the differences of the peripheral neural mechanism between itch and pain by recoding peripheral C-fibers using microneurography (Handwerker et al., 1991). They, however, did not find any difference in discharge patterns encoding itch and burning pain evoked by histamine and mustard oil, respectively. Jinks et al. reported that most superficial dorsal horn neurons responding to histamine were also excited by capsaicin, mustard oil and noxious heat (Jinks et al., 2000). Conversely, itch specific neural pathway has been found in recent animal and human studies. Schmelz observed specific C-receptors for itch in human skin (Schmelz et al., 1997). Andrew et al. found the spinothalamic lamina I neurons selectively sensitive to histamine (Andrew et al., 2001). Thus, discussion over the neural pathway of itch and pain is still controversial.

Recent neuroimaging techniques such as positron emission tomography (PET) have enabled visualization of the functional cerebral network involved in the processing of itching. Hsieh et al. first reported that histamine-evoked itch sensation was associated with activations of the frontal, the parietal and the cingulate cortex, the supplementary motor area and the premotor area (Hsieh et al., 1994). Similar cortical regions were also activated by painful stimulus (Treede et al., 1999). Darsow et al. demonstrated that the itch intensity ratings correlated mainly to activation of the sensory and motor areas (Darsow et al., 2000). In accordance with the finding, it was also found that the somatosensory cortex correlated to the intensity of painful stimulus (Duncan et al., 1994), suggesting that the central mechanism of itch sensation was similar to that of pain. On the contrary, Drzezga et al. indicated the difference of central neural processing between itch and pain. They reported that itch and pain seemed to share common central pathways but that the presence or lack of thalamic activation was likely to reflect a true difference between pain and itch (Drzezga et al., 2001).

These previous reports demonstrate that itch and pain have some differences, but also indicate that the neural processing of itching has some similarities to that of pain. Therefore it could be assumed that the mechanism of itch modulation by cooling is partly similar to that of pain in the human brain (e.g. PAG). The itch modulation system

in the human brain, however, has not been studied using neuroimaging techniques until now. In the present study, we investigated the mechanism of itch modulation by cooling in human brains using PET and $H_2{}^{15}O$.

2. Methods

2.1. Subjects

Fifteen healthy male volunteers (mean ± SD of age, 22 ± 2.3 years old) were included in the present study. Subjects with a history of allergy, atopic eczema or other dermatological diseases were excluded from the study. None of the subjects participating in the present study were under any medication nor had any previous history of psychiatric disorders. All subjects were evaluated as right-handed based on the Edinburgh inventory (Oldfield, 1971). They were not allowed to take any medication, alcohol and any other drugs the day before and the day of the experiment. Written informed consent was obtained from each subject and the study was performed in compliance with the relevant laws and institutional guidelines.

2.2. Experimental design

In most of previous studies, itch sensation was modulated by cooling on the same or near site of itch. However, if cold stimulus were given to the site of itch, it would become hard to exclude the possibility that itching was already inhibited at the spinal cord level. The purpose of the present study was to investigate the inhibitory mechanism of itch sensation by cooling in the human brain. Thus, we gave cold pain stimulus to the contralateral side of itch. Afferent inputs from peripheral nerves are unilaterally conveyed to the brain via the spinal cord (Bear et al., 2001; Nieuwenhury et al., 1988). That is why itching and cold stimuli were given to the right and left feet, separately in the present study. In our pilot study, innoxious cold stimulus (20 °C) given to the contralateral side of the itching stimulus did not inhibit itch sensation while noxious cold stimulus (5 °C) did. Thus we employed cold pain stimulus of 5 °C.

In the present study, PET measurement was conducted under 7 different conditions as follows: Condition 1) saline stimulus, Condition 2) mild itching stimulus with 0.001 % histamine solution, Condition 3) intense itching stimulus with 0.01 % histamine solution, Condition 4) dual stimulations of intense itching (0.01 % histamine) and cold pain (5 °C) (dual stimuli), Condition 6) cold pain stimulus (5 °C), and the resting condition (condition 7).

Itch sensation induced by histamine tends to increase in a dose-dependent fashion (Simone et al., 1987). Therefore two different concentrations of histamine solution were used in the present study to verify the dose-dependency. The histamine solutions (0.01 % and 0.001 %) were prepared by dissolving histamine to saline. Two ml of the histamine solution was infiltrated into a square electrode pad (2 cm x 2 cm), which was attached to the back of the right foot. Itch sensation was elicited by the electrical subcutaneous penetration of the histamine solution with iontophoresis system (UI-2060, Uniflows, Japan). In the present study, the electrical current given by the iontophoresis was fixed at 1 mA in order to eliminate the possibility that the brain activity changed due to different intensities of the current (Torquati et al., 2002). The duration of the iontophoretic stimuli was 2 min (total charge: 120 mC, 1 mA x 120 sec). A saline condition served as a control for the itching stimuli where the saline solution (2 ml) was applied to the subjects in the same way as itching stimulus conditions using iontophoresis. No stimulus was given to the left foot in the following three conditions: 1) saline, 2) mild and 3) intense itching stimulus conditions.

In the dual stimuli condition, the intense itching and cold pain stimuli were simultaneously applied to the right and left feet, respectively. For cold pain stimulus, thermocooler (Thermal cycler, Japan) was used to keep the skin temperature of the back

of the left foot at 5 °C, where the areas to be stimulated by iontophoresis and cold pain were controlled to be equal (2 cm x 2 cm). The cold pain stimulus was given to the left foot for 2 min simultaneously with the intense itching stimulus. The sequence of conditions 3 (intense itching stimulus) and 4 (dual stimuli) were randomized among the subjects.

We employed the cold pain stimulus condition in order to examine whether the regional cerebral blood flow (rCBF) changes observed in the dual stimuli was attributable to the cold pain stimulus to the left foot or not. A control for the cold pain stimulus condition was the resting condition. Details of conditions employed in the present study are shown in Table 1.

The stimulus was applied to the subjects for the duration of 50 sec just before PET investigation started to let the subjects adapt to the stimuli. Then PET measurement started and lasted for 70 sec under the presence of the continuous stimulus to the end of the PET measurement. All subjects closed their eyes during PET scanning. Time intervals between scans were more than 10 min in order to eliminate the effect of previous itch and/or cold pain sensations. After each scanning, intensity and unpleasantness of subject's itch sensation was scaled with visual analog scales ranging from 0 to 10. When subjects feel no itch sensation on their right foot, the scale will be

0". When the itch intensity and unpleasantness is the worst in their past experience, the score will be "10".

2.3. PET measurements and data analysis

The cerebral blood flow (CBF) images were obtained at whole brain level using a PET scanner (Shimadzu SET-2400W, Japan), with an average spatial resolution of 4.5 mm the full-width half-maximum (FWHM) and with sensitivity of a 20 cm cylindrical phantom of 48.6 k.c.p.s. KBq^{-1} ml^{-1} in the 3D-mode. PET measurement was performed for 70 sec. Subjects were injected with approximately 5.4 mCi (200 MBq) of $[^{15}O]$-H_2O through antecubital vein for each scan.

The CBF images obtained were processed and analyzed by Statistical Parametric Mapping (SPM) software (SPM99; Welcome Department of Cognitive Neurology, London, U.K.) (Friston et al., 1995a; Friston et al., 1995b). After realignment for intra-subject motion correction, all images were stereotaxially normalized, using linear and non-linear transformations into a standard space of Talairach and Tournoux (1988). The normalized images were then smoothed using a 16 x 16 x 16 mm Gaussian filter. The values of rCBF were expressed as ml 100 g^{-1} min^{-1}, adjusted using ANCOVA and scaled to a mean of 50 ml / 100 g / min. The significant increase or decrease in rCBF

was evaluated according to the general linear model at each voxel.

To test the hypotheses on specific rCBF changes, the estimates were compared using linear contrasts. The resulting set of voxel values for each contrast constitutes a statistical parametric map of the *t*-statistics. To discover brain regions related to the histamine stimulus, CBF images during the intense itching stimulus were compared to those during the saline stimulus. CBF images during the intense itching stimulus were compared to those during the dual stimuli to detect any rCBF difference between the conditions. The effect of cold pain stimulus on the brain activity was investigated by comparing CBF images in the cold pain stimulus condition to those in the rest. The *t*-value of each voxel was transformed into normally distributed Z-statistics. For each comparison, voxels with a Z-value higher than 2.99, corresponding to $p<0.001$ (uncorrected), were considered to represent regions with significant change in rCBF.

The changes of subjective feelings of itch intensity and unpleasantness were compared among the mild itching, the intense itching and the dual stimuli conditions with ANOVA and multiple comparison (Tukey). A probability of less than 0.05 was considered to be statistically significant.

2.4. VOI analysis

We performed volume of interests (VOI) analysis with SPM to compare the brain activity related to itching among the conditions such as the mild itching, the intense itching and the dual stimuli conditions. We determined the localization of the peak activation related to the intense itching stimulus as compared to the saline stimulus condition. Mean voxel values were calculated among the voxels including the peak and also exceeding a threshold of $Z > 2.99$. Mean of these voxel values reflected rCBF since all voxel values in the CBF images were scaled to a mean of 50 ml / 100 g / min. The rCBF changes in the mild itching, the intense itching and the dual stimuli conditions in comparison to the saline stimulus condition were examined by ANOVA and multiple comparison (Tukey). A probability of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Itch intensity and unpleasantness

There were significant effects of stimulus on subjective feelings of itch sensation (ANOVA): itch intensity [$F(2,42) = 6.75$, $p = 0.003$] and itch unpleasantness [$F(2,42) =$

4.30, p = 0.02]. Subjective feelings of itch intensity and unpleasantness increased with the increment of histamine concentration (Fig. 1 A and B). The increase of itch intensity during the dual stimuli was significantly lower than that during the intense itching stimulus.

3.2 Brain regions activated by the intense itching stimulus

The significant increases in rCBF during the intense itching stimulus was observed in the left anterior cingulate cortex (ACC) (Brodmann area 24, BA24), the left thalamus, the right anterior parietal cortex (BA 40), the right posterior parietal cortex (BA 7), the bilateral dorsolateral prefrontal cortex (DLPFC) (BA 46) and the right premotor cortex (BA6) (Fig. 2 and Table 2).

3.3. Comparison of brain activity among the conditions

There were significant effects of stimulus on the rCBF change related to itching (ANOVA): The left BA 24 [F(2,42) = 5.89, p = 0.006], the right BA 46 [F(2,42) = 1.53, p = 0.23], the left BA 46 [F(2,42) = 4.86, p = 0.012], the left thalamus [F(2,42) = 6.73, p = 0.003], the right BA 6 [F(2,42) = 5.84, p = 0.006], the right BA7 [F(2,42) = 6.42 p = 0.004] and the right BA 40 [F(2,42) = 3.95, p = 0.026]. The rCBF increased

significantly in the left BA 24, the right BA 7 and the right BA 6 and tended to increase in the left BA 46 ($p = 0.052$) with higher histamine concentration (Fig. 3). The activation in the left BA 24, the left thalamus, the right BA 7, and the left BA 46 significantly attenuated and that in the right BA 6 ($p = 0.071$) tended to decrease in the dual stimuli condition as compared to the intense itching stimulus condition (Fig. 3). On the other hand, the rCBF in the bilateral secondary somatosensory area (S2) (BA 43/ BA 40) and in the right thalamus increased significantly (Fig. 4 and Table 3). In addition, the activity in the midbrain ((x, y, z) = (-16, -10,2), Z = 2.84, P =0.002) tended to increase during the dual stimuli as compared to the intense itching stimulus. The significant rCBF increase during the cold pain stimulus was observed in the right thalamus as compared to the resting ((x, y, z) = (6, -16, 4), Z score = 3.11, P = 0.001), but not observed in the S2 and the midbrain. These findings indicated that the rCBF increase in the right thalamus during the dual stimuli was attributable to the cold pain stimulus on the left foot.

4. Discussion

Several investigators have proposed hypotheses to account for the inhibitory mechanism of itch sensation by cooling in the central nervous system (CNS). However, it has been still unclear whether such a system exists in the human brain or not. On the other hand, the central modulation system for pain has been studied more extensively (Mayer, 1984; Casey et al., 2000; Bantik et al., 2002). PAG is one component of the central pain modulation system. The neural mechanism of itching is similar to that of pain. Both sensory signals are conveyed to the brain via C-fibers and spinothalamic tracts, and activate the same brain regions such as the frontal, parietal and cingulate cortex (Schmelz, 2001; Hsieh et al., 1994). Therefore we have hypothesized that the central inhibitory mechanism of pain by PAG would also work for the inhibition of itch sensation. In the present study, we investigated the itch modulation system in the human brain using positron emission tomography.

4.1. Itch intensity and unpleasantness

Subjective feelings of itch intensity and unpleasantness increased with the increment of histamine concentration, and the itch intensity during the dual stimuli was significantly lower than that during the intense itching stimulus (Fig.1). These results suggested that itch sensation was suppressed by the cold pain stimulus simultaneously

given to the contralateral side of the itching stimulus. These results supported the presence of the itch modulation mechanism in the human brain (Murray et al., 1975).

4.2. ACC, DLPFC, parietal cortex and premotor cortex

The rCBF in the anterior cingulate cortex (ACC), the dorsolateral prefrontal cortex (DLPFC), the posterior parietal cortex and the premotor cortex increased with the increment of histamine concentration (Fig.3). Our results partly supported the report by Drzezga et al. that several brain regions including the anterior cingulate cortex, the frontal cortex, the parietal cortex and the insula had significant correlations to the logarithm of the histamine concentration and itch unpleasantness (Drzezga et al., 2001). We did not observe any changes in the somatosensory areas although the itch intensity ratings correlated to activation of the sensory cortex (Darsow et al., 2000). Drzezga et al. observed the rCBF change in the somatosensory area, but Hsieh et al. did not. There could be several interpretations for this discrepancy because the PET studies on itching, including the present study, had substantial methodological differences. For example, Hsieh et al. performed intracutaneous injection of histamine, Drzezga et al. and Darsow et al. elicited itching by skin prick test and the present study by iontophoresis. The most probable reason, why Darsow and Drzezga observed rCBF changes in the

somatosensory area and Hsieh and the present authors did not, might be associated with the histamine concentration which was 0.01% in the present study. Hsieh et al. used 10 μg/ml, corresponding to 0.01 %, of histamine. It was over 0.03 % in the reports of Darsow et al. and Drzezga et al. The concentration of histamine of 0.01 % would be too weak to observe the significant rCBF increase in the somatosensory area.

ACC is involved in nociceptive processing (Vaccarino et al., 1989). The role of ACC in pain is thought to be the affective-evaluation dimension (Vogt et al., 1993). ACC is functionally divided in two regions. The rostral part of ACC (rACC) is related to cognitive division and the caudal part of ACC (cACC) is emotional division (Bush et al., 2000). Both rACC and cACC are activated by noxious stimulus (Derbyshire et al., 1998). In our experiments, the activated areas in the ACC were mostly localized to rACC associated with cognitive division of itching (Fig.2).

It was reported that dispersed attention from pain suppresses the pain sensation. In such conditions, pain-related activations of rACC and cACC decreased and increased, respectively (Frankenstein et al., 2001). In line with the pain modulation, the rACC responsed during the itching sensation were significantly attenuated by simultaneous stimulation of cold pain as shown in Fig.3. It was also demonstrated that the activity in rACC reflected the subjective evaluation of pain (Rainville et al., 1997). Therefore, the

decreased activation of the rACC observed in the present study might be related to the diminished subjective feelings of itch intensity.

The DLPFC, the premotor cortex and the posterior parietal cortex are frequently observed in experiments involving attention, working memory, and goal-directed processes (Casey et al., 1998; Corbetta et al., 1993; Coull et al., 1998; Fink et al., 1997; Gitelman et al., 1996; Klingberg, 1998; Lewin et al., 1996; Nobre et al., 1997). The DLPFC and the premotor cortex are mainly associated with motor planning and programming, and the posterior parietal cortex processes spatial cognition and attention with movement (Corbetta et al., 1993). It is proposed that the DLPFC and the premotor cortex are related to the motor reactions for withdrawal or avoidance from pain and that posterior parietal cortex is associated with spatial cognition of the body (Ingvar, 1999). The activation of the DLPFC, the premotor cortex and the parietal cortex observed in the present study might reflect the organization of the motor response to itch, such as scratching. Their decreased activities in the dual stimuli condition would reflect the attenuation of the desire to scratch.

4.3. Thalamus

We observed significant activation of the left thalamus during the strong sensation of

itching when compared to the control, although it was reported that itching did not activate the thalamus significantly (Drzezga et al., 2001) (Fig. 2 and Table 2). The conceivable explanation for the difference between our study and the previous studies was the methodology. In the previous studies reported by Drzezga et al. and Darsow et al., the itching sensation was elicited by a skin prick test, in which, histamine was slowly infiltrated into the skin slightly injured by a puncture (Pepys, 1975). Hsieh et al. elicited itch sensation by the intracutaneous injection of histamine. The area to be directly stimulated was pinpoint in the previous studies. On the other hand, we used the histamine iontophoresis to elicit an itching sensation. This improved method is often used for eliciting itch sensation in clinical studies (Darsow et al., 1996; Schmelz et al., 1997). Here, histamine was electrically injected into the skin and the area to be directly stimulated was 4 cm^2, much larger than that in the previous studies. Thus, the significant rCBF increase of the left thalamus was observed in the present study, but not in the previous studies. Since the thalamic activation is important in pain and itch perception, reproducibility should be examined in future investigations.

The significant rCBF increase was observed in the right thalamus during the simultaneous stimulation of itching and cold pain as compared to itching stimulus alone (Fig. 4). The right thalamus was also activated in the cold pain as compared to the rest

((x, y, z) = (6, -16, 4), Z score = 3.11, P = 0.001) while it was not observed in the intense itching stimulus condition (Fig. 2). It has been found that the thalamus is activated by pain stimulation (Tracey et al., 2000). Thus it was suggested that the activation of the right thalamus in the dual stimuli condition was attributable to the cold pain stimulus on the left foot.

4.4. Midbrain

Midbrain including the periaqueductal gray matter (PAG) was activated during the dual stimuli as compared to the intense itching stimulus alone as shown in Fig.4. The midbrain did not show even any tendency toward increased rCBF in the cold pain stimulus condition or in the intense itching stimulus condition. The findings indicated that the midbrain was activated in the presence of both itching and cold pain, but not in the presence of single modality of itching or cold pain. PAG is known as the central pain modulation system. In pharmacological studies, PAG is thought to be one of the targets for analgesia. Microinjection of morphine, an opioid receptor agonist, into the midbrain reduces pain sensation (Manning et al., 1998). It was also reported that electrical stimulation of PAG attenuated pain (Fields, 2000). PAG neurons project axons down to the dorsal horns of the spinal cord via medulla and raphe nuclei, where they suppress

the activity of nociceptive neurons (Mayer, 1984). Interestingly, in the animal study, it was demonstrated that spinal neuronal responses to histamine were markedly suppressed by electrical stimulation to the midbrain PAG (Carstens, 1997). Andrew et al. reported that spinothalamic tract (STT) neurons responded to histamine with the same temporal profile as the activity in histamine selective C-fibers and as the corresponding perception of itch (Andrew et al., 2001), suggesting that the decreased activity in the STT neurons reported by Carstens et al. would be related to the inhibition of itch sensation. In views of the previous reports, it was suggested that the activation of PAG was associated with the attenuation of the itch intensity and of the itch related-brain activity during the dual stimulations of itching and cold pain. Our results supported the hypothesis that the descending inhibitory mechanism of PAG for pain would also work for itch modulation.

4.5. Secondary somatosensory cortex

The bilateral secondary somatosenosry cortex (S2) was significantly activated during the dual stimuli. S2 did not manifest rCBF increase in the cold pain stimulus condition as compared to the rest. The rCBF increases in S2 in the intense itching condition were not observed at any threshold of p value. Our results were consistent with the previous

report that the S2 did not respond to histamine stimuli at any concentrations (Drzezga et al., 2001). Therefore it was suggested that S2 was not involved in the central processing of itch. Our result could not explain why the significant increases of rCBFs in the bilateral S2 were observed in the dual stimuli condition only. One reason for this would be that dual stimulation of cold pain and intense itching might alter the activity of S2. Further investigation would be needed to explain the activation of S2 in the dual stimuli condition.

5. Conclusion

We examined the neural correlates of the itching sensation using $H_2{}^{15}O$-PET and histamine iontophoresis. The subjective feelings of itching were accompanied by significant rCBF increase in the left anterior cingulate cortex (BA24), the left thalamus, the right anterior parietal cortex (BA40), the right posterior parietal cortex (BA7), the bilateral dorsolateral prefrontal cortex (BA46) and the right premotor cortex (BA6). The dual stimulation with cold pain and itching resulted in significant reduction in the rCBF increase in these areas and in the subjective feelings. We observed the rCBF increase in the midbrain including the periaqueductal gray matter (PAG) (known as the pain

modulation system) in the itch-modulating conditions. These findings indicate that the activation of PAG and accompanying deactivation of the cortico-subcortical network might be associated with the itch modulation in the CNS. Further investigation would be needed to explain the activation in S2 and thalamus.

The whole findings indicated that the activation of the PAG and the decreased brain activity related to itching would be associated with the reduction of itch sensation by cooling.

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Fig. 1. The increases in subjective feelings of itch intensity (A) and unpleasantness (B) (mean and SD) in the mild itching stimulus (MiI), the intense itching stimulus (InI) and the dual stimulations of intense itching and cold pain (IP) conditions in comparison to the saline stimulus condition are shown. *: $p < 0.05$ by ANOVA and post-hoc multiple comparison (Tukey).

Fig. 2. Areas of significant rCBF increase during the intense itching stimulus as compared to the saline stimulus (uncorrected p value < 0.001). Red arrow shows the left thalamus on a transaxial slice of the PET template.

Fig. 3. The change in rCBF (mean and SD) from the baseline (saline stimulus) in each brain region related to itching. Abbreviations: MiI = mild itching stimulus, InI = intense itching stimulus, IP = dual stimulations of intense itching and cold pain, L = left hemisphere and R = right hemisphere. *: $p < 0.05$ by ANOVA and post-hoc multiple comparison (Tukey).

Fig. 4. Areas of rCBF increase during the dual stimuli as compared to the intense itching stimulus (uncorrected p value < 0.005). Red arrow shows midbrain on a

transaxial slice of the MRI template.

Table 1
Conditions and stimulations

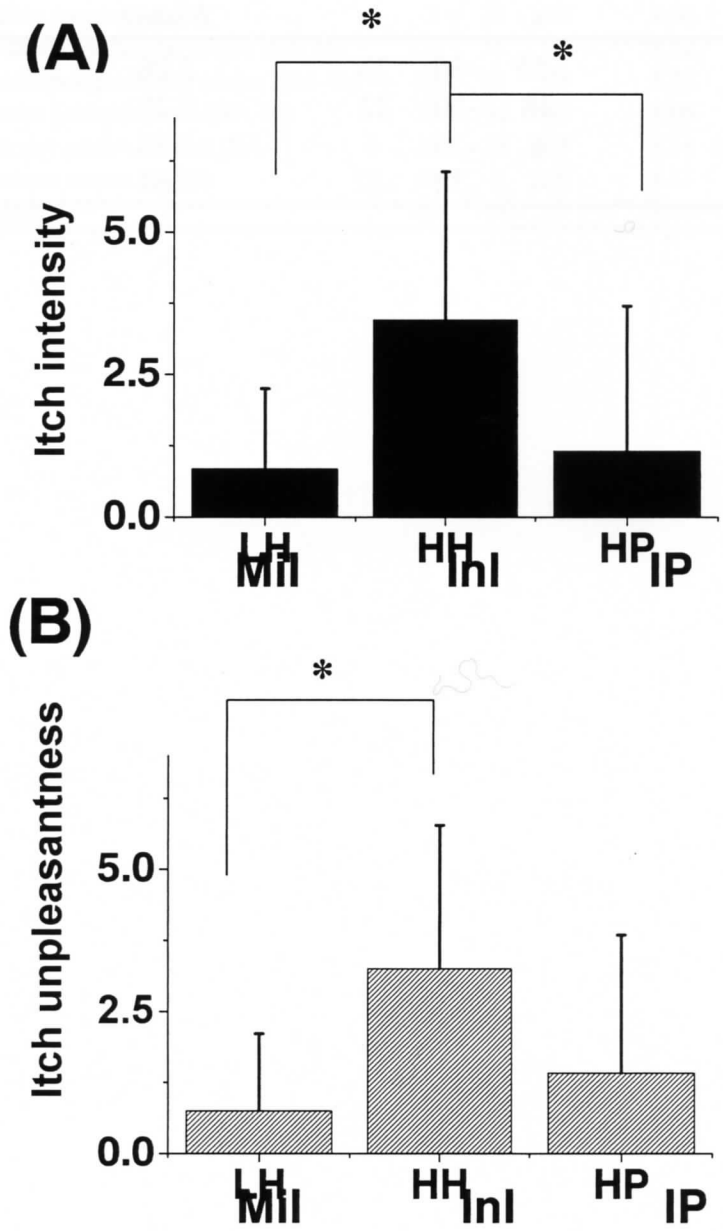
Conditions	Stimulation on the right foot by iontophoresis		Cold pain stimulus on the left foot	
	Charge (mC)	Concentration of histamine (%)	Duration (min)	Tempareture (°C)
Saline (a control for itching stimuli)	120 (1mA x 120 sec)	0		
Mild itching	120 (1mA x 120 sec)	0.001		
Intense itching	120 (1mA x 120 sec)	0.01		
Dual stimuli (intense itching and cold pain)	120 (1mA x 120 sec)	0.01	2	5
Rest (a control for cold pain stimulus)				
Cold pain			2	5

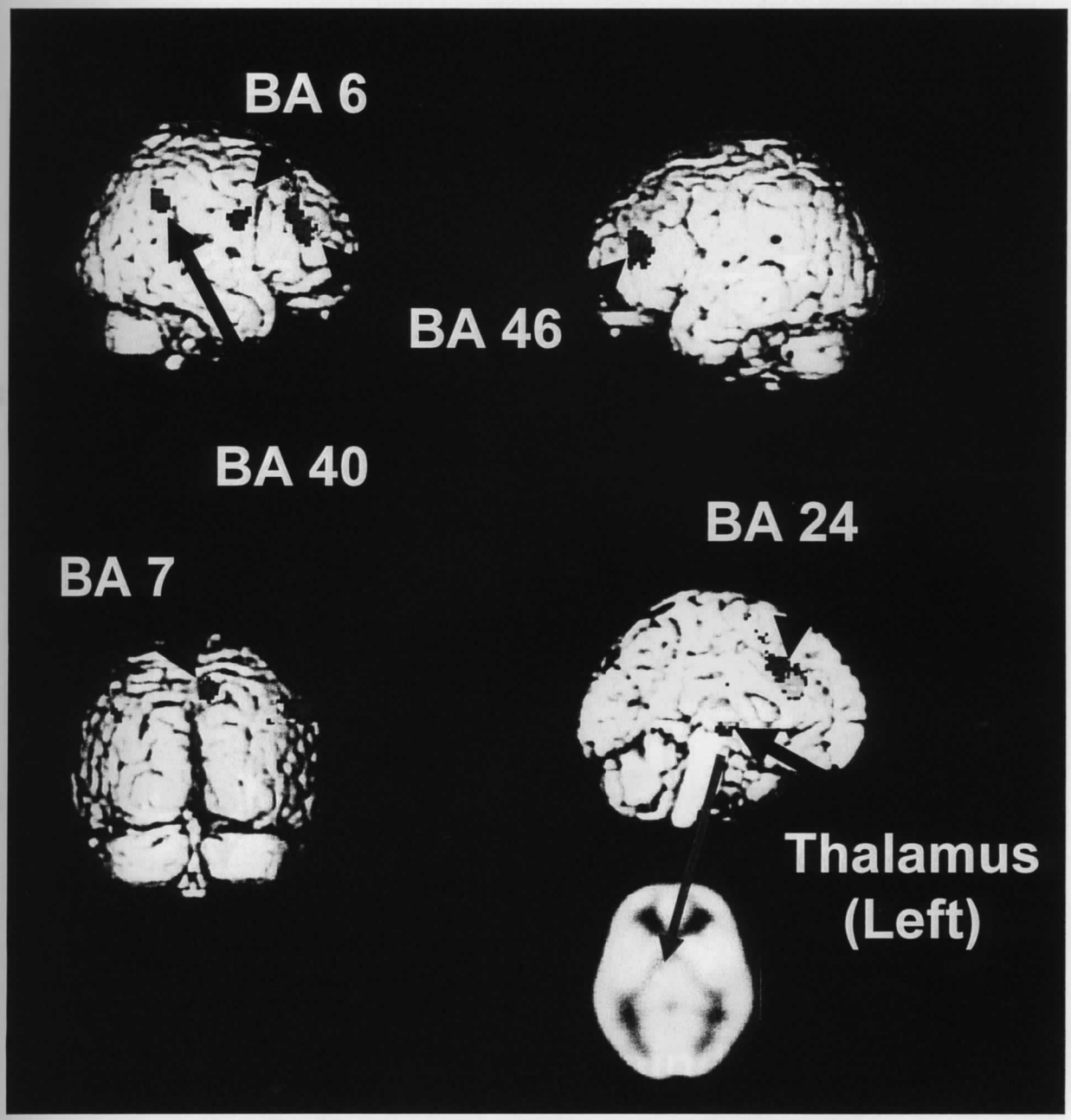
Table 2
Brain regions significanlty activated during the intense itching stimulus as compared to the saline

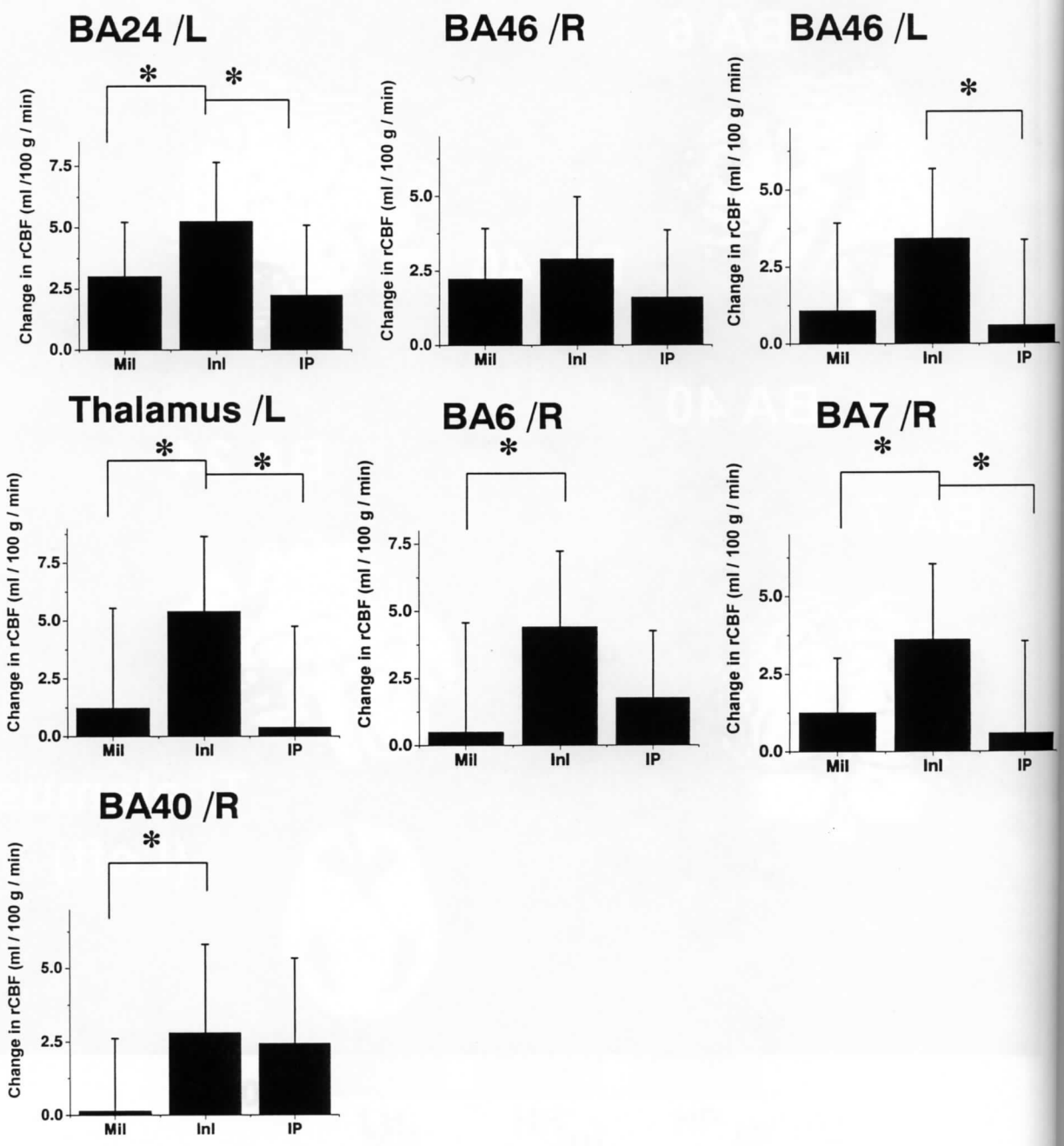
Brain regions	x	y	z	Z score
Left cingulate cortex (BA 24)	-2	28	28	4.00
Left dorsolateral prefrontal cortex (BA 46)	-30	34	24	4.02
Right dorsolateral prefrontal cortex (BA 46)	32	32	28	3.39
Right anterior parietal cortex (BA 40)	33	-48	40	3.08
Right posterior parietal cortex (BA 7)	10	-78	54	3.14
Right premotor cortex (BA 6)	48	-2	26	3.11
Left thalamus	-10	-16	2	3.98

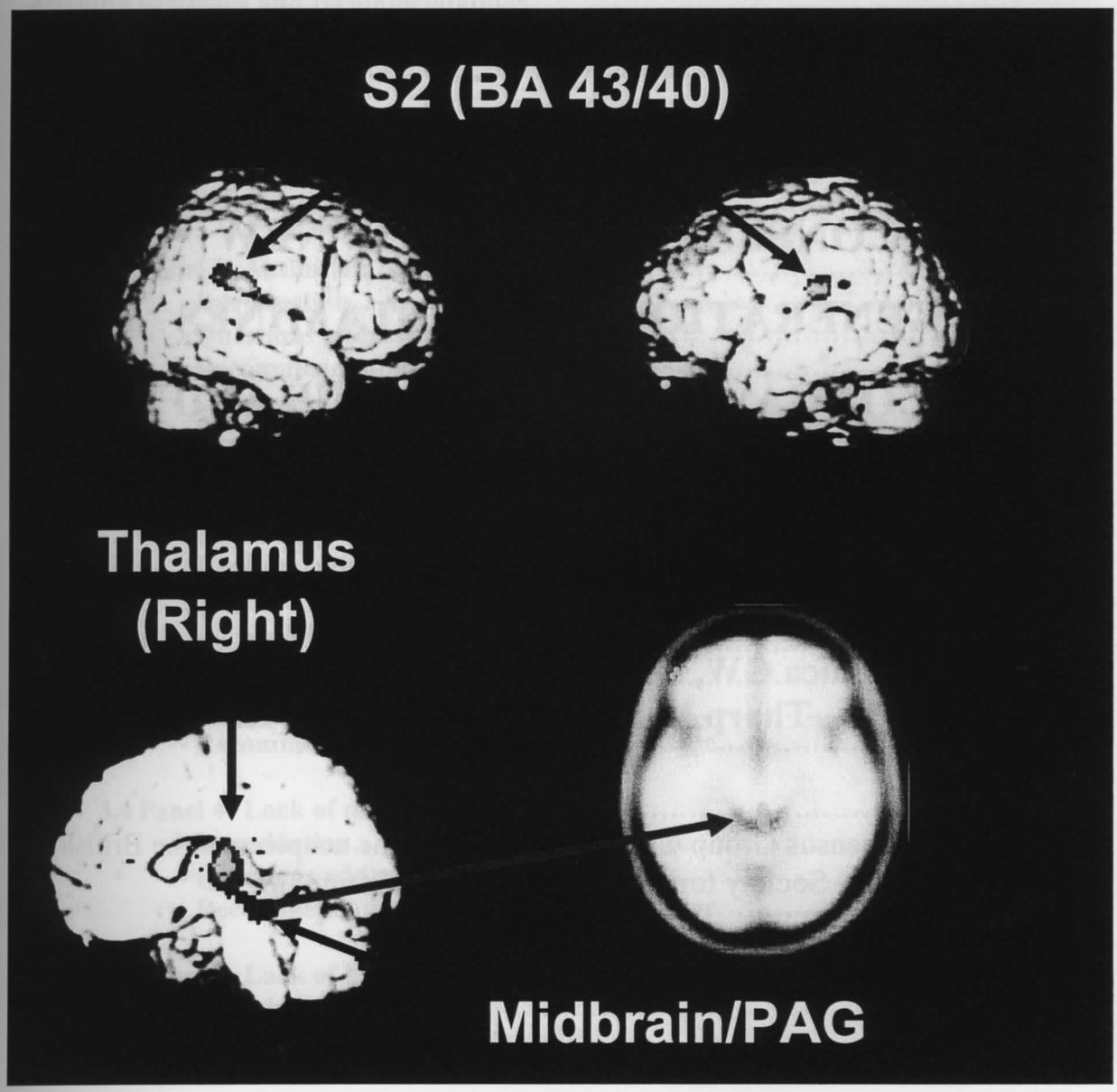
Table 3
The rCBF increase in the dual stimuli condition as compared to the intense ithcing stimulus condition

Brain regions	x	y	z	Z score
Left S2	-55	-30	20	3.08
Right S2	48	-30	22	3.28
Right thalamus	6	-16	4	3.56
Midbrain	4	-32	-12	2.84


(A)
*
*
Itch intensity
5.0
2.5
0.0
LH
Mil
HH
InI
HP
IP
(B)
*
Itch unpleasantness
5.0
2.5
0.0
LH
Mil
HH
InI
HP
IP


BA 6
BA 46
BA 40
BA 7
BA 24
Thalamus
(Left)


BA24 /L
BA46 /R
BA46 /L
Thalamus /L
BA6 /R
BA7 /R
BA40 /R
Change in rCBF (ml /100 g / min)
Change in rCBF (ml / 100 g / min)
Mil
Inl
IP
*


S2 (BA 43/40)
Thalamus
(Right)
Midbrain/PAG

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