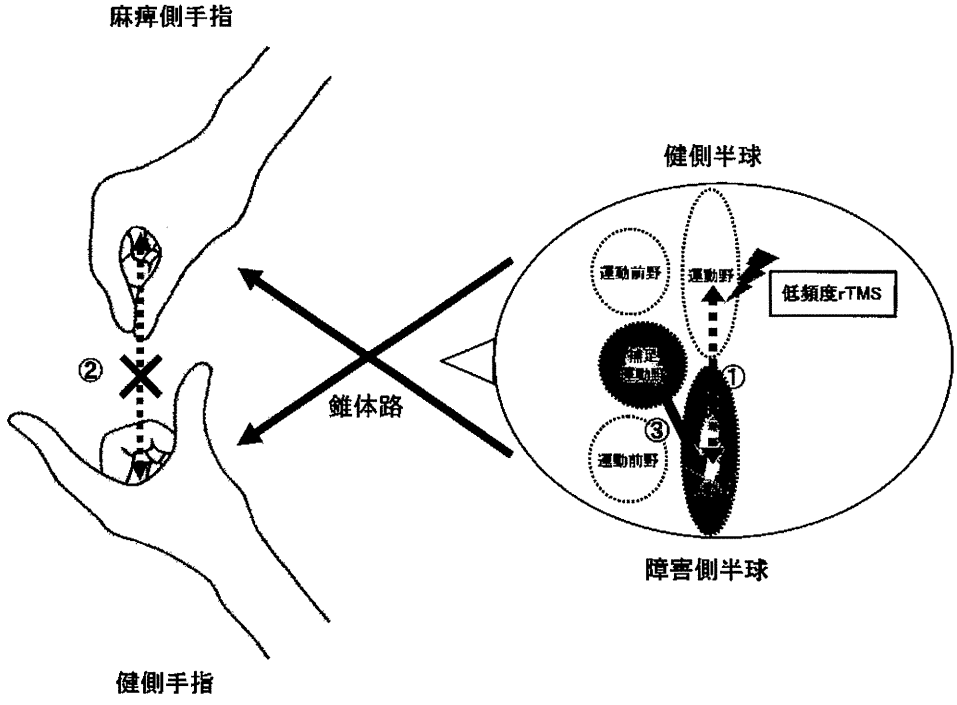

図7　rTMS後の両側運動機能変化メカニズム

①健側半球運動野へ低頻度rTMSを行うと，両側半球間の機能結合が減少し，②両側手指の協調性が低下する．③補足運動野と障害側運動野の機能結合が強い場合は両側協調性低下が起こりにくい．

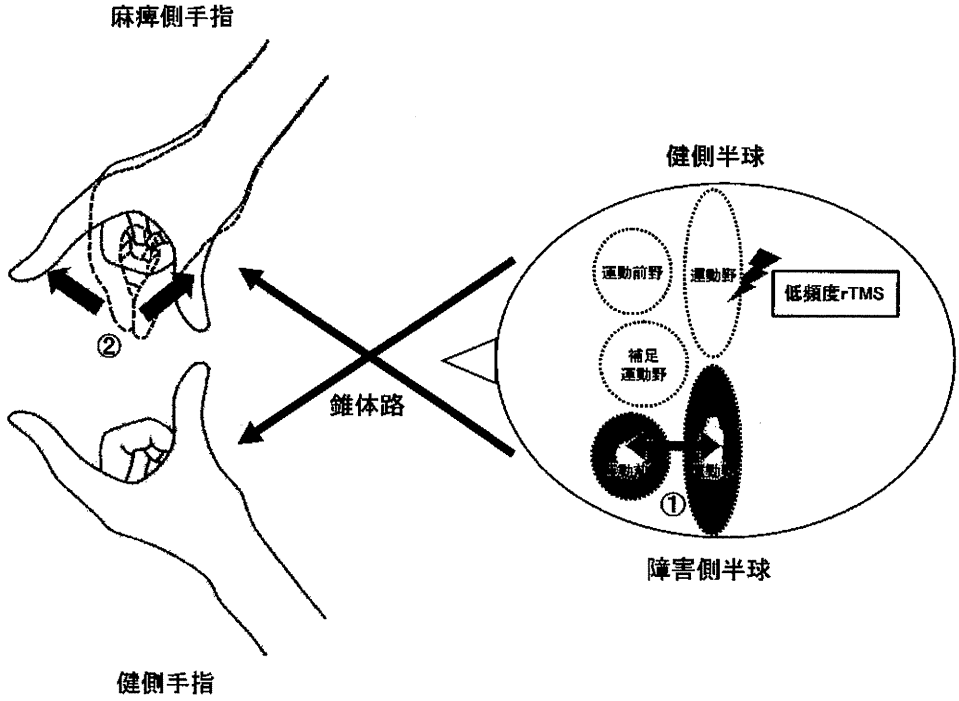

図8　rTMS後の麻痺側機能改善メカニズム

①障害側半球運動前野と障害側半球運動野の機能結合が，②健側半球運動野への低頻度rTMS後における麻痺側手指機能の改善に関与している．

スク脳波関連コヒーレンスの関係も含め，対照群を含めた詳細な検討が今後必要と思われる．また健常者の運動野に低頻度rTMSを行い両側運動を検討した報告はなく，健常人と脳卒中患者におけるrTMS後の両側運動機能変化を比較検討することにより，脳卒中後の可塑性および運動メカニズムの新たな研究につながる可能性がある．

過去の報告と同様に補足運動野，運動前野の部位は脳波電極を基準に解析を行ったが[19,23-26]，これらの運動関連領域の役割の理解をさらに深めるためには，脳画像の活用および脳波電極数を増やすことなどによって運動関連領域の正確な位置を同定し解析を行う必要があると考えられる．また運動機能の評価としてメトローム音からのタッピング運動開始時間を計測し，rTMSが両側協調度や麻痺側及び健側運動機能へ与える影響を詳細に検討することが今後必要と思われる．

本研究は両側手指のタッピング運動が実施できる麻痺の軽い症例を対象とした．脳卒中後における機能画像の研究から，機能障害が強い脳卒中患者は麻痺側運動に健側半球が関与していることが報告されている[36]．麻痺が重度の症例でも健側運動野への低頻度rTMSによって麻痺が改善した報告を認めてはいるが[37]，麻痺の強い患者に抑制作用を持つ低頻度rTMSを健側運動野に行うことにより麻痺側運動機能が悪化する可能性を本研究から否定することはできない．また脳梁抑制は麻痺の重症度により強さが異なることが報告されているため[3,38]，麻痺の程度に応じてrTMS後の両側運動協調性の反応が異なる可能性がある．そのため脳卒中患者全体におけるrTMSの麻痺側機能および両側運動に与える影響を結論づけるためには，機能障害が強い症例を含めた研究が今後必要と思われる．

rTMS直後に認めた両側協調度の低下はrTMS 30分後には両側半球間の脳波コヒーレンスと共に正常化したが，rTMSを継続的に実施する報告も散見するため[9]，rTMSを継続的に実施することにより両側運動協調性の低下が持続する危険性がある．本研究のタスク関連脳波コヒーレンス解析から補足運動野－障害側運動野がrTMS後の両側運動協調度低下に対し代償作用を持つ可能性があるため，興奮性作用を持つ高頻度rTMSまたは陽極直流経頭蓋電気刺激などを障害側運動野または補足運動野に行い興奮性を増大させる方法が，健側運動野への低頻度rTMSによる両側協調性低下を防止できるか検討が必要と考えられる．

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■原著

皮膚冷刺激が脳卒中症例における下肢運動訓練に与える影響

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要旨　脳卒中症例の筋力訓練に対する皮膚冷刺激の効果を検討するため，脳卒中入院症例9例（男7例，女2例）に対し，大腿四頭筋部に皮膚冷刺激を加え，10 m歩行時間および下肢伸展モーメントを評価した．全症例における検討では非刺激値との間に有意差はなかったが，65歳未満では非刺激時に比し，皮膚冷刺激時に有意の膝伸展モーメントの増強が認められた．冷刺激付加は脳卒中症例における筋力訓練による筋力の改善を促進する可能性があることが示唆された．

Abstract　To clarify the effects of cold skin stimulation on physical exercise for cerebral stroke patients, we compared muscle leg strength and walking ability in cerebral stroke subjects with cold skin stimulation to those without cold skin stimulation. For nine stroke patients (7 males and 2 females) who were able to gait without cane, we evaluated maximal extension torque of leg muscle on affect side and 10-m-gait-time with cold skin stimulation at their anterior thigh and control condition (no cold skin stimulation). As a result, maximal extension torque with cold stimulation was significantly higher than that without cold stimulation in the subjects less than 65 years old, although we could not find significant differences in maximal extension torque or 10-m-gait-time between all subjects with and without cold stimulation. These findings suggest that low-intensity muscle training with cold skin stimulation is useful for relatively young cerebral stroke patients.

Key words：脳卒中片麻痺（hemiplegic stroke），皮膚冷刺激（cold skin stimulation），筋力訓練（muscular exercise）

はじめに

骨格筋における筋線維タイプの加齢性変化はタイプⅡ（速筋）線維が大きいことが知られている．一方，持続筋であり姿勢を保つなど疲労しにくい特徴を有するタイプⅠ線維（遅筋）は高齢になっても比較的保たれる傾向にあるとされている．歩行の俊敏性や歩行バランス，転倒回避のための下肢筋活動などはタイプⅡ線維の動員がより多くかかわっており，高齢者ではその動員低下が転倒，骨折リスクの重要な一つの因子となっている[12]．

The effects of cold skin stimulation on leg muscular exercise for stroke patients

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表1　症例の内訳

	年齢	性別	脳卒中のタイプ	Br. stage	高次脳機能障害
1	59歳	男性	脳幹梗塞	下肢Ⅵ	軽度注意障害
2	63歳	男性	脳内出血	下肢Ⅵ	軽度注意障害
3	51歳	男性	くも膜下出血	下肢Ⅵ	軽度注意・記憶障害
4	61歳	男性	脳梗塞	下肢Ⅴ	(－)
5	80歳	男性	脳梗塞	下肢Ⅵ	(－)
6	83歳	女性	脳幹梗塞	下肢Ⅴ	軽度注意・記憶障害他
7	49歳	男性	脳内出血	下肢Ⅴ	(－)
8	81歳	女性	くも膜下出血	下肢Ⅵ	軽度注意障害
9	38歳	男性	くも膜下出血	下肢Ⅵ	軽度注意・記憶障害他

そのため，高齢者にはタイプⅡ線維動員の訓練がより必要なるものと考えられる．しかし，タイプⅡ線維に対する通常の運動訓練では最大随意筋収縮の60～80%程度の高いトレーニング負荷が必要であり，高齢者では骨関節損傷や筋損傷などが危惧され，実際の診療現場では実施にさまざまな困難を伴う．さらに内部障害やそれらを合併しやすい脳卒中後の高齢者では心循環イベント発症のリスクも伴う．

近年，筋力訓練中に皮膚冷刺激を付加するとタイプⅡ筋線維の閾値低下が起こり，低負荷トレーニングにおいても高負荷トレーニングと同等の筋力増強効果が得られるとの報告がある[11]．また，筋力訓練中の皮膚冷刺激は高齢者においても有効であるとの報告もなされている[12]．しかしながら，脳卒中症例に対し低負荷トレーニングにおける皮膚冷刺激の効果を検討した研究は報告されていない．本研究の目的は回復期リハビリテーション中の脳卒中症例に対する運動訓練における皮膚冷刺激の効果を明らかにすることである．

対象および方法

対象は回復期リハビリテーション病棟に入院している脳卒中症例9例（男性7例，女性2例）である．これら対象の内訳は脳梗塞4例（脳幹梗塞2例を含む），脳出血2例，くも膜下出血3例であり，平均年齢は62.8歳（38～83歳），下肢Brunnstrom stageがⅤ 3例，Ⅵ 6例であり，全例独歩が可能であった（表1）．また全例，心循環器系の重篤な合併症はなく，糖尿病や高血圧などを有する例はそのコントロールも良好であった．また，運動器疾患などで強い関節痛を有するものは対象から除外した．神経心理学的評価において重篤な認知低下はなく，実験の目的ならびに方法を理解できた症例を対象とした．なお，調査に先立ち，インフォームドコンセントを得た．

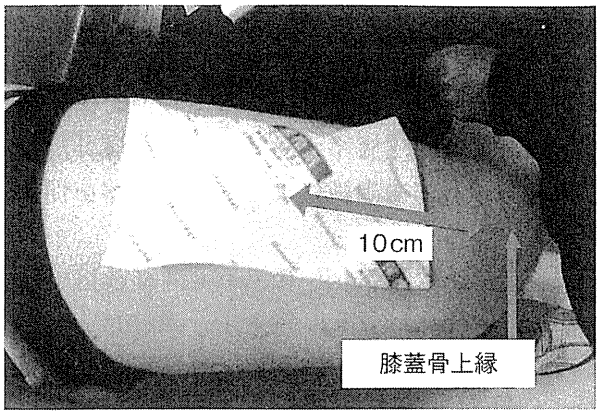

図1　大腿部前下面に施行した皮膚冷刺激（アイスパック）．アイスパック（12×8 cm）の中心が膝蓋骨上縁正中から10 cm近位に来るように設定した．

以上の調査対象に対しその患側の大腿部前

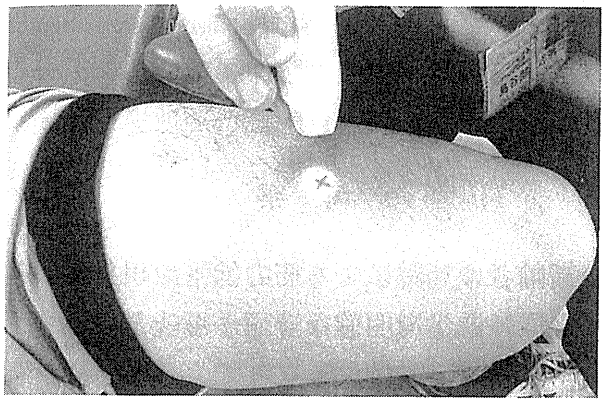

図2　皮膚温の測定
赤外線サーモメーターを用いて目標皮膚温23℃になるまで冷却した．

図3　ストレングスエルゴによる最大下肢伸展筋トルク測定の様子．骨盤をシートベルトに固定，測定脚をサドルに固定する．背もたれの角度，リフトの高さ，膝屈曲角度はアームが斜め45°の位置で，実際の膝関節が30°に屈曲するように調節，設定した．

面下部にアイスパック（アイスノン：12×8 cm）の中心が膝蓋骨上縁正中から10 cm近位に位置するように装着することにより皮膚冷刺激を与えた（図1）．その後，皮膚赤外線体温計（日本テクニメッド社サーモフォーカスプロ，図2）を用いて冷却部の皮膚温が23℃になるように皮膚および皮膚周辺を冷却した．23℃に達したら，最大下肢伸展モーメント（伸展筋トルク値）および10 m歩行時間を測定した．さらに同一被験者に対し，常温である33～34℃下で同様の方法で最大下肢伸展モーメント（伸展筋トルク値）

表2　10 m歩行時間および下肢伸展筋トルクにおける冷刺激と非冷刺激の比較

	10 m歩行時間 (sec.)		下肢伸展筋トルク値 (Nm)	
	冷刺激	非冷刺激	冷刺激	非冷刺激
1	5.43	5.40	1.083	1.167
2	5.39	5.93	1.020	0.900
3	4.08	4.92	0.770	0.580
4	8.77	8.65	1.135	0.955
5	5.47	4.98	1.160	1.171
6	8.36	8.74	0.395	0.501
7	7.98	7.41	0.985	0.820
8	8.29	7.97	0.350	0.350
9	3.86	4.37	1.200	0.905

および10 m歩行時間を測定した．最大下肢伸展筋トルク測定にはストレングスエルゴ（三菱運動療法システム—ストレングスエルゴ240）を使用し下記の方法で行った．つまり，筋トルク測定にあたっては体幹・骨盤をシートベルトに固定，測定脚をエルゴのサドルに固定し非測定脚は足底全体を機械フロアに全面接触させ，背もたれの角度，リフトの高さ，膝屈曲角度は先行研究による諸家の方法に準じ膝屈曲角はアームが斜め45°の位置で，実際の膝関節が30°に屈曲するように調節，設定した（図3）．測定回数にあたっては冷刺激，非冷刺激ともに最大下肢伸展モーメント計2回（1日1回，2日連続），10 m歩行時間は計6回（1日3回，2日連続）行った．各セクションの測定順序は無作為に行い，疲労の影響を避けるために十分なセクション間の休息時間（10分以上）を取りつつ各測定を施行した．得られた被験者の測定値群から平均値を算出し，おのおのの最大下肢伸展モーメント，10 m歩行時間とした．また年齢による影響を検証するために，最近日本で発表された最も症例数が多い転倒骨折患者の分析の報告[4]を参考に，65歳で2群に分けてそれぞれの測定値を比較した．統計処

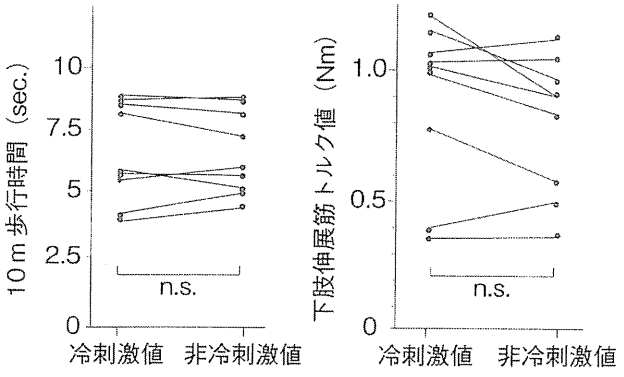

図4　10 m 歩行時間と下肢伸展筋トルクにおける冷刺激と非冷刺激の比較．10 m 歩行時間と最大下肢伸展モーメントにおいて冷刺激の効果を認めなかった．

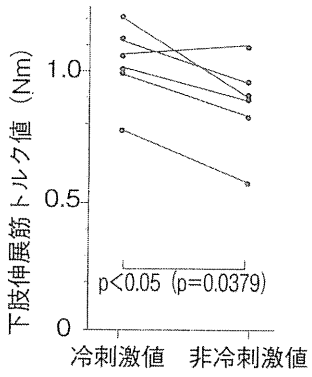

図5　年齢における冷刺激と非冷刺激の比較
65 歳未満の患者において皮膚冷刺激は最大下肢伸展モーメントを有意に増加させた．

理の方法は paired t-test（Excel 統計 ver. 6）を用い，有意水準を 5%未満とした．

結　果

各被験者の冷刺激，非冷刺激おのおのの 10 m 歩行時間および最大下肢伸展筋モーメントを表 2 に示した．10 m 歩行時間に関しては冷刺激の有無は有意の効果を認めなかった（図 4）．また，最大下肢伸展モーメントにおいても冷刺激の有無は有意差を認めなかった（図 4）．対象を比較的若年の 65 歳未満と 65 歳以上の被験者に分けて検討したところ，10 m 歩行時間は冷刺激の有無は有意の効果を認めなかったものの，最大下肢伸展モーメントにおいて 65 歳未満の症例では皮膚冷刺激付加は最大下肢伸展モーメントを有意に増加させていた（図 5）．

考　察

高齢者の加齢による筋力低下の現象は最近の画像の進歩で明確な評価が可能となっており，また組織学的検討などでもその特徴，傾向が報告されている．Lexell[7,8] らは大腿外側広筋の加齢性変化を組織学的に研究しており，それによると筋断面積，筋容積ともに 24 歳で最高値を示しており，以降経年的に低下し特に 60 歳台以降高い減少率を示したと報告している．さらに大腿外側広筋の筋線維数における加齢性変化も調査しており，同様に 24 歳でピークを迎え，高齢になるに従ってその減少率が加速する．また加齢による筋線維タイプ別の変化の検討では，タイプ別での線維数の比率に差違は認められないものの，高齢になるに従ってタイプⅡ線維の萎縮が著明になると報告している．Larsson[6] らも同じく大腿外側広筋を使って若年者と高齢者のタイプⅡ線維の直径による萎縮度を調査し，やはり高齢者ではタイプⅡ線維の萎縮が顕著であったと述べている．タイプⅡ線維，特にタイプⅡb 線維は速筋であり，素早い動作や歩行バランス，転倒回避動作に重要な意味をもっていると考えられる．したがって先にも述べた高齢者の転倒リスクの増加はこのタイプⅡb 線維の動員減少が重要な因子となっている．

近年，高齢者の筋力低下は不可逆的なものではなく，運動訓練によって回復することが報告されている[5,12]．しかし，高齢者への高負荷トレーニングの日常診療への応用は筋組織などの軟部組織損傷や骨関節疾患の悪化，循環系，特に虚血性心疾患などの点より，多

くの困難性を有する．また，従来型の低負荷トレーニングではその筋力増強効果は高いといえず，長期間の訓練を必要とするため，モチベーションの維持はきわめて困難なことが予想される．

高齢者の運動筋のタイプⅡb線維を効率良く動因する方法としては加圧筋力訓練[10]，振動刺激を用いた筋力訓練[1]，皮膚冷刺激を付加した筋力訓練などが試みられている．これらの訓練のなかで皮膚冷刺激は安価で簡便であり，至適温度25℃で最も効果が期待できる非常に安全性にも優れた方法である．その機序については，皮膚の局所的な冷却で冷受容器が刺激され，多シナプス結合による運動単位の活動様式が変化を受け[2]，25℃で最もタイプⅡb線維で動員閾値が下がるとされている．

今回の研究では全例を対象にした大腿四頭筋部の皮膚冷刺激の有無は10 m歩行時間および下肢伸展トルクとも有意の効果を認めることはできなかった．しかし，65歳未満の対象では，少なくとも最大下肢伸展筋トルク値で明らかに皮膚冷刺激付加群が有意差をもって高値であった．これは比較的若年者の場合，十分な運動ニューロン数が活動でき，さらに皮膚冷刺激が冷受容器を介して高閾値運動単位（タイプⅡb線維の動員）の選択的促通が容易に引き出せた可能性がある．すなわち筋トルク値測定中の運動神経プールの迅速な興奮や高閾値運動単位の高い同期性などが関与した可能性がある[9]．一方，高齢者の筋ではタイプⅡb線維の萎縮とともに運動神経プールの興奮性低下，高閾値運動単位の同期性低下が起こっていると考えられる．もし皮膚冷刺激でそれらを比較的早期に改善し筋力増加要因の改善を引き起こすことができれば，より高齢の脳卒中症例でも筋力増強訓練に応用できる可能性があろう．

本研究はあくまでも実験的なものであり，臨床に応用するにはいくつかの問題点がある．一つは実際の訓練にあたって冷刺激の有効温度を持続する工夫が必要な点，もう一つは今回Brunnstrom病期 stage Ⅴ，Ⅵの独歩可能な脳卒中症例を対象としたが，重症度，選択筋，発症からの期間などにより主に活動する筋線維タイプが異なる可能性があり[3]，今後，より重篤な麻痺を有する症例や脳卒中発症からの期間の影響などの検討も行う必要がある．

結　語

脳卒中症例の下肢運動訓練における皮膚冷刺激付加の効果を調べるために，9例の脳卒中患者に対し大腿部前面下部での皮膚冷刺激および非刺激での患側下肢伸展モーメントと10 m歩行時間を比較した．その結果65歳未満の被験者では皮膚冷刺激付加群において最大下肢伸展モーメントが有意差をもって増加した．今後は必要な有効温度の持続方法，より麻痺が重度な症例での効果の有無，発症からの期間の影響などを検討する必要があり，それらにより皮膚冷刺激付加による低負荷筋力増強訓練の臨床応用が可能になると思われる．また，より高齢な患者についても継続した研究が必要である．

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Painful muscle stimulation preferentially activates emotion-related brain regions compared to painful skin stimulation

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ABSTRACT

Skin pain and muscle pain are categorically distinct from each other. While skin pain is a sharp, spatially localized sensation, muscle pain is a dull, poorly localized and more unpleasant one. We hypothesized that there are specific brain regions preferentially activated by muscle pain compared to skin pain. To test this hypothesis, brain responses were recorded from 13 normal male subjects in response to repeated painful electrical stimulation of the muscle and skin of the left leg, using 3-T magnetic resonance imaging scanner. The common brain regions that responded to painful stimulations of both skin and muscle were the thalamus, anterior cingulate cortex, bilateral insula, contralateral primary and secondary somatosensory cortices, and ipsilateral cerebellum. Brain regions specifically activated by muscle stimulation were the midbrain, bilateral amygdala, caudate, orbitofrontal cortex, hippocampus, parahippocampus and superior temporal pole, most of which are related to emotion. Regions except the midbrain showed contralateral preference. These results suggest that dull sensation, which is characteristic of muscular pain, is related with processing in these brain regions.

1. Introduction

Muscle pain, such as shoulder pain and low back pain, are common clinical problems which impair the quality of patient's life. Although actual prevalence of musculoskeletal pain is not clear, it is suggested that such pain is common not only among adults, but also among the adolescent population (McBeth and Jones, 2007). In Japan, 21.4 million people, which is 24.3% of the population aged 30 years or older, were estimated to have low back pain in 2005 (Suka and Yoshida, 2009), and 9.1 million (9% of the total population) were estimated to have musculoskeletal pain that interferes with daily life (Suka and Yoshida, 2005). As often discussed, skin pain and muscle pain are categorically distinct from each other (Henderson et al., 2006; Kupers et al., 2004; Niddam et al., 2002; Schreckenberger et al., 2005; Svensson et al., 1997a): While skin pain is often described as sharp and spatially localized sensation, muscle pain is usually dull, poorly localized and more unpleasant than cutaneous pain (Ikemoto et al., 2006). These distinct characteristics easily lead us to hypothesize that corresponding brain activities should be in some respect different between muscle and skin pain.

Earlier studies on the central mechanism of pain have predominantly dealt with skin pain using contact thermode (Peyron et al., 2000). Against this background, several researchers have laid stress upon the necessity of studies on the central mechanism of the muscle pain (Henderson et al., 2006; Kupers et al., 2004; Niddam et al., 2002; Schreckenberger et al., 2005; Svensson et al., 1997b). Although little difference has been reported between the brain activity responsible for muscle pain and that for skin pain in earlier studies (Svensson et al., 1997b), recent studies are revealing such differences. Niddam et al. (2002) and Schreckenberger et al. (2005), for example, have reported increased neural activities in response to painful muscle stimulation at inferior/middle frontal gyrus, with electric stimulation and with acidic buffer injection, respectively. Activity at the caudate nucleus, a part of the basal ganglia known to be implicated in motor functions, has been also reported (Kupers et al., 2004; Niddam et al., 2002). Kupers et al. (2004) compared brain activities induced by hypertonic saline injection to the muscle with those induced by tactile stimulation of the skin with a von Frey hair. Furthermore, Henderson et al. (2006) showed muscle specific response at the ipsilateral anterior insula using hypertonic saline injection. In addition, they found that activity in the perigenual cingulate cortex, which is implicated in emotional response, was significantly decreased in muscle pain than in cutaneous pain.

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Other brain regions that are associated with aversive emotion include hippocampus (Viveros et al., 2007), amygdala (Fanselow and Gale, 2003), midbrain (Brandao et al., 2003) and orbitofrontal cortex (Rolls, 2000). So far, brain regions responsible for the dull sensation, which is the special characteristic of the muscle pain compared to the skin pain, are not clear.

In this study we used electrical stimulation of the skin and the muscle of the similar subjective intensity levels, and it was synchronized with fMRI scans so that the analysis is statistically more robust and accurately pinpoints finer differences between the respective brain regions responsible for painful muscle and skin stimulation. In addition ROI analysis was performed focused on the brain areas that are considered to be related to emotion.

2. Materials and methods

2.1. Subjects

We studied 13 healthy male volunteers (aged 20–36 years, mean ± S.E.M.: 26 ± 1 years) with the approval of both the Ethical Committee for Human and Genome Research of Research Institute of Environmental Medicine, Nagoya University and the Ethical Committee of the National Institute for Physiological Sciences, Japan. Informed written consent was obtained from all subjects and the study adhered to the tenets of the Declaration of Helsinki.

2.2. Stimulus

Electrical stimulation was used to induce pain (electrical stimulator: Nihon Kohden SEN-3301, Japan; isolator: Nihon Kohden SS-102J, Japan). While subjects lay supine on the MRI scanner bed, a fine stainless steel needle electrode (length: 48 mm, diameter: 0.18 mm) that was insulated except for its tip and served as a cathode, was inserted 20 mm down through the skin into the rostral belly of the left anterior tibial muscle for the muscle stimulation. For the skin stimulation, the needle was bent perpendicular at 2 mm from the tip and inserted into the skin near the muscle stimulation site. The part of the needle left above the skin was taped onto the skin surface. The surface electrode serving as an anode was then taped onto the skin surface about 30 mm proximal from this point. An experiment consisted of two sessions: the skin pain and the muscle pain sessions, and both were performed in all subjects. Schematic diagram of stimulus application is shown in Fig. 1. We defined a pain scale in which 0 represented minimum pain and 10, maximum pain imaginable, and chose three stimulus intensities inducing pain levels, 0, 5 and 7, for use. At the scale 0 level, subjects received minimum electric current intensity which caused barely noticeable pain sensation (0.5 mA for all the subjects). Stimulus intensities corresponding to pain scales 5 and 7 were determined both for the skin and the muscle in each subject at the beginning of each session by applying electric pulses of 1 ms duration and current intensities in ascending order.

Muscle twitch was observed in response to muscle stimulation, even at the pain scale 0. After determining the current intensity, the subject was positioned in the MRI scanner and received 90 stimuli consisting of the 3 pain levels (30 stimuli each) in random order. Subjects received no cues regarding stimulus intensity, such as visual or audio signs, so anticipation was excluded. The electric stimulation was synchronized with fMRI scans using the Presentation software (Neurobehavioral Systems, Inc.), that is, event-related fMRI study. The interval between stimuli was also randomized between 14 and 18 s to avoid anticipation and habituation. In the middle of a session, the pain scale determination procedure described above was repeated to check if adaptation to the stimulation has occurred. The stimulus intensity corresponding to each pain scale was shown in Table 1. The order of cutaneous and muscle pain sessions was randomized in each subject. Subjects were not familiar with the electrical-induced pain prior to this study.

Table 1
Stimulus intensity for the muscle and cutaneous stimuli.

Stimulation	Skin		Muscle	
Pain scale	5	7	5	7
1st session	2.38 ± 0.20	4.15 ± 0.23	2.59 ± 0.31	4.22 ± 0.26
2nd session	2.40 ± 0.16	3.92 ± 0.21	2.51 ± 0.33	4.26 ± 0.23

Stimulus intensities are in mA (mean ± S.E.M.).

2.3. Imaging procedure

fMRI was performed using a 3.0 T scanner system (The Magnetom Allegra, Siemens Co., Erlangen, Germany) with a standard head coil. Each session consisted of one anatomical scan and two functional scanning runs. The anatomical scans were recorded using a high-resolution T1-weighted anatomical protocol (3D gradient-echo pulse, modified driven equilibrium Fourier transform, TR 88.1 ms, TE 4.12 ms, TI: 650 ms, FOV 250 mm, 256 × 256 × 256 matrix). The functional scans were collected using a blood oxygen level-dependent (BOLD) protocol with a T2*-weighted gradient echo-planar imaging (EPI) sequence (TR 1500 ms, TE 30 ms, θ 90°; FOV 250 mm, 64 × 64 × 16 matrix, slice thickness 6 mm, gap 1.5 mm). The scanning planes covered the whole brain from the top of the cortex to the base of the cerebellum. Each session consisted of 728 whole brain volume acquisitions. Extra baseline conditions (14 s) with no stimulation were added at the beginning of each scanning run. The first eight images were discarded to account for spin saturation effects. All subjects were instructed to give attention to the stimuli and to refrain from movement as much as possible. To further prevent movement artifacts, the subject's head was immobilized with padded earmuffs and a foam headrest. Each subject was provided with earplugs to decrease the noise generated by the MRI machine.

2.4. Image processing and analyses

Functional data were motion-corrected and low-pass filtered with an 8-mm FWHM Gaussian kernel in order to increase the signal-to-noise-ratio. All images were realigned and stereotactically normalized into the standard anatomic space by means of linear and nonlinear transformation. Activation maps were generated using SPM5 software developed at the Wellcome Department of Imaging Neuroscience, London. This analysis yields *t*-statistics based on a linear model using random field theory. Evoked fMRI responses from all runs were modeled using a canonical HDR function (Friston et al., 1998). In the single-subject analysis, the design matrix contained two task-related regressors (the muscle pain and surface pain conditions), and two regressors for parametric modulation due to the pain intensity. The presentation of each stimulus was embedded in a series of delta functions. The task-related regressor was modeled by convolving it with a canonical hemodynamic response function (HRF). To construct the regressor for parametric modulation, the interaction between the trial and the parameter variable was first calculated for each face condition as follows. The delta function for each stimulus was modulated by the pain intensity. In other words, the height of the delta function was changed as a function of the pain intensity. Next, the trial × parameter interaction term was convolved with the HRF, giving the regressor for the parametric modulation. Finally, the regressor for each pain condition was orthogonalized with respect to the corresponding task-related regressor. We used the high-pass filter, which was composed of the discrete cosine basis function with a cut-off period of 128 s, in order to eliminate the artifactual low-frequency trend. Serial autocorrelation assuming a first-order autoregressive model was estimated from the pooled active voxels using the restricted maximum likelihood (ReML) proce-

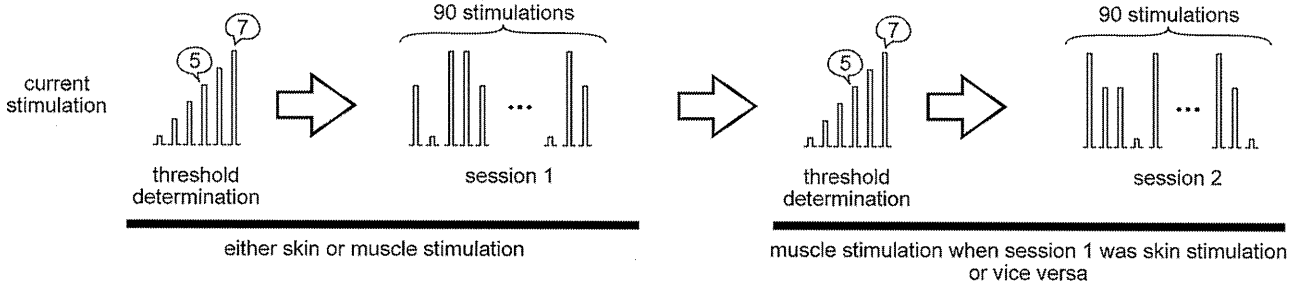

Fig. 1. Schematic diagram of stimulus application. Electric current stimulation was applied to the left leg for each subject. Subjects received two fMRI scanning sessions (skin and muscle stimulations. The order was randomized). Before each session, determination of pain threshold was carried out. Balloons indicate the subjective pain scales the subject mentioned. Note that the same stimulus intensities at which the subject mentioned as pain scales 5 and 7 were used in the successive scanning session. See text for details.

Table 2
Predefined contrasts for fMRI analysis.

	Muscle pain		Surface pain	
	Constant	Modulation	Constant	Modulation
MI	1	0	0	0
MP	0	1	0	0
SI	0	0	1	0
SP	0	0	0	1
MI > SI	1	0	−1	0
SI > MI	−1	0	1	0

Brain areas responded to the painful muscle stimulation irrespective of (MI) or proportional to (MP) its intensity, and to the painful skin stimulation irrespective of (SI) or proportional to (SP) its intensity. MI > SI: greater activity during the muscle pain than surface pain, SI > MI: greater activity during the surface pain than muscle pain.

dure, and was used to whiten the data and the design matrix (Friston et al., 2002). To give the estimated parameters, the least-square estimation was performed on the high-pass filtered and pre-whitened data and design matrix. The weighted sum of the parameter estimates in the individual analysis constituted contrast images that were used for the second-level analysis. The predefined contrasts are shown in Table 2. We constructed appropriate contrast images to examine brain areas showing effects in the four conditions: areas that responded to the painful skin stimulation irrespective of (SI) or proportional to (SP) its intensity, and areas responded to the painful muscle stimulation irrespective of (MI) or proportional to (MP) its intensity. Then we created additional contrasts: Greater activity during the muscle pain than surface pain (MI > SI) and vice versa (SI > MI). The areas commonly responded to both muscle pain and surface pain were depicted by means of conjunction analysis with conjunction null hypothesis (MI&SI) (Nichols et al., 2005). The brain coordinates based on the Montreal Neurological Institute (MNI) system. Voxels with uncorrected p-values less than 0.001 were clustered to best describe inter-subject variability. Region of interest (ROI) analyses was carried out using the MarsBaR toolbox and ROIs defined from the probabilistic atlas of SPM5 to test the region-specific hypothesis (Brett et al., 2002). Using this software, statistical tests were performed on the mean time course of the voxels within the defined ROIs.

3. Results

3.1. Pain perception

Despite similar pain intensities, there were clear differences in the sensory descriptors ascribed to muscle versus skin pain. Subcutaneous electric current evoked pain that was localized to the skin immediately surrounding the needle insertion site. In contrast, intramuscular electric stimuli evoked a deep, dull and unpleasant

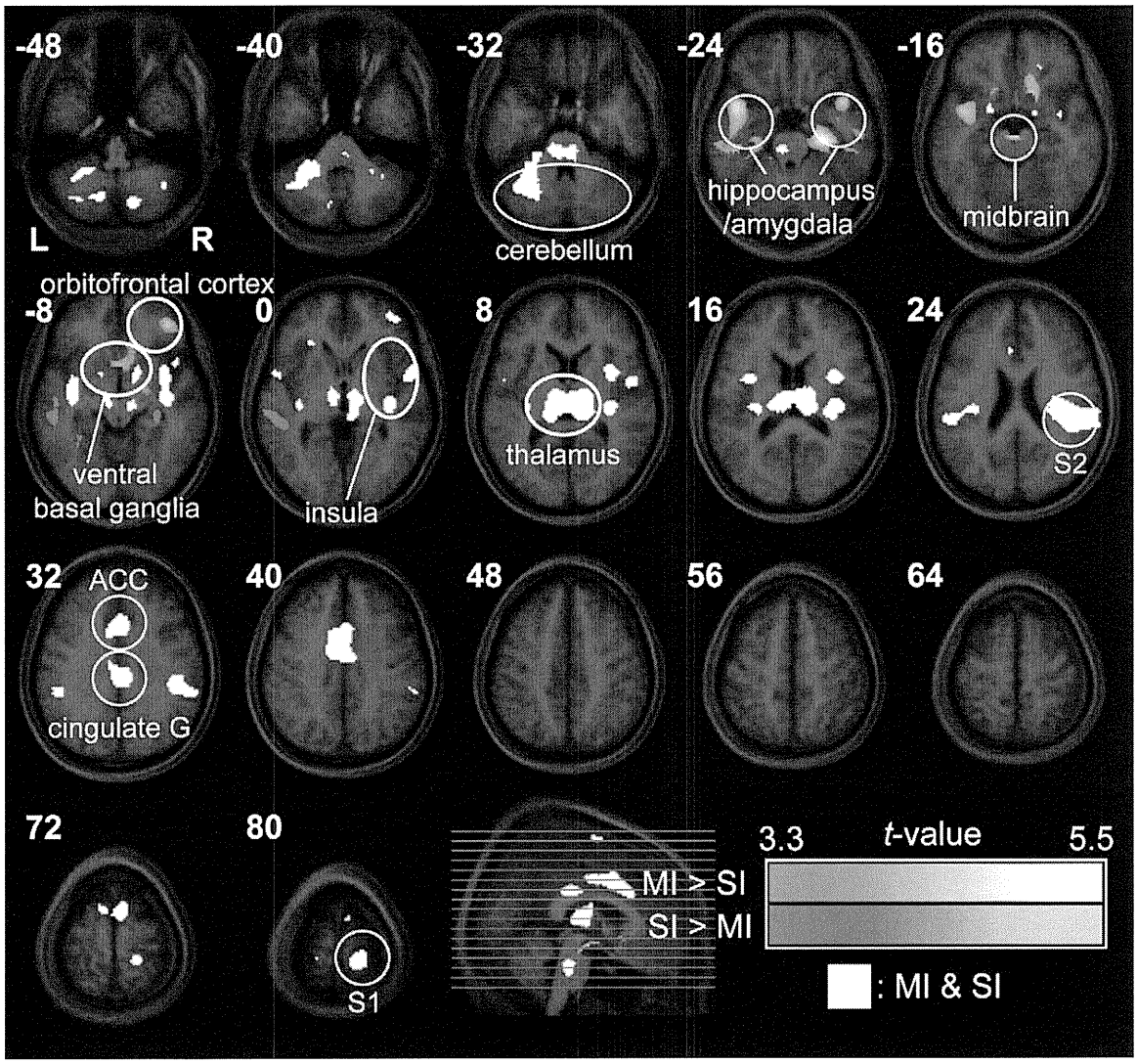

Fig. 2. Sequential brain maps of t-scores representing brain activities in response to electrical painful stimuli. Color scales indicate signal intensity increases during painful stimuli. *Red-yellow*: specific response to painful muscle stimulation; *Blue-green*: specific response to painful skin stimulation. The overlapped regions (white) responded both to the muscle and skin stimuli (conjunction analysis, family-wise error corrected $p < 0.05$). The figure presents axial slices taken every 8 mm from $z = -48$ to $z = +80$. ACC: anterior cingulate cortex, S1: primary somatosensory cortex, S2: secondary somatosensory cortex. MI: painful stimulation for the muscle, SI: for the skin. See text for detailed description of predefined fMRI contrasts.

sensation, that is spatially more diffuse compared to the case of the subcutaneous stimulation. Painful sensations induced by electrical stimulation of the skin or muscle were different from what we experience in natural settings, but main basic features were retained as mentioned above. There was no radiation of the deep pain to remote areas in the present experimental condition. Pain perception usually lasted a few seconds following both muscle and skin stimulation.

3.2. Response to painful muscle stimulation

Cortical neuronal response to the painful muscle stimulation unrelated to stimulus intensity (MI) was observed in bilateral ventral insula/amygdala, mid and posterior insula, ventral basal ganglia and secondary somatosensory cortex (S2) (Fig. 2 and Table 3). Midline activity was found in the anterior cingulate (Brodmann's Area [BA] 32, Fig. 2) and cingulate gyrus (BA23, 24). Significant

Table 3
Brain regions responding to painful electric stimuli.

Anatomic region	BA	x	y	z	Z-score
Response to muscle pain (unrelated to intensity, MI)					
R ventral insula/amygdala	28/34	−36	2	−18	6.83*,†
L ventral insula/amygdala	28/34	38	0	−20	6.05*,†
R posterior insula/S2	13	34	−24	22	6.77*,†
R mid-insula	13	34	4	14	5.82*,†
L mid-insula	13	−36	2	14	5.15*,†
L posterior insula	13	−36	−24	24	4.89*,†
R ventral basal ganglia	NA	12	10	−12	6.41*,†
L ventral basal ganglia	NA	−16	18	−14	5.96*,†
R thalamus	NA	10	−22	12	6.39*,†
L thalamus	NA	−10	−18	10	5.55*,†
R S1	1,2,3	16	−44	80	6.38*,†
R supplementary motor cortex	6	8	−8	76	6.34*,†
L/R midbrain	NA	−2	−16	−16	6.16*,†
R middle frontal gyrus	10	38	50	−6	6.03*,†
L cerebellum	NA	−34	−56	−32	5.82*,†
L S2	40	−50	−38	28	5.43*,†
L/R anterior cingulate cortex	24	−6	8	38	5.32*,†
L/R cingulate gyrus	23	6	−28	30	5.21*,†
Response to muscle pain (proportional to intensity, MP)					
R inferior frontal gyrus	47	30	34	−6	4.04
R posterior insula	13	40	−16	−8	3.75
R insula	13	34	2	18	3.60
L pons	NA	−8	−22	−20	3.45
L supramarginal gyrus	40	−56	−38	32	3.36
R M1	4	18	−34	86	3.34
R cingulate gyrus	23	10	−24	32	3.33
L cerebellum (declive)	NA	−36	−58	−28	3.60
L cerebellum (culmen)	NA	−8	−40	−26	3.50
L cerebellum (inferior semi-lunar lobule)	NA	−22	−66	−48	3.43
L cerebellum (cerebellar tonsil)	NA	−24	−50	−50	3.34
R cerebellum (uvula)	NA	24	−70	−32	3.31
Response to skin pain (unrelated to intensity, SI)					
R posterior insula/S2	13	34	−22	24	6.93*,†
L/R cingulate gyrus	7	8	−32	30	6.24*,†
L/R thalamus	NA	10	−22	10	6.16*,†
R S1	5	18	−46	78	6.01*,†
R cerebellum	NA	36	−58	−50	5.12*,†
L superior temporal gyrus	22	−54	0	6	5.10*,†
R middle frontal gyrus	10	42	52	2	5.06*,†
L middle frontal gyrus	10	−28	52	16	4.88*,†
L/R midbrain	NA	−8	−28	−32	5.02*,†
L precuneus	7	−20	−60	36	4.84*,†
R precuneus	7	4	−76	52	4.51*,†
R superior frontal gyrus	11	22	40	−20	4.74*,†
R middle frontal gyrus	8	46	10	46	4.72*,†
R middle temporal gyrus	19	34	−56	16	4.71*,†
R inferior parietal lobule	40	52	−50	56	4.69*,†
L inferior parietal lobule	40	−54	−44	54	4.67*,†
Response to skin pain (proportional to intensity, SP)					
L/R supplementary motor cortex	6	4	−4	74	4.30
R S1	1,2,3	18	−48	78	3.97
R lentiform nucleus	NA	20	−10	−4	3.79
R posterior insula	13	36	−24	2	3.46
L/R thalamus	NA	−2	−12	−4	3.33
L/R cingulate gyrus	24	−2	−10	38	3.75
L/R cingulate gyrus	32	2	24	28	3.57
R cerebellum	NA	6	−46	−36	3.74
L superior frontal gyrus	11	−22	40	−22	3.74
L middle frontal gyrus	10	−30	60	14	3.69

MNI coordinates at the peak activations are indicated (uncorrected $p < 0.001$). Because activated regions often spread out to contiguous areas as seen in Fig. 2, some regions are titled as "L/R" even though the coordinates indicate either hemisphere. S1: primary somatosensory cortex, S2: secondary somatosensory cortex. M1: primary motor cortex.

* False discovery rate corrected $p < 0.05$.

† Family-wise error corrected $p < 0.05$. BA: Brodmann's area.

activation in the thalamus extended into both hemispheres centered on midline, with much greater response in the right thalamus (contralateral to the stimulated site). Broad activation in the cerebellum also extended into both hemispheres, but with much greater response in the ipsilateral side. Unilateral response to painful muscle stimulation was observed in the primary somatosensory cortex (S1) and orbitofrontal cortex contralateral to the stimulation. Brain regions that showed greater response proportional to the intensity of electric painful stimulation to the muscle (MP) included contralateral inferior frontal gyrus, insula, primary motor cortex, cingulate gyrus and cerebellum. Ipsilateral responses were observed in the pons, supramarginal gyrus and cerebellum.

To test if the painful muscle stimulation activates the brain regions related to emotion, we carried out ROI analyses. In the contralateral amygdala, the volume of regions that significantly (family-wise error corrected p value <0.05) responded to the painful muscle stimulation was 1016 mm^3 (Table 4). Within this region, the response to the painful muscle stimulation was significantly greater than that to the painful skin stimulation (corrected $p=0.0087$). This result was supported further by the regional time-activation plot, which showed greater BOLD response to the painful muscle stimulation than to the painful skin stimulation (Fig. 3A). Similarly, the bilateral caudate, orbitofrontal (inferior, middle and superior) cortices, hippocampus and parahippocampus showed significantly greater response to the painful muscle stimulation with contralateral preference (Table 4 and Fig. 3B–D. Graphs are shown only for the contralateral side). The response in the medial orbitofrontal cortex was significant only in the contralateral side. The superior temporal pole showed bilateral activation to the painful muscle stimulation, but with ipsilateral preference (Fig. 3E). With regard to the midbrain where no ROI template was available, its location was determined by the averaged anatomical image from the subjects. There was 24 mm^3 cluster at the MNI coordinates (−1, −12, −14) that showed greater response to the painful muscle stimulation than to the painful skin stimulation (t-value ≥ 4.8). Within this cluster, the time-activation plot showed clearly larger response to the painful muscle stimulation than to the painful skin stimulation (Fig. 3F).

3.3. Response to painful skin stimulation

Distinct activations were observed at the typical pain neuromatrices such as the S1, S2, insula, anterior cingulate cortex and thalamus in response to painful skin stimulation (Fig. 2: MI&SI and Table 3). The cerebellum, midbrain, precuneus and inferior parietal lobule also responded to the painful skin stimuli. Subtraction analysis was carried out to search brain regions that showed greater response to the painful skin stimulation than to muscle stimulation (SI > MI). Although statistically significant activities were observed at MNI coordinates (20, −6, −6) (globus pallidus), (−54, −34, −6) (middle temporal gyrus), (32, −28, −8) (hippocampus) and (−34, −50, −6) (parahippocampal gyrus), responses to painful skin stimulation were obviously small compared to the responses to painful muscle stimulation in Fig. 3, even though the responses were taken at the points that showed local maximum t-values (Fig. 4). Thus the statistical difference seems to be rather due to decreased response to painful muscle stimulation.

Brain regions that showed greater response proportional to the intensity of electric painful stimulation to the skin (SP) included the insula, S1, cerebellum, superior frontal gyrus, middle frontal gyrus and lentiform nucleus contralateral to the stimulation; bilateral cingulate gyrus and thalamus (Table 3).

As well as in the painful muscle stimulation, ROI analyses were carried out to test if the painful skin stimulation activates the brain regions related to emotion (Table 4). The volumes of regions that significantly responded to the painful skin stimulation were generally fewer than those to the painful muscle stimulation. For

Table 4
Region of interest analyses for the brain regions related to emotion.

		Response to painful muscle stimulation		Response to painful skin stimulation	
		Volume (mm^3)	p-Value for m ⊁ s	Volume (mm^3)	p-Value for s ⊁ m
Amygdala	L	552	0.0099**	0	–
	R	1016	0.0087**	912	0.9403
Caudate	L	1576	0.0111*	1624	0.1132
	R	1752	0.0086**	2128	0.1426
Putamen	L	616	0.0894	520	0.1705
	R	928	0.1837	2560	0.1493
Inferior orbitofrontal Cortex	L	568	0.0106*	0	–
	R	2776	0.001**	8	0.6736
Middle orbitofrontal Cortex	L	0	–	0	–
	R	712	0.0056**	0	–
Middle orbitofrontal Cortex	L	352	0.0351*	0	–
	R	2752	0.0012**	232	0.8623
Superior orbitofrontal Cortex	L	968	0.0111*	0	–
	R	2096	0.0087**	48	0.5908
Hippocampus	L	712	0.0223*	360	0.2800
	R	1208	0.0027**	584	0.1790
Parahippocampus	L	832	0.0007***	0	–
	R	1488	0.0002***	0	–
Superior temporal Pole	L	2312	0.0002***	184	0.5401
	R	2262	0.0046***	376	0.6054

Volume of the brain region that showed significant response (family-wise error corrected $p<0.05$) to the painful stimulation unrelated to its intensity within each anatomical ROI template was indicated in mm^3. Within this statistically significant region, the probability that the response to the painful muscle stimulation was not greater than to the painful skin stimulation (null hypothesis: m ⊁ s), and vice versa, were calculated. All p values are corrected for multiple comparison.

* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

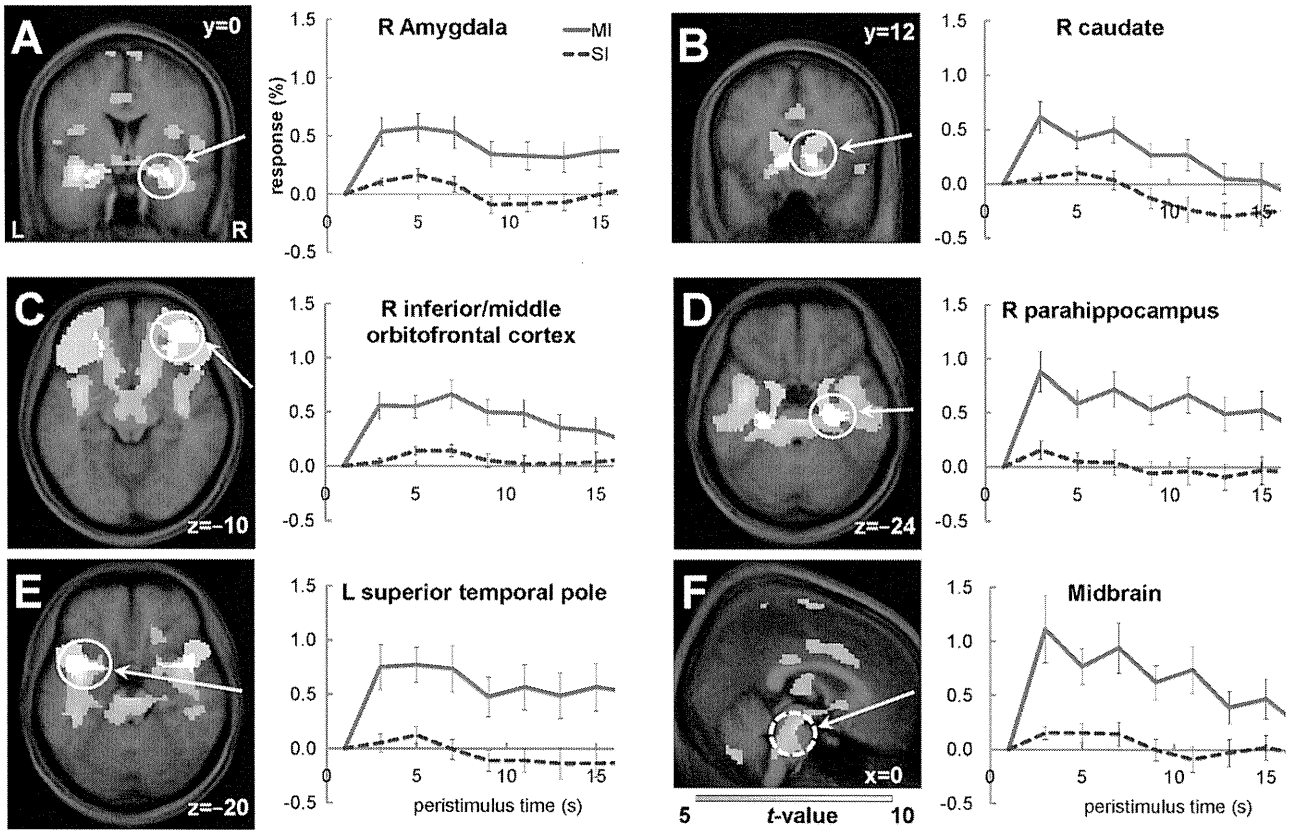

Fig. 3. Activation maps and peristimulus time–response curves at the brain regions that showed greater activities for painful muscle stimulation than for painful skin stimulation. (A) amygdala, (B) caudate, (C) inferior and middle orbitofrontal cortex, (D) parahippocampus, (E) superior temporal pole, and (F) midbrain. These anatomical regions are indicated by blue regions in the activation maps (except midbrain which does not have the *MarsBaR* anatomical templates). Brain activations were indicated by orange (MI). White regions indicate overlap with anatomical templates. Red lines in the time–response curves indicate brain response to painful muscle stimulation (MI); blue broken lines, response to painful skin stimulation (SI). Bars indicate S.E.M. MI: painful stimulation for the muscle, SI: for the skin. See text for detailed description of predefined fMRI contrasts.

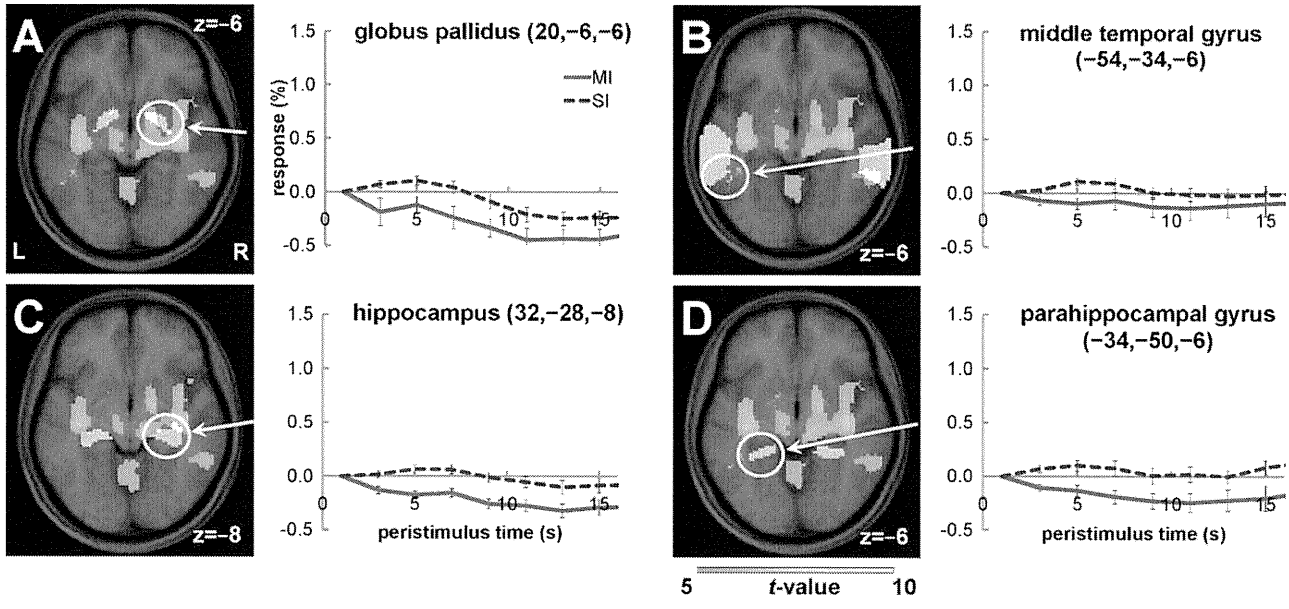

Fig. 4. Peristimulus time–response curves at brain regions that showed greater response to painful skin stimulation than to muscle stimulation. (A) globus pallidus, (B) middle temporal gyrus, (C) hippocampus, and (D) parahippocampus. These anatomical regions are indicated by blue regions in the activation maps. Brain activations were indicated by orange (SI). White regions indicate overlap with anatomical templates. Red lines in the time–response curves indicate brain response to painful muscle stimulation (MI); blue broken lines, response to painful skin stimulation (SI). Bars indicate S.E.M. MI: painful stimulation for the muscle, SI: for the skin. See text for detailed description of predefined fMRI contrasts.

example, while significant response was observed in 552 mm^3 cluster in the ipsilateral amygdala in response to the painful muscle stimulation, no significant response was seen to the painful skin stimulation. Moreover, even in the regions that showed significant response to the painful skin stimulation, such as the contralateral caudate, the probability that the response to the painful skin stimulation was greater than to the painful muscle stimulation was insignificant in any regions. This forms a striking contrast to that the painful muscle stimulation significantly activated the amygdala, caudate, orbitofrontal cortices, hippocampus, parahippocampus and superior temporal pole.

4. Discussion

In addition to activation of areas that are well established as pain neuromatrices (Peyron et al., 2000) such as the primary and secondary somatosensory cortex, insula, anterior cingulate cortex and thalamus, we found that the midbrain, amygdala, caudate, orbitofrontal cortices, hippocampus, parahippocampus and superior temporal pole responded preferentially to painful muscle stimulation. Most of these areas are thought to be involved in emotion. Increased activities in response to painful muscle stimulation at inferior/middle frontal gyrus have already been reported by Niddam et al. (2002) and Schreckenberger et al. (2005). Our finding in the present study is that this region responds more intensely to painful muscle stimulation than to skin stimulation. Activities we observed at anterior cingulate/cingulate gyrus and insula are in line with previous studies (Henderson et al., 2006; Kupers et al., 2004; Niddam et al., 2002; Schreckenberger et al., 2005; Svensson et al., 1997b). Henderson et al. showed muscle specific activity at the anterior insula, but only on the ipsilateral side (Henderson et al., 2006). While activity at the caudate nucleus was reported by Niddam et al. (2002) and Kupers et al. (2004), we found that the more ventral part of the basal ganglia responded to the painful muscle stimulation and this region was not activated by the painful skin stimulation. Several brain regions (globus pallidus, middle temporal gyrus, hippocampus and parahippocampal gyrus) showed greater response to painful skin stimulation than to muscle stimulation. However, responses to painful skin stimulation in these areas were small and the statistical difference seems to be rather due to decreased response to painful muscle stimulation.

There is a possibility that the predominant brain activities in response to painful muscle stimulation in this study reflect artifacts, e.g. motor response due to muscle twitch, or the attention to an uncommon muscle stimulation compared to the skin. Peyron et al. (2007) reported the presence of a motor component in response to painful stimulation, which includes vermis, MI, SI, and paracentral cortices bilaterally, right premotor, right SII and posterior cingulate cortices. Brain regions related with emotion in our study do not overlap with these regions, suggesting that their activation by painful muscle stimulation is not due to motor response. To our knowledge, this is the first report of a more intense neuronal response to painful muscle stimulation than to skin stimulation at the midbrain, parahippocampal gyrus, insula-amygdala junction and ventral basal ganglia.

4.1. Midbrain

Blood oxygenation level-dependent (BOLD) activations produced by painful stimulation of the muscle showed significant activations in the medial midbrain. To the best of our knowledge, this is the first report to document the predominant neuronal activity in this region in response to the painful muscle stimulation compared to the skin stimulation in humans. Multiple regions in the midbrain are found to be involved with aversive emotional response. For example, the periaqueductal gray (PAG) is involved in fear and defense response (Brandao et al., 2003), while ventral tegmental area, the midbrain raphé nuclei, central gray and Gudden's nuclei with stress response (Morgane et al., 2005). Moreover, PAG is suggested to mediate anxiogenic actions via cholecystokinin receptors, and to be implicated in the development of both acute pain and chronic hyperalgesic states (Lovick, 2008). Our data in the current study suggests that the posterior part of the midbrain, probably including the PAG, is preferentially activated by painful muscle stimulation. The PAG is also known as an important nucleus of origin for the descending pain modulating system.

Interestingly, Hentall et al. have reported that noxious cutaneous stimuli did not modify the activity of interpeduncular nucleus in rats. This supports our finding that the medial midbrain specifically responds to painful muscle stimulation, not to painful skin stimulation. Other midbrain regions are also known to be implicated in pain. For instance, activations in the posterior hypothalamus, dorsal rostral pons and ventrolateral midbrain (which straddle red nucleus and substantia nigra) are observed in patients suffering from continuous headache (Matharu et al., 2004). Noxious stimulation of muscle or skin induces cardiovascular responses (Sato et al., 1997), and their centers locates in the brain stem. We did not monitor heart rate, and subjects in the current study did not particularly mention cardiovascular change such as increased heart rate during the experiments. However, there is a possibility that cardiovascular change occurred and the activity seen in the midbrain was related with this change.

4.2. Amygdala/hippocampal regions

In this study, painful muscular stimulation activated ventral part of the medial temporal lobe bilaterally, which include the amygdala and resides in the vicinity of the ventral part of the insula. On the other hand, ROI analyses in this study showed relatively small number of volumes in the right amygdala (contralateral to the stimulus) showed statistically significant activity in response to painful skin stimulation (Table 4). Peyron et al. (2007) reported that painful electric stimulus activated the right amygdala (contralateral to the stimulus). Taken together, it is suggested that both painful skin and muscle stimulations activate the amygdala, but the painful muscle stimulation does so to a larger extent.

There are a number of studies devoted to show the relationship between the amygdala and emotion. For example, conditions that induce negative emotions, such as fear, or unpleasant, aversive stimuli activate amygdala (Davidson, 2002). Furthermore, a direct link between the affective aspects of pain and the activity in the amygdale has been reported by Schneider et al. (2001). On the other hand, significant preference of painful muscle to painful skin stimulation was observed in neural activity in the parahippocampal gyrus in the current study. Parahippocampal regions and amygdala are known to mediate evaluative processing of emotion (Wood et al., 2005). Taken together, brain activation in the ventral part of the medial temporal region in response to painful muscle stimulation may represent aversive emotional response.

There are some reports that indicate skin pain and muscle pain evoke different emotional responses even though they have the same intensity. For example, Schreckenberger et al. (2005) reported that intramuscular infusion of low pH buffer caused more unpleasantness than intracutaneous infusion, even though pain intensity was set to equal for both cases. Similar example is that intramuscular hypertonic saline injection evoked gnawing sensation more frequently than subcutaneous injection despite that the pain intensity was the same (Henderson et al., 2006). Therefore, it is likely that painful muscular stimulation preferentially activated brain regions responsible for aversive emotional response compared to painful skin stimulation in this study.

4.3. Orbitofrontal cortex

We observed a neuronal activity in response to painful muscle stimulation in the middle frontal gyrus, a part of the orbitofrontal cortex. Interestingly, Schreckenberger et al. (2005) reported that medial frontal gyrus, a part of the orbitofrontal cortex, showed greater response to intramuscular painful stimulation than to intracutaneous one, closely resembling our results. The fact that they and we obtained the same results despite using different pain induction methods (low pH buffer infusion and electrical stimulation, respectively) strongly suggests that the orbitofrontal cortex is activated more preferentially by painful muscle stimulation compared to skin stimulation.

The orbitofrontal cortex is known to have connections with hypothalamus, brainstem autonomic areas and amygdala, and to be able to influence autonomic aspects of emotional expression (Rempel-Clower, 2007). Other evidence that the orbitofrontal cortex is related to the affective aspect of sensation is that it responds to painful and nonpainful gastric stimulation (Vandenberghe et al., 2007), distension of the lower gastrointestinal tract (Derbyshire, 2003), and pleasant and painful touch stimulation to the hand (Rolls et al., 2003). In this connection, the brain activities observed in response to painful muscle stimulation in this area may reflect stronger affective and aversive component of muscle pain than cutaneous pain (Svensson et al., 1997a).

4.4. Ventral Basal ganglia

Basal ganglia are traditionally considered to play a role in motor function, and are now known to respond to various kinds of painful stimulation. For example, activity in the caudate head and putamen in response to painful gastric stimulation was reported (Lu et al., 2004). Visceral pains evoked by balloon distention at the esophagus (Strigo et al., 2003) and stomach (Lu et al., 2004) activate the putamen and caudate body/globus pallidus respectively. Also supporting the notion that basal ganglia are associated with pain is the fact that they have high opioid binding potential (Baumgartner et al., 2006).

Neuronal activity at the caudate nucleus in response to painful muscle stimulation was described by Kupers et al. (2004) with PET. They used hypertonic saline injection of the jaw muscle for the painful stimulation. Our finding is that the ventral basal ganglia (seemingly ventral part of the caudate nucleus) respond more to painful muscle stimulation than to painful skin stimulation of the leg. While the caudate nucleus was reportedly activated during a spatial discrimination task of painful heat stimulation of the skin (Oshiro et al., 2007), no significant activity in basal ganglia in response to painful skin stimulation was reported in other previous studies using electrical stimulation (Peyron et al., 2007), low pH infusion (Schreckenberger et al., 2005) and hypertonic saline injection (Henderson et al., 2006).

4.5. Superior temporal pole

In the present study, significantly greater response to the painful muscle stimulation than to the skin stimulation was observed in the superior temporal pole. Again, no statistically significant activity in this region in response to painful skin stimulation was reported in other previous studies using electrical stimulation (Peyron et al., 2007), low pH infusion (Schreckenberger et al., 2005) and hypertonic saline injection (Henderson et al., 2006). This fact suggests that the superior temporal pole hardly plays a role in processing of skin pain. Recently, this region was reported to be involved with negative reward information (Liu et al., 2007). The muscle pain might be processed as negative reward in the brain.

4.6. Brain regions preferentially respond to skin pain

As mentioned in result section, no brain region showed significant increase in activity in response specifically to painful electrical skin stimulation (Fig. 4). This result is in agreement with the studies that showed no significant increase in brain activity in any region, in which low pH buffer infusion (Schreckenberger et al., 2005) and hypertonic saline injection (Henderson et al., 2006) were used as painful stimulus. This result is in striking contrast with the fact that spinal and thalamic neurons that have muscle nociceptive inputs almost always have convergent input from cutaneous structure (Kniffki and Mizumura, 1983; Taguchi et al., 2008), but not vice versa. Possibility of absence of skin pain specific region must be carefully scrutinized in the future studies.

4.7. Limitation of the present study

Electrical stimulation that was used to induce pain in this experiment has some limitations such as that not only nociceptors but also various kinds of A-fiber mechanoreceptors and thermoreceptors are excited at the same time, and that quality of pain is in some respects different from ordinary pain experienced in natural conditions. This different character of sensation might be induced by different temporary pattern of impulse discharges (only one pulse was given in this experiment) and difference in fibers excited. However, this method synchronized with fMRI scans allowed us to analyze statistically more robust and accurately pinpoint finer differences between the respective brain regions responsible for painful muscle and skin stimulation. Therefore, to have better knowledge about which brain regions are responsible for muscle or skin pain, it is essential to compare results obtained by various stimulation methods.

In conclusion, the present experiment showed that brain regions specifically activated by muscle stimulation were the midbrain, bilateral amygdala, caudate, orbitofrontal cortex, hippocampus, parahippocampus and superior temporal pole, most of which are related to emotion. Regions except the midbrain showed contralateral preference. These results suggest that dull sensation, which is characteristic of muscular pain, is related with processing in these brain regions.

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REVIEW

Pain perception in humans: use of intraepidermal electrical stimulation

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ABSTRACT

The choice of a system specific stimulus is difficult when investigating the human nociceptive system, in contrast with the tactile, auditory and visual systems, because it should be noxious but not actually damage the tissue. The discomfort accompanying system specific stimulation must be kept to a minimum for ethical reasons. In this review, recent progress made in the study of human pain perception using intraepidermal electrical stimulation (IES) is described. Also, whether IES is a viable alternative to laser stimulation is discussed. IES selectively activates Aδ nociceptors, elicits a sharp pricking sensation with minimal discomfort and evokes cortical responses almost identical to those produced by laser stimulation. As IES does not require expensive equipment, and is easy to control, it would seem useful for pain research as well as clinical tests.

INTRODUCTION

Pain, particularly its emotional component, is essential for survival. However, excessive pain is distressful. Therefore, pain research in humans is important for uncovering the underlying mechanisms of this essential function as well as for establishing treatment for pain relief. The recent development of non-invasive techniques has enabled us to examine directly the human brain, and the number of reports on pain perception using functional brain imaging techniques has progressively increased in the past 20 years. In general, studies using non-invasive techniques, such as electroencephalography, magnetoencephalography (MEG), positron emission tomography and functional MRI (fMRI) have found that noxious stimuli activate several areas of the brain, including the thalamus, basal ganglia, primary (S1) and secondary (S2) somatosensory cortex, insula and cingulate cortex (figure 1A).

The choice of an appropriate stimulus is another important aspect of pain research in humans because research into the human nociceptive system is limited by ethical constrains because of possible tissue damage and the discomfort evoked by a noxious stimulus. There are various ways to activate the nociceptive system, including chemical, thermal, electrical and mechanical stimulation. Each method has its own advantages and disadvantages but, ideally, the stimulation should be safe, reproducible and quantifiable.[1] In addition, it should stimulate Aδ or C nociceptors selectively if one wants to specifically investigate activation of the nociceptive system. For research or clinical testing that requires precise information of latency, such as evoked potentials, a steep rise in the intensity of the stimulus is also important. From an ethical point of view, the discomfort accompanying system specific stimulation should be as weak as possible.

Electrical stimuli fulfil many of these requirements but lack selectivity. Because mechanoreceptors have a lower electrical threshold than nociceptors, electrical stimuli always coactivate mechanoreceptors of the tactile system at a noxious intensity. Mechanical stimuli, such as pinpricks, which are often used for clinical tests, lack selectivity as well as the steepness. For a similar reason, the usefulness of chemicals for pain research is limited.[2]

Laser stimuli delivered as a brief pulse with a steep rise in intensity can activate cutaneous nociceptors without the concomitant activation of mechanoreceptors.[3] Therefore, laser stimulation is the best means of activating the human nociceptive system at present. In fact, lasers are used in research as well as clinical testing.[4 5] One problem with laser stimulation however is that the equipment needed is expensive.

Here we review studies using intraepidermal electrical stimulation (IES) developed for the selective activation of cutaneous nociceptors. An electrical method that can selectively stimulate nociceptors would clearly be useful for pain research or clinical tests.

INTRAEPIDERMAL ELECTRICAL STIMULATION

Electrode

This method is based on the fact that nociceptive fibre terminals are located in the epidermis and superficial layer of the dermis, while other fibres end deep in the dermis. When the superficial layer of the skin is electrically stimulated, the localised current is expected to selectively activate nociceptors. For this purpose, we made a pushpin-like electrode with a stainless steel needle, 0.2 mm in length.[6] Although it successfully stimulated cutaneous Aδ nociceptors,[6 7] the range of current at which there was no concomitant activation of Aβ mechanoreceptors was narrow—that is, as the current increased in intensity, it reached far enough to activate mechanoreceptors located deeper than nociceptors. Then we improved the method by employing a concentric bipolar configuration (figure 1B). The cathode used was an outer ring, 1.2 mm in diameter, and the anode was an inner needle that protruded 0.1 mm from the outer ring.[8] The effective range for the selective activation of nociceptors widened because less current spread to undesired skin layers (figure 1C). We confirmed the effectiveness of the concentric configuration at reducing undesired loop current in rats.[9]

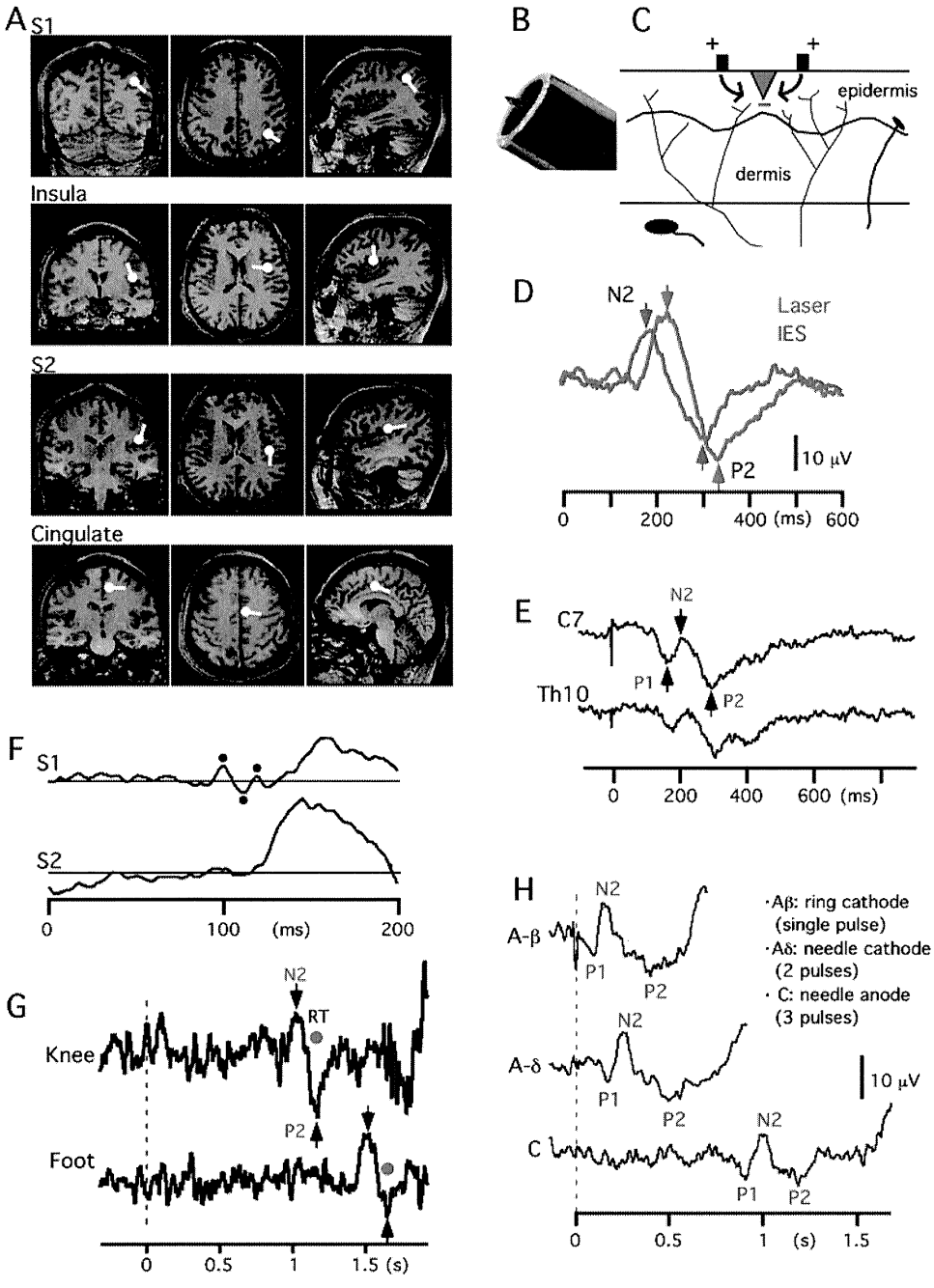

Figure 1 Use of intraepidermal electrical stimulation (IES) for studies on human pain perception. (A) Cortical activity detected by magnetoencephalography (MEG) in the primary somatosensory cortex (S1), secondary somatosensory cortex (S2), insula and cingulate cortex following IES to the left hand. (B) Photograph of the concentric bipolar needle electrode for IES. (C) The current passing through the electrode is spatially restricted to the superficial part of the skin where nociceptive free nerve endings are located. (D) Comparison of evoked potentials following stimulation of the hand between IES (blue) and laser (red) stimulation. Note the similar waveform and a 40 ms delay for laser stimulation. (E) Estimation of conduction velocity (CV) in the spinal cord. Very similar waveforms are evoked by IES to the back midline at the C7 and Th10 levels. (F) Primary responses to IES in S1 recorded by MEG. Note the triphasic waveform of the early S1 activity with polarity reversals at a 10 ms interval. (G) Estimation of CV of C fibres of the lower limb. The calculated CV was 1～1.1 m/s for N2, P2 and RT (reaction time). (H) Activation of Aβ, Aδ and C receptors by one electrode. By using different parameters, different receptors can be stimulated at the same site.

Stimulation

When the electrode is gently pressed against the skin, the needle tip is inserted adjacent to the nerve endings of the thin myelinated fibres in the epidermis and superficial part of the dermis. As there is no blood in the epidermis, the IES electrode cannot cause bleeding. Although we have never had an infection due to insertion of the electrode, the skin is first disinfected with alcohol and the electrode is for single use only. Unlike with laser stimulation, there is no undesired skin effect, such as heat burn or erythema. The electric stimulus can be a conventional square wave pulse of 0.5～1.0 ms but a slowly rising pulse, such as a triangular wave,[10] is better. Double pulses with a 10～25 ms interval are usually used to obtain clear responses but a single pulse is also used when a precise response latency is necessary (eg, see Inui *et al*[11]). The current is of an intensity that produces a definite sensation of pain, 2～6 on the visual analogue scale (0～10). IES can be applied to any area of the body. To augment the response, two or three electrodes, 10 mm apart, are used. In recent studies, a triple electrode type (NM-980W; Nihon Kohden, Tokyo, Japan) has been used.

Sensations

When a weak current, of approximately 0.1～0.5 mA, is applied by IES, a sharp pricking sensation, an indication of Aδ nociceptor activation, is elicited without any other sensations. The magnitude of the pricking sensations increases with an increase in stimulus intensity, number of pulses and pulse duration. The intensity of the painful sensation increases slightly with the use of multiple electrodes. These results suggest that for painful sensations, the contribution of temporal summation is greater than that of spatial summation. The pricking sensation is abolished by the local application of lidocaine.[12]

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