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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]

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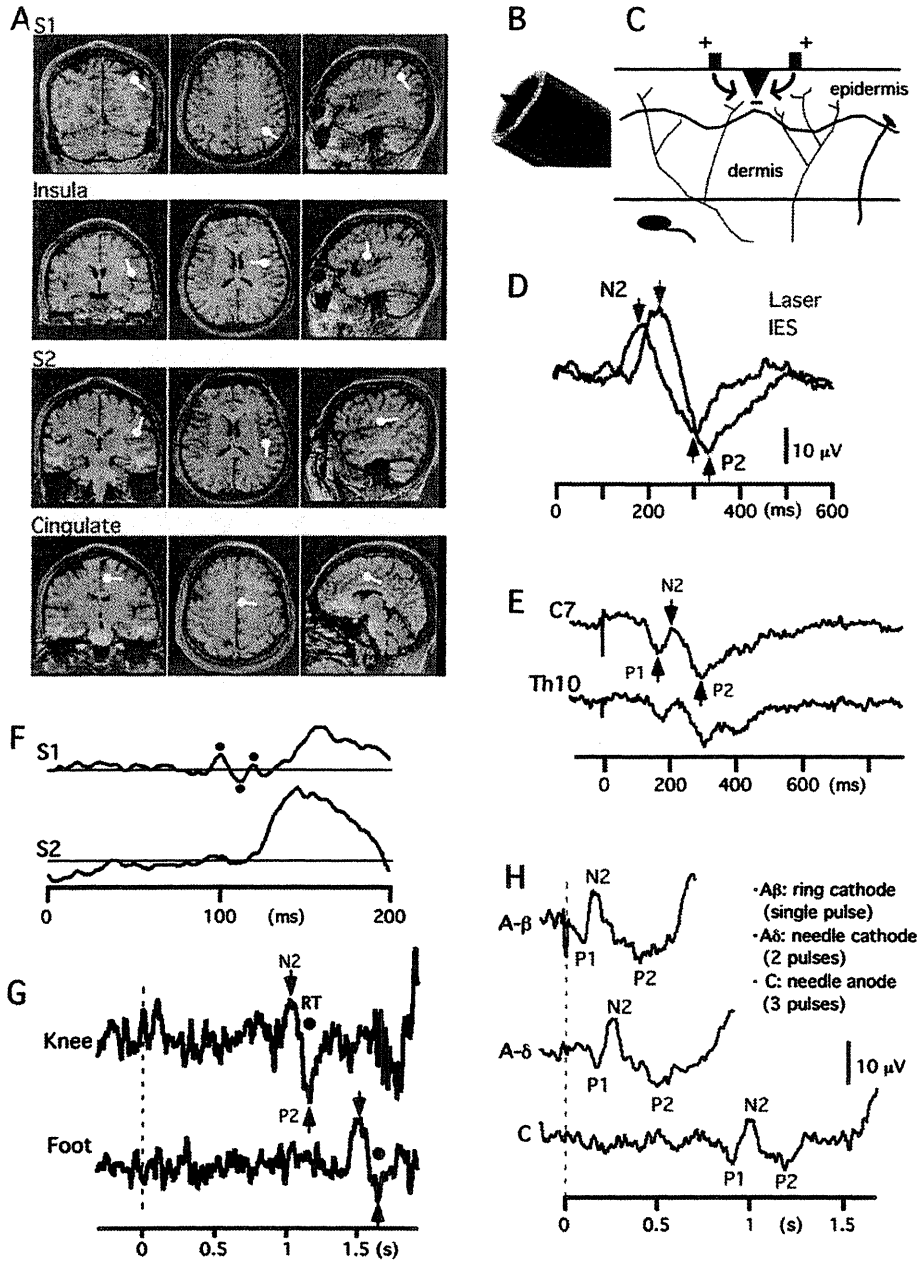

Figure 1 Use of intraepidermal electrical stimulation (IES) for studies on human pain perception. (A) Cortical activity detected by magnetoencephalography (MEG) in the primary somatosensory cortex (S1), secondary somatosensory cortex (S2), insula and cingulate cortex following IES to the left hand. (B) 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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Cortical responses to IES and conduction velocity

Cortical responses to IES are recorded using an evoked potential (EP) as large vertex potentials consisting of a negativity (N2) and a positivity (P2),[6] sometimes preceded by an earlier positivity (P1).[13] Figure 1D compares N2/P2 following IES of the hand and following stimulation of Aδ nociceptors by a CO_2 laser at the same site. The waveform is very similar but with a latency difference of 40 ms due to the temperature conduction time in the skin for laser stimulation.[3] Therefore, IES and laser evoked potentials are almost the same. As the latency of the somatosensory vertex potentials depends on the time taken for the signals to reach the brain, the type of peripheral nerve can be roughly estimated based on N2/P2 latency. For example, in the hand area, stimulation of Aβ fibres results in N2 peaking at 140 ms while N2 for Aδ and C nociceptor stimulation peaks at about 200 ms and 800~900 ms, respectively.

Responses in the somatosensory cortex are also very similar between IES and laser stimulation—that is, in studies using MEG, S1 in the hemisphere contralateral to the stimulation and S2 of both hemispheres are activated.[11 14 15] When the time delay for laser beams due to temperature conduction is taken into consideration, the response latency of each cortical activity is almost the same. In addition, the temporal profile of the IES induced cortical response is similar to that evoked by high intensity electrical stimulation in a patient who has no Aβ fibres due to sensory neuropathy.[16]

As the latency of EP components is longer following IES of a distal rather than a proximal site due to the distance travelled, peripheral conduction velocity (CV) can be calculated by dividing the difference in latency between the EP components by the distance between the two sites. With this method, mean CV was 15.1 m/s using EPs (hand and upper arm)[6] and 15.6 m/s using MEG (hand and elbow).[7] Both values are within the range for Aδ fibres (4~30 m/s), as measured by microneurographic studies.[17 18]

In summary, IES elicits a sharp pricking sensation without other sensations via peripheral signals ascending through Aδ fibres, and produces cortical responses that are almost the same as those evoked by laser stimulation. Both the painful pricking sensation and evoked brain responses (Aδ fibre latency) are abolished by local application of lidocaine. These findings suggest that IES selectively activates Aδ nociceptors. Recently, a European group verified this by showing that: (1) after the selective denervation of capsaicin sensitive nociceptors by 72 h application of a capsaicin cream, IES evoked cortical responses were almost abolished, and the threshold for detecting IES increased markedly (0.09 vs 0.6 mA) compared with controls; (2) when the conduction of myelinated nerve fibres was selectively blocked by compression, the time course of the blockade of responses to IES followed closely the time course of the blockade of Aδ fibre responses to laser stimulation; and (3) IES with a high current (2.5 mA) coactivated Aβ-fibres.[19] In some studies, a similar concentric bipolar electrode without a needle was used to elicit a painful sensation.[20] Stimulation with this electrode is easy to control and less invasive than IES but it activates Aβ fibres.

MERITS OF IES

IES has several advantages over other noxious stimuli and should contribute to progress in pain studies. We next present some of the studies taking advantage of IES.

Selective activation of Aδ nociceptors without the need for expensive equipment

The stimulus is easy to control and requires no specialised skills. In certain clinical patients, the assessment of small fibre function is important. However, because few hospitals have a laser stimulator, mechanical stimuli are often used even though they lack nociceptive selectivity. As IES is very simple, it seems useful for clinical tests. A new portable stimulator weighing just 290 g and specialised for IES (PNS-7000; Nihon Kohden) should enhance its use in clinics. When starting IES with a current of 0.01 mA and increasing the stimulus in steps of 0.01 mA, a weak pricking sensation occurs at the threshold. As there is no other sensation below the threshold, the threshold for Aδ nociceptor activation can be easily assessed. Therefore, IES is expected to detect functional changes in peripheral small fibres. To test this possibility, we examined the effects of lidocaine tape on pain threshold and EPs.[12] As expected, local application of lidocaine significantly elevated the pain threshold, and almost abolished EPs while effects on tactile sensation and tactile EPs were very small.

One possible use of IES is for so-called small fibre neuropathy. The diagnosis of small fibre neuropathy is often difficult because small fibres are invisible in routine nerve conduction studies. For example, in diabetic patients, disturbances begin in small fibres in the distal part of the limb. Our recent study[21] confirmed the usefulness of IES for evaluating small fibre function in diabetic patients. As another example, Obayashi *et al*[22] recently reported a case of domino liver transplantation induced amyloid neuropathy. The patient, due to sclerosing cholangitis, underwent a domino liver transplantation reusing a resected liver from a patient with familial amyloid polyneuropathy. When thermohypesthesia and hypoalgesia appeared 7 years after the transplantation, results of neurological examinations, including tests of tendon reflexes, vibration sense, proprioception and nerve conduction, were all normal but the Aδ nociceptor pain threshold by IES was elevated. This report suggests that a follow-up examination of small fibre function is important for such patients and IES can serve this purpose.

IES can be applied to any part of the body

With most MEG or fMRI machines, applying laser beams to areas other than the limbs is difficult. Therefore, if we want to stimulate these cutaneous sites and record brain responses, IES is useful. A recent study showed that cortical magnetic responses are clearly recorded following IES to various areas, including the neck, face and back (Omori and Isose, unpublished data). The use of IES would stimulate pain studies using fMRI. Yoshino *et al*[23] reported that IES could be used safely in an fMRI room and evoked clear brain activity detectable as haemodynamic changes.

By recording EPs following stimulation of two cutaneous sites, we can measure the CV of the periphery as well as in the spinal cord. This information may be useful for certain clinical cases such as demyelinating diseases. The CV in the spinothalamic tract can be estimated by stimulating two different levels of the back midline. As the peripheral conduction distance is short and similar between two sites, the latency difference is due to the conduction time difference in the spinal cord. However, because N2/P2 is an endogenous EP component, its latency and amplitude are affected by the subject's internal state. To reduce this undesired effect, random stimulation of the two sites is useful. Figure 1E shows an EP recording following stimulation of the back at the C7 and Th10 vertebral spinous process levels. In this case, very similar waveforms were evoked although that for the distal site had a longer response latency. The calculated CV is 13.5 m/s for P1 and 13.0 m/s for N2. The results indicate that the CV is similar between the periphery and spinal cord, which is consistent with the results of studies using laser stimulation.[24–26] Although N2/P2 is an endogenous component

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common to all sensory modalities,[14 27] it is easily recorded and useful for estimating CV or task related EP components.[28]

Steep rise in stimulus intensity

As IES is an electrical method, it provides a good time locked stimulus, which is important when analysing responses in the order of milliseconds. Several studies have taken advantage of this. In an MEG study investigating early responses to IES in S1,[11] the early S1 activity was a sharp transient of approximately 80~100 ms following IES and reversed its polarity once or twice after a 10 ms interval similar to the 20/30 ms component evoked by tactile stimulation of the hand (figure 1F), a common feature of the primary response among sensory modalities (for visual and auditory systems, see Inui *et al*[29 30]). Because of the polarity reversing nature of primary cortical responses with a 10 ms interval, a small latency jittering of 10 ms of peripheral activation among each trial is enough to cancel out the response. In fact, no studies using laser stimulation have detected early cortical activation except one by Wang *et al*[31] in which jittering of the response was corrected for each trial. As another example, there are two studies using a pair stimulation paradigm.[8 32] When one wants to deliver two different stimuli at various conditioning test intervals, the timing of the onset of peripheral activation is particularly important. In an MEG study by Inui *et al*,[8] cortical responses to paired noxious (IES) and innocuous (conventional transcutaneous electrical stimulation) stimuli applied to the back at 11 conditioning test intervals of −500~500 ms were recorded to reveal cortical mechanisms underlying pain relief by tactile inputs. Results showed that IES induced responses were markedly inhibited when transcutaneous electrical stimulation was applied 20~60 ms later and 0~500 ms earlier than IES. Based on the time taken for each signal to reach the spinal cord and the cortex, we concluded that cortical responses to IES can be inhibited by innocuous tactile stimuli at the cortex without a contribution at the spinal level.

Minimal discomfort and possible use in animal studies

IES evokes clear cortical responses at a weak current around twice the threshold. However, at this intensity, some subjects report that the stimulus is not painful at all (visual analogue scale 0~1). This means that the IES evoked sensation is a pure noxious sensation with minimal discomfort—that is, pain. Indeed, IES evoked sensations are very weak compared with the uncomfortable feeling caused by conventional transcutaneous electrical stimulation at a painful intensity. Unless the aim of the study is discomfort, a less uncomfortable stimulus is better with respect to the ethical restrictions of studies on the nociceptive system. In an MEG study by Wang *et al*[33] investigating the effects of sleep on IES induced cortical responses, subjects were rarely awakened by IES at an intensity high enough to obtain clear cortical responses before sleep.

In animal studies, the use of mechanical stimuli, such as pinching the tail, is common. However, such a stimulus inevitably coactivates Aβ mechanoreceptors. Therefore, if possible, selective stimulation is desirable. In spite of its usefulness for pain studies in humans, laser stimulation is rare in animal studies. One reason is the cost of laser machines but another may be the difficulty of applying laser beams to freely moving animals. In addition, adjusting the laser energy to an appropriate strength is difficult. IES can be applied at any time without immobilising the animal once the electrode is attached to the skin.

For research into the animal nociceptive system, one Australian group used IES. In awake dogs, van Oostrom *et al*[34] recorded EPs following IES to the hind paw. Results showed that: (1) the amplitude of the N2/P2 components increased with an increase in stimulus intensity (0.2~1.0 mA); (2) CV was 5~20 m/s; and (3) when the stimulus intensity was increased, there were mild behavioural reactions, withdrawal of the stimulated hind paw and lip licking. It is worth noting that a clear EP recording is possible in awake animals and, in addition, the behavioural response is mild when IES evokes clear cortical responses.

WEAK POINTS OF IES

As nociceptive free nerve endings are located in the epidermis while the other thicker fibres run more deeply in the dermis, the current passing through the electrode should be spatially restricted to the superficial layer of the skin. In other words, IES activates tactile mechanoreceptors in the dermis when the current is too strong. In fact, results of a study by Mouraux *et al*[19] using a nerve conduction blockade showed that IES at 2.5 mA activates Aβ mechanoreceptors in addition to Aδ nociceptors. Therefore, one cannot use a strong current even when intense sensations of pain are necessary. Usually, the threshold for stimulation of Aδ nociceptors by IES with double pulses is below 0.1 mA, and 2~3 times the threshold is enough to obtain clear cortical responses. At around this intensity, IES selectively activates Aδ nociceptors. However, one would consider the painful sensation to be too weak at this intensity. For a stronger sensation, spatial summation by use of multiple electrodes or temporal summation by a long duration pulse or pulse train should be considered instead of an increase in intensity.

STIMULATION OF C FIBRE BY IES

Now we shall describe our recent attempt to selectively stimulate C nociceptors by IES. Because of the very high electrical threshold, it is difficult to stimulate C nociceptors selectively by conventional transcutaneous electrical stimulation. Although in isolated nerves of animals a specific method such as anodal blocking[35] can be used for this purpose, such an invasive technique cannot be applied to humans. Based on differences in the distribution of C and Aδ nociceptors (Aδ <C), Plaghki's group reported the successful stimulation of C nociceptors by laser beams for the first time.[36] They stimulated a tiny area of the skin with a laser beam that is expected to hit C nociceptors exclusively, and the results supported this. The difference in the threshold of the response to thermal stimuli between C (40°C) and Aδ (46°C) nociceptors is also useful for the selective activation of C nociceptors by laser beams. Tran *et al*[37] successfully stimulated C nociceptors by employing a low energy laser beam to a tiny skin area.

As for electrical stimulation, the selective activation of C nociceptors seems impossible because of the high electrical threshold. However, the higher density of C nociceptors might be an advantage if the current passing through the concentric electrode is limited to a very small area. In addition, there are several reports of factors that are effective at activating C fibres: a pulse of long duration, a slowly rising pulse, temporal and spatial summation, and anodal stimulation (see Otsuru *et al*[10]). Based on these reports, we tested IES for the selective stimulation of C nociceptors under the following conditions: (1) the anode was the inner needle and the cathode was the outer ring; (2) the electric pulse was a triangular wave with a rise and fall time of 1 ms; (3) the stimulus was a train of three pulses at 50 Hz; and (4) three electrodes 10 mm apart were used.[10]

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Sensations

IES elicits weak sensations with a reaction time of approximately 1 s following stimulation of the hand area. The sensory threshold is approximately 0.04 mA. The sensation evoked varies among subjects or among penetrations but is usually the feeling of a light touch and sometimes pricking or slight burning, which is similar to the sensations evoked by a low intensity laser beam applied to a tiny area of the skin.[37 38] A warm or itchy sensation is very rare. Under these conditions, there is no clear axonal reflex flare reaction mediated by mechanoinsensitive C nociceptors.[39] We consider IES to activate polymodal C nociceptors.[40] Therefore, the electrical threshold for the stimulation of C nociceptors is not as high as generally considered.

Conduction velocity

Similar to Aδ nociceptors, CV can be measured by recording EPs. Because of the long reaction time (RT) for C nociceptor stimulation, RT can be also used to estimate CV. Figure 1G shows an example of EPs following stimulation of the foot and knee. Note the very late N2/P2 component compared with Aδ stimulation. The distance between the two stimulated sites was 43 cm and the latency difference was 392 ms for N2, 404 ms for P2 and 440 ms for RT, which yielded a conduction velocity of 1.0~1.1 m/s. The mean CV following stimulation of the hand and forearm among eight subjects was 1.5 m/s.[10]

Stimulation of Aβ, Aδ and C fibres through the same electrode

As IES stimulates Aδ nociceptors when the inner needle is the cathode, Aδ and C nociceptors can be stimulated with one electrode by switching the polarity. Figure 1H shows an example of hand stimulation. First, we recorded C responses with anodal stimulation. The peak latency of N2 was about 1 s. Then the polarity was switched. The cathodal stimulation now evoked Aδ responses with N2 peaking at 200 ms.

As the outer ring of the IES electrode is attached to the skin, Aβ mechanoreceptors can be activated by standard monopolar stimulation through the outer ring. The waveform in response to Aβ stimulation in figure 1H has a well known N2 component peaking at 140 ms. In this example, each type of stimulation evoked very similar potentials, consisting of P1, N2 and P2 components. If we want to stimulate different nerve fibres at the same cutaneous site, this method seems useful and easy.

FUTURE PERSPECTIVES

We have reviewed studies using IES. We believe that it is useful for basic research as well as clinical tests, and will help us to better understand the physiology of the nociceptive system, pathology of pain related disorders or mechanisms of the analgesic effects of drugs. To validate the usefulness of IES and to establish normative data, however, studies using a large group of normal subjects are necessary. For clinical testing, IES can be used for pain disorders such as fibromyalgia as well as small fibre neuropathies such as diabetic neuropathy. IES seems particularly suitable as a screening test because it can be used easily in a clinical setting. As for the stimulation of C nociceptors by IES, however, we feel that there is still room for improvement to obtain responses good enough for clinical testing.

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Contributors Both authors contributed to this study and qualify as authors in accordance with the ICMJE guidelines for authorship.

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総説

痛みと痒みの脳内認知機構

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Mechanisms of Intracerebral Pain and Itch Perception in Humans

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Abstract

Electrophysiological studies involving techniques such as magnetoencephalography (MEG) and hemodynamic studies involving techniques such as functional magnetic resonance imaging (fMRI) have recently been intensively used to elucidate the mechanisms underlying pain and itch perception in humans. The MEG results obtained after A-delta fiber (first pain) and C fiber (second pain) stimulation were similar, except for longer latency in the case of C fibers. Initially, the primary somatosensory cortex (SI) contralateral to the stimulation is activated, and the secondary somatosensory cortex (SII), insula, amygdala, and anterior cingulate cortex (ACC) in both hemispheres are then activated sequentially. The fMRI findings obtained after the stimulation of C fibers and those obtained after the stimulation of A-delta fibers both showed activation of the bilateral thalamus, bilateral SII, right (ipsilateral) middle insula, and bilateral Brodmann's area (BA) 24/32, with most of the activity being detected in the posterior region of the ACC. However, the magnitude of activity in the anterior insula on both sides and in BA 32/8/6, including the ACC and pre-supplementary motor area (pre-SMA), after the stimulation of C nociceptors was significantly stronger than that after the stimulation of A-delta nociceptors.

We have recently developed a new stimulation electrode that causes an itching sensation via electrical stimulation applied to skin. The conduction velocity (CV) of the signals caused by this stimulation is approximately 1 m/sec in a range of CV of C fibers. The findings obtained after itch stimulation were similar to those obtained after pain stimulation, but the precuneus may be an itch-selective brain region. This unique finding was confirmed by both MEG and fMRI studies.

Key words：pain, itch, magnetoencephalography (MEG), functional magnetic resonance imaging (fMRI)

はじめに

ヒト脳内での痛みと痒みの認知機構の研究は，極めて重要なテーマであるにもかかわらず，種々の技術的困難のために遅々として進まなかった。ヒトを対象とする場合，非侵襲的検査を用いなければならないことが最大の理由である。しかし，近年の科学技術の急速な進歩によって，従来から行われてきた脳波に加え，ポジトロン断層撮影（positron emission tomography：PET），機能的磁気共鳴画像（functional magnetic resonance imaging：fMRI）および脳磁図を用いた研究発表が増加してきた。脳磁図は時間分解能が高いため初期反応の時間的情報を得るのに適しており，fMRIは空間分解能が高いため詳細な活動部位の解析に適している[1,2)]。本稿では，痛みと痒みに分けて，それらに関連した脳活動について

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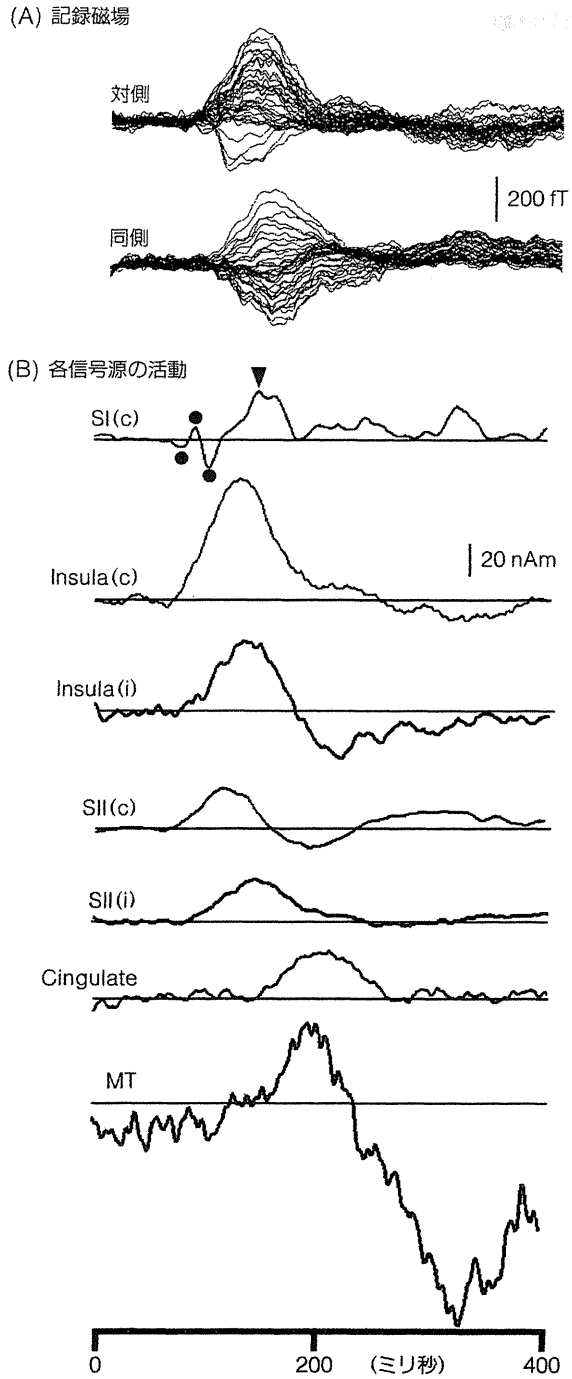

Fig. 1　Aδ線維を上行する信号による脳磁図反応（SI，SII，島，前部帯状回および内側部側頭葉の活動）

A：記録磁場波形，B：各信号源の活動時間経過。
〔略語〕SI：第1次体性感覚野，SII：第2次体性感覚野，MT：mid-temporal area（内側部側頭葉；扁頭体，海馬付近），c：刺激対側半球，i：刺激同側半球
Inui K, Tran DT, Qiu Y, Wang X, Hoshiyama M et al: A comparative magnetoencephalographic study of cortical activations evoked by noxious and innocuous somatosensory stimulations. Neuroscience **120**: 235-248, 2003 より転載

脳磁図とfMRIを用いた筆者らの最近の研究成果を紹介したい。

Ⅰ．痛みの脳内認知機構

1．脳磁図を用いた研究

まず，Aδ線維を上行するファーストペイン（first pain）についてまとめる。最近筆者らは表皮内の自由神経終末（痛覚刺激を受容する）だけを選択的に刺激する方法，皮内電気刺激法（intra-epidermal stimulation：IES法）を考案したので，本稿ではこれを用いた研究を紹介したい[3,5]。IES法は，針の部分が約0.2ミリの押しピン型電極を用いて表皮内を電気刺激（0.5ミリ秒，0.1～0.3 mA）する方法で，表皮内に位置する自由神経終末を選択的に刺激することができる。自由神経終末が表皮内に分布するのに対し，触覚に関わる機械受容器は表皮最深部もしくは真皮に分布するためである。従来から行われているレーザー光線を用いた方法に比し，特殊な機器が必要ではないこと，電気刺激であるのでタイムロック（time-lock）が非常によいこと，刺激電極が表皮内にとどまるため，刺入時の痛みや出血がほとんどないことなどの長所があり，今後は広く普及していくことが予想される。

手背刺激によって約100ミリ秒を頂点とする微弱な活動が第1次体性感覚野（primary somatosensory area：SI）領域に認められる（Fig. 1）。触覚刺激に対するSI反応に比べて反応が非常に小さい。おそらく主に刺激部位の同定のみに関わっていると考えられる。このSI初期成分に続いて，約20ミリ秒遅れて第2次体性感覚野（secondary sematosensory area：SII）が活動を始める。両側反応であり，刺激同側の反応が10～20ミリ秒遅れる。SIIの活動と平行して島の活動（両側性）がみられる。したがって視床—SI—SIIの経路と，これとは別の視床—島の経路が存在することになる。それぞれの機能についてはまだ明らかにされていないが，異なる役割を担っているものと考えられる。

例えばSIIに病変のある患者では刺激が痛みであることが判別できないのに対し，島に病変のある患者では，刺激が痛みであることはわかるにもかかわらずそれに応じた情動反応や刺激部位を刺激から遠ざける行動が欠落している。したがってSIIは侵害性刺激の性質認知に関わり，島はその情動的認知に関わるのではないかと推察される。

刺激後200～300ミリ秒の脳活動を解析すると，前部帯状回（anterior cingulate cortex：ACC）と内側部側頭葉（mid-temporal area：MT；扁桃体，海馬を含む）に活動が推定される（Fig. 1）。前内側部側頭葉の活動は島

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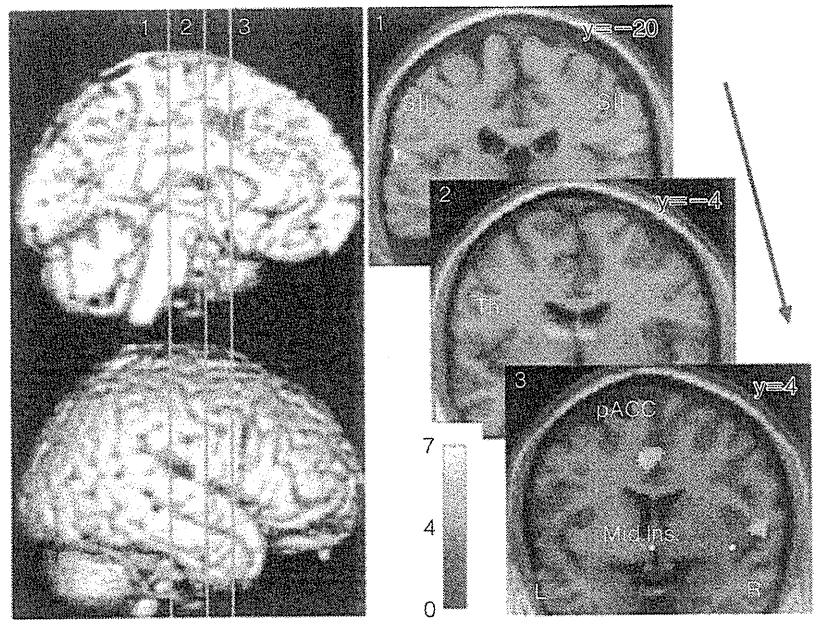

Fig. 2　Aδ線維刺激とC線維刺激に共通して有意差を示した部位
左図の3本の垂直線は各々の冠状断面を示す。
〔略語〕SII：第2次体性感覚野，Th.：視床，pACC：前部帯状回の後部，Mid. Ins.：島中央部
Qiu Y, Noguchi Y, Honda M, Nakata H, Tamura Y et al: Brain processing of the signals ascending through unmyelinated C fibers in humans: an event-related fMRI study. Cereb Cortex **16**: 1289-1295, 2006 より転載

の活動の頂点付近で開始しており，またこの部位は島からの強い投射を受けていることから，われわれは，視床―島―前部帯状回および前内側部側頭葉，の経路を推定している。視床―SI―SII の経路が刺激の意味判別的な側面(刺激の部位，強さ，種類)に関わり，視床―島―前部帯状回および前内側部側頭葉，の経路が情動面や刺激に対応する行動に関わるのではないかと考えられる。痛覚情報処理経路を2分する古典的な概念に従えば，前者がlateral systemに，後者がmedial systemに相当する。

次にC線維を上行するセカンドペイン(second pain)について述べる。C線維の特徴として，Aδ線維に比して興奮閾値が低く末梢皮膚での受容体密度がはるかに高いことが挙げられる。最近われわれは，特殊なアルミニウム製の薄いプレートを作成した。これは，厚さ0.1 mmで，プレート中央部の25 mm四方の部分に，1 mmごとに縦横26列の小さな穴(直径0.4 mm，面積0.125 mm^2)を穿ったものである。これを皮膚上に置いてレーザー光線を照射することにより，容易にC線維を選択的に刺激することが可能となった[6,7]。計算された末梢神経伝導速度は約1～2 m/秒であり，脊髄伝導速度は約1～4 m/秒であった。

脳磁図記録では，ほぼAδ線維刺激による場合と類似の反応を示し，SI，SII―島，帯状回，内側部側頭葉(MT)の活動がみられた。もちろん伝導速度が遅いため初期反応の頂点潜時は約750ミリ秒とかなり長い。C線維刺激による脳波，脳磁図反応の特徴的な変化は，覚醒度の変化と注意効果による変化が極めて大きいことである。この結果は，セカンドペイン，すなわち内臓痛や癌性疼痛に対して心理療法の効果が大きいことを示唆する興味ある所見である。

2．fMRIを用いた研究

前述した刺激方法を用いて，Aδ線維とC線維を記録し，事象関連fMRIを記録した[9]。驚くべきことに，C線維刺激による場合のほうがAδ線維刺激時よりも活動が大きかった。2種類の刺激に対して共通して活動する部位は，両側の視床，SII，右側の中部島，両側のBrodmannの24/32野［前部帯状回の後部（posterior part of anterior cingulate cortex：pACC）が主］であり，これらが痛覚刺激に対して常に活動する部位と考えられた（**Fig. 2**）。次に2種類の刺激間に有意な差がみられた部位を解析したところ，右側半球のBrodmannの24/32/8野[前部帯状回の前部（anterior part of anterior cingulate cortex：aACC）の背側と補足運動前野（pre supplementary motor area：pre-SMA)］と両側の島前部において，C線維刺激の場合に有意に活動が大きいことがわかった（**Fig. 3**）。セカンドペインに関連すると考えられるC線維刺激に対してpACCの背側の活動が有意に大きい，という結果は，セカンドペイン認知がファーストペイン認知よりも情動に関係が強いことを示唆している。

最近，われわれは，情動と痛覚認知に関してfMRIを用いて研究を行っている。例えば，実際に痛みを与えられなくても，注射のような「痛そうな画像」をみただけで，pACCと島が活動することを明らかにした[10]。これは

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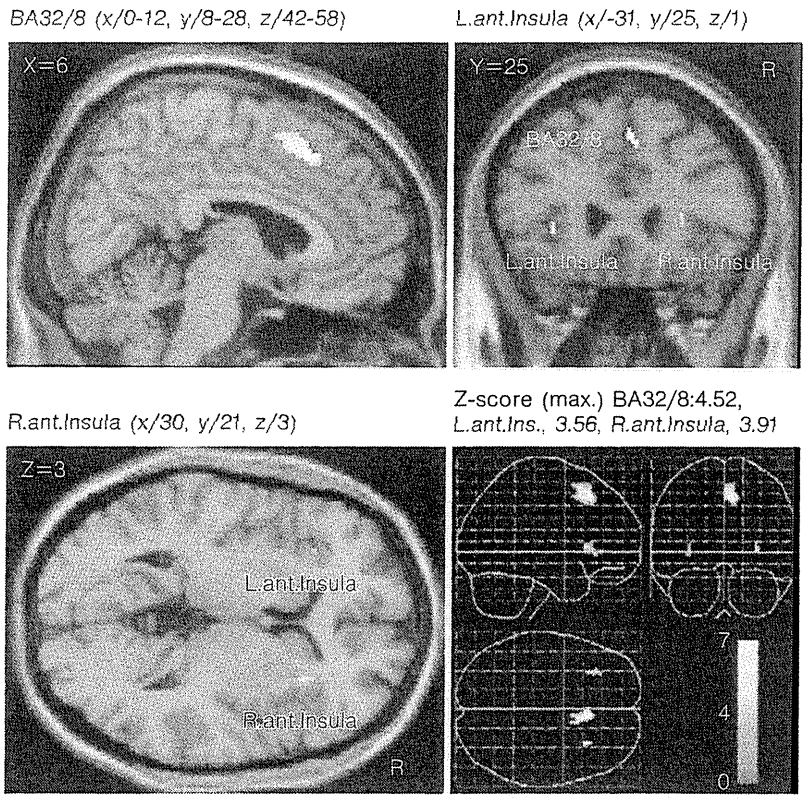

Fig. 3　C線維刺激による活動がAδ線維刺激による活動より有意に大きかった部位

両側の島全部，前部帯状回と pre-SMA に有意差がみられた。

〔略語〕BA：Brodmann 野，L. ant. insula：左側前部島，R. ant. insula：右側前部島

Qiu Y, Noguchi Y, Honda M, Nakata H, Tamura Y et al: Brain processing of the signals ascending through unmyelinated C fibers in humans: an event-related fMRI study. Cereb Cortex **16**: 1289-1295, 2006 より転載

「心の痛み」と「実際の痛み」は辺縁系では同じように活動することを示しており興味深い。また，瞑想中には痛みを感じないヨガの達人では，瞑想中に痛み刺激を与えても，視床，SII，島，帯状回の活動はみられず，前頭葉，頭頂葉，中脳に活動がみられた[11]。これらの部位，特に中脳は下行性痛覚抑制系に重要な部位と考えられており，ヨガの達人では，瞑想中はなんらかの機序によって下行性痛覚抑制系が最大限に活性化されるために，痛みを感じないのだろうと推測した。

3．そのほかの最近の研究

痛覚認知に関しては，上記のようなイメージングを利用した研究以外にも，最近，いくつかの興味ある知見を得ている。そのうちのいくつかを簡単に紹介したい。

触覚刺激が痛覚認知を抑制するという，いわゆる関門制御説（gate control theory）は，発表当初から，その責任部位に関して議論が続いてきた。提案者である Melzack と Wall[11] は脊髄を責任部位としているが，われわれは脳磁図を用いた詳細な研究によって，大脳皮質が責任部位だと考えられる結果を得た[12]。

動物実験では，脊髄視床路の伝達経路が複数あることが報告されてきたが，ヒトでは証明されていなかった。脳磁図を用いた実験によって，ヒトの Aδ 線維を上行する痛覚信号を伝導する脊髄視床路の伝達経路には少なくとも 2 種類があり，伝導速度が約 17 m/秒のものと，約 10 m/秒のものがあることを証明した。前者は SI に到達し，後者はシルヴィウス裂周辺に到達すると考えられる知見を得た[13,14]。

動脈の圧受容器が痛覚認知に影響するか否かを痛覚関連誘発脳波を用いて解析した。収縮期には脳波の振幅は拡張期よりも有意に低下していることがわかり，動脈の圧受容器が痛覚認知に影響を及ぼすという仮説が立証された。これは英国バーミンガム大学との共同研究の成果である[15]。

ヒトでの痛覚認知における頭頂葉後部（posterior parietal cortex：PPC）の役割について，SI と SII の活動との関連を含めて詳細に解析した。PPC の活動はおそらく SI の活動に引き続いて現れ，PPC の中でも下頭頂小葉（inferior parietal lobule）（Brodmann の 40 野）が痛覚認知に重要であることを発見した[16]。

喫煙（ニコチン）には鎮痛効果があることが，動物実験では推測されていたが，ヒトではいまだ証明されていなかった。われわれは，痛覚関連誘発脳波を用いて，血中ニコチン濃度，自覚的な痛みの程度などを詳細に解析

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した。すると，喫煙（ニコチン）はAδ線維を上行するファーストペインに対しては有意に鎮痛効果を示したが[17]，C線維を上行するセカンドペインに対しては無効であることがわかった[18]。世界で初めて明らかにされた興味深い結果であった。

特殊な針電極（IES電極）を用いた実験に関しては既述したが，最近われわれはさらに研究を進めており[19]，C線維を選択的に刺激できるようになった[20]。現在，実用化に向けて準備を進めている。

Ⅱ．痒みの脳内認知機構

痒みは掻きむしらずにはいられない不快な体性感覚である。特に，アトピー性皮膚炎など難治性の痒みの場合，慢性的に生じる激しい痒みが患者の心身に大きなダメージを与える。現在，このような難治性の痒みを抑える効果的な治療法開発を目指したさまざまな研究が行われている。その多くは皮膚など末梢に注目したものであり，これまで脳はほとんど注目されてこなかった。しかしながら，脳が痒みの認知において重要な役割を果たすことから，脳も痒みの治療法開発につながる重要な研究対象であると考えられるようになった。このような背景のもと，近年，PET，fMRI，脳波や脳磁図などを用いた痒みの脳科学的研究が急速に発展してきた。本稿では，これまでの研究で明らかになった痒みの脳内認知機構について概説する。

1．PETとfMRIを用いた痒みの研究

ヒスタミンなどの掻痒物質がC線維を刺激すると，その情報が，脊髄視床路を介して，脳へ伝達される[21,22]。さらに，その情報はさまざまな脳部位で情報処理を受け，“痒み”という感覚がつくり出される。これまでのPETやfMRIを用いた研究から，前頭前野，帯状回，島，体性感覚野，運動関連領域（運動野，運動前野，補足運動前野），頭頂葉，線状体，視床，小脳などの脳部位が，痒み刺激によって活動することが明らかとなった[23-26]。

これらの脳部位は，痛み刺激によっても活動することから，痒みと痛みの脳内メカニズムは多くの類似点を持つと考えられている。刺激が加わった身体部位やその強さの認知は，主に，SIとSIIが関係すると考えられている。頭頂葉や運動関連領野の活動は，痒みを感じる身体部位を把握し，掻きむしることによって痒みを取り除こうとする運動準備を反映すると考えられる。

また，痒みや痛みによって生じる不快感は，主に，前部帯状回や前部島が関係すると考えられている。大変興味深いことに，後部帯状回と後部島は，痛みよりも痒みに対してより強く反応することが報告されている[18]。また，これら脳部位の神経活動は痒みの主観的スコアと有意な相関関係を示したが，痛みではそのような相関関係を示さなかったという報告もある[27]。

一方，痒みよりも痛みにより選択性のある脳部位もある。例えば，Drzezgaら[24]は，視床やSIIは，痒み刺激に対してあまり反応しないと報告している。実際，これら脳部位の痒み刺激に伴う神経活動を観測した先行研究は少ない。一方，視床とSIIは，痛みの認知において重要な役割を果たすと考えられており，ほとんどの痛みの脳機能画像研究で，これら脳部位の痛み刺激に伴う神経活動が観測されている[28]。Herdeら[29]は，前部帯状回膝下部や扁桃体は痛み刺激によって活動亢進するが，痒み刺激では活動低下すると報告している。後部帯状回，後部島，視床，SII，前部帯状回膝下部や扁桃体の痒み刺激と痛み刺激に対する反応の違いは，一部，痒みと痛みの感覚の違いに関係すると推察される。

2．アトピー性皮膚炎患者を対象としたPET，fMRI研究

以上の研究は，主に，健常者を対象として行われた研究であるが，アトピー性皮膚炎患者を対象とした痒みの脳機能画像研究も行われている。例えば，Schneiderら[30]は，痒み刺激に対する線条体や視床の反応は，健常者よりも，アトピー性皮膚炎患者のほうが強いと報告している。線条体は運動制御や報酬系に関係することから，アトピー性皮膚炎患者における線条体の過活動は，“掻破をとめられない”，あるいは，“掻きむしりたいという欲求を止められない”といった現象（イッチ・スクラッチ・サイクル）に関係すると推察されている。

Ishiujiら[31]は，arterial spin labeling（ASL）法というMRI装置を用いた新しい撮影方法を用いて，痒み刺激時の脳活動を健常者群とアトピー性皮膚炎患者群で比較し，痒み刺激に伴う後部帯状回と楔前部の反応において，両群に有意差があることを報告した。後部帯状回と楔前部の痒みの認知における機能的役割はまだ不明であるが，後部帯状回は痒みに選択性があること，後述するように楔前部も痒みに選択性を持つことを考慮すると，アトピー性皮膚炎患者における痒みの認知機構を理解するうえで，これらは今後注目すべき脳部位かもしれない。

3．脳波と脳磁図を用いた痒みの研究

PETやfMRIを用いた脳機能画像研究は，どの脳部位が痒み刺激によって活動するかを明らかにした。しかし

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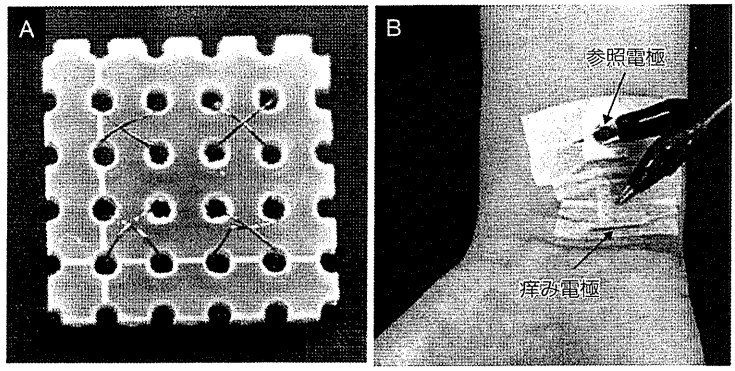

Fig. 4　通電性痒み刺激
A：痒み電極，B：痒み電極を使って手首を通電刺激している様子。
Mochizuki H, Inui K, Yamashiro K, Ootsuru N, Kakigi R: Itching-related somatosensory evoked potentials. Pain **138**: 598-603, 2008 より転載

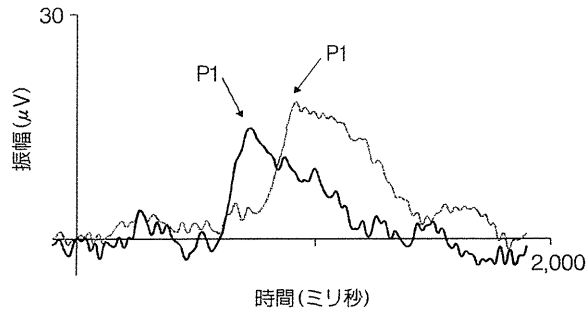

Fig. 5　痒み刺激誘発電位
通電性痒み刺激を用いて手首（灰色）と前腕前面（黒色）を刺激したときの痒み刺激誘発電位。記録部位：正中中心部（電極：Cz）。P 1：振幅の頂点。
Mochizuki H, Inui K, Yamashiro K, Ootsuru N, Kakigi R: Itching-related somatosensory evoked potentials. Pain **138**: 598-603, 2008 より転載

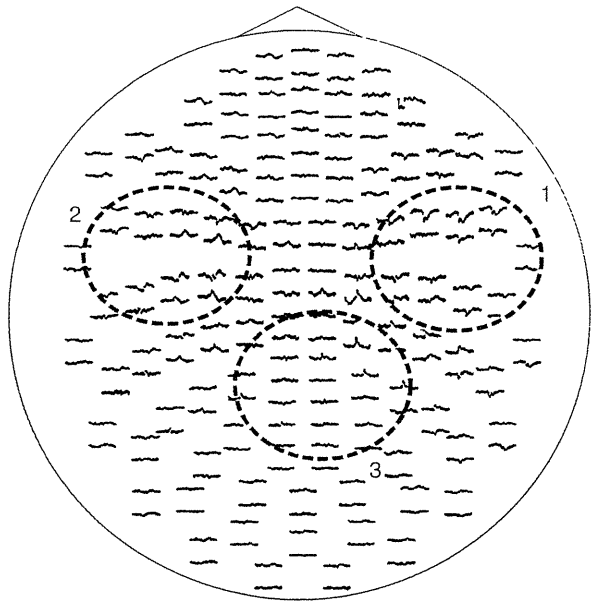

Fig. 6　痒み刺激誘発磁場
1：刺激対側の前頭一側頭領域（SII一島）（右半球）の磁場反応，2：刺激同側の前頭一側頭領域（SII一島）（左半球）の磁場反応，3：頭頂一後頭領域（楔前部）の磁場反応。
Mochizuki H, Inui K, Tanabe H, Akiyama LF, Otsuru N et al: Time course of activity in itch-related brain regions: a combined MEG-fMRI study. J Neurophysiol **102**: 2657-2666, 2009 より転載

ながら，それら同定された脳部位がどのような機能的なつながりを持っているのか，すなわち，痒みの脳内ネットワークについてはほとんどわかっていない。痒みの脳内ネットワークをとらえるためには，ミリ秒単位で脳内の神経活動を計測する必要がある。時間分解能が分あるいは秒単位の PET や fMRI ではそのような計測は不可能であるが，脳波や脳磁図であればミリ秒単位で脳活動を計測することができる。しかしながら，技術的制約のため，脳波や脳磁図を用いて痒みに関係する脳活動をとらえるためには，数十回以上繰り返し痒み刺激を与えなければならない。痒みの脳機能画像研究で主に用いられているヒスタミンでは，短時間に数十回の痒み刺激を繰り返し与えることは極めて困難である。

最近になって，Ikoma ら[32]が皮膚に電気刺激を与えることで痒みを誘発する刺激法を確立した。この刺激法の場合，1 秒程度の短い痒みを誘発することができるため，短時間に痒み刺激を繰り返し与えることができる。そこで，筆者らは，この方法を応用して痒み電極を作成し（Fig. 4），通電性痒み刺激に伴う脳反応（痒み刺激誘発電位）が計測できるのかどうかを脳波を用いて調べた。Fig. 5 に示すのは，痒み刺激誘発電位である。手首刺激時と前腕前面刺激時の頂点潜時（Fig. 5 の P 1）は，それぞれ，963 ミリ秒と 772 ミリ秒であった。

頂点潜時の時間差と，手首と前腕前面の距離から，通電性痒み刺激によって生じる痒みの伝導速度を推定した。その結果，約 1 m/秒であることがわかった[33]。一般的な C 線維の伝導速度が 0.4〜2.0 m/秒であることから，生理的な痒みと同様に，通電性痒み刺激による痒みの

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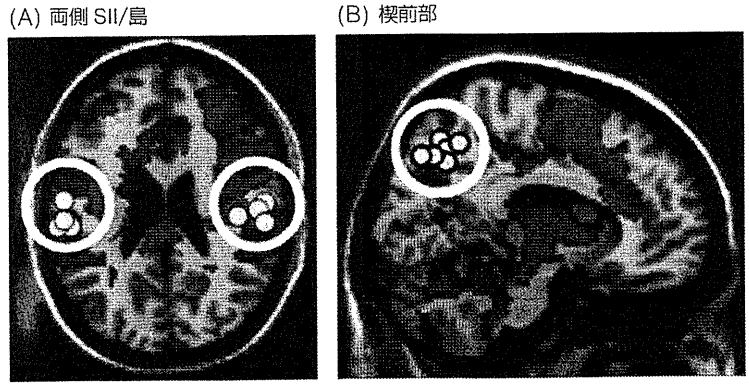

Fig. 7　MEG 実験で推定されたダイポールと fMRI 実験で活動した脳部位
A：両側前頭側頭領域の磁場反応に関係するダイポール（白丸）は両側の SII/島の神経活動に関係し，B：頭頂領域の磁場反応に関係するダイポール（白丸）は楔前部の神経活動に関係すると示唆された。MRI 画像中の濃灰色部分は fMRI 実験で痒み刺激に伴って活動亢進した脳部位。
Mochizuki H, Inui K, Tanabe H, Akiyama LF, Otsuru N, et al: Time course of activity in itch-related brain regions: a combined MEG-fMRI study. J Neurophysiol **102**: 2657-2666, 2009 より転載

情報がC線維によって伝達されることが明らかとなった。

さらに，筆者らは，痒みの脳磁図と fMRI 実験を行い，痒みに関係する脳部位の活動をミリ秒単位で計測した[34]。脳磁図実験では，2 つの痒み電極を左手首に取りつけて，通電時間 400 ミリ秒の痒み刺激を 100 回与えた。fMRI 実験では，通電時間を 5 秒とし，刺激回数を 30 回とした。今回の MEG 実験で計測された痒み刺激に関連する脳磁場反応(痒み刺激誘発磁場)は，両側の前頭一側頭領域と頭頂一後頭領域に認められた（**Fig. 6**）。

両側の前頭一側頭領域の磁場反応は，主に，SII や島(SII/島)の神経活動に関係し，頭頂一後頭領域の磁場反応は楔前部の活動に関係することが，脳磁図と fMRI データの解析で明らかになった（**Fig. 7**）。

一方で，痛みの脳磁図研究では，頭頂一後頭領域の磁場反応は SI や後部一頭頂葉の活動に関係すると報告されている[35]。痒みの脳磁図実験で観測された楔前部の活動は，この部位が痛みよりも痒みに選択性を持っていることを暗示している。

磁場反応の頂点潜時を脳部位間で比較した検討から，刺激対側の SII/島の潜時は同側 SII/島の潜時よりも有意に短いことがわかった[34]。この時間差は，脳梁を介した刺激対側 SII/島から刺激同側 SII/島への情報伝達に要した時間と考えられる。この結果から，視床一刺激対側 SII/島一刺激同側 SII/島といった神経ネットワークの存在が示唆された。また，楔前部の潜時は刺激対側 SII/島と刺激同側 SII/島の中間であった。解剖学的な線維連絡を考慮すると，楔前部の活動は，視床一刺激対側 SII/島一刺激同側 SII/島とは別の，独立した神経ネットワークを形成している可能性がある。

おわりに

痛みや痒みの研究はこれまでは末梢受容体と脊髄レベルでの動物実験が主流であったが，今後はヒト脳内での認知機構の研究がより盛んになっていくものと思われる。痛みや痒みの認知は極めて主観的であり，ヒトを対象としなければ理解が困難な点が大きいからである。

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In vivo patch-clamp analysis of dopaminergic antinociceptive actions on substantia gelatinosa neurons in the spinal cord

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ABSTRACT

To elucidate the mechanisms of antinociception mediated by the dopaminergic descending pathway in the spinal cord, we investigated the actions of dopamine (DA) on substantia gelatinosa (SG) neurons by *in vivo* whole-cell patch-clamp methods. In the voltage-clamp mode ($V_H = -70$ mV), the application of DA induced outward currents in about 70% of SG neurons tested. DA-induced outward current was observed in the presence of either Na^+ channel blocker, tetrodotoxin (TTX) or a non-NMDA receptor antagonist, CNQX, and was inhibited by either GDP-β-S in the pipette solution or by perfusion of a non-selective K^+ channel blocker, Ba^{2+}. The DA-induced outward currents were mimicked by a selective D2-like receptor agonist, quinpirole and attenuated by a selective D2-like receptor antagonist, sulpiride, indicating that the DA-induced outward current is mediated by G-protein-activated K^+ channels through D2-like receptors. DA significantly suppressed the frequency and amplitude of glutamatergic spontaneous excitatory postsynaptic currents (EPSCs). DA also significantly decreased the frequency of miniature EPSCs in the presence of TTX. These results suggest that DA has both presynaptic and postsynaptic inhibitory actions on synaptic transmission in SG neurons. We showed that DA produced direct inhibitory effects in SG neurons to both noxious and innocuous stimuli to the skin. Furthermore, electrical stimulation of dopaminergic diencephalic spinal neurons (A11), which project to the spinal cord, induced outward current and suppressed the frequency and amplitude of EPSCs. We conclude that the dopaminergic descending pathway has an antinociceptive effect via D2-like receptors on SG neurons in the spinal cord.

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1. Introduction

Dopamine (DA) is well known as a neurotransmitter and neuromodulator in the brain, not just as a precursor in the synthesis of other catecholamines. DA receptors are classified into two families: D2-like, incorporating D2 and the closely-related D3 and D4 receptors and D1-like which includes D1 and the closely-related D5 receptor (see reviews [33,50]). DA controls a variety of functions including locomotors activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation. Compared with the enormous literature devoted to DA actions in the brain, little is known about the role of DA in the spinal cord.

The first-order neurons transmitting pain impulses from the skin are of two types; small-diameter myelinated fibers of the Aδ group that are responsible for a fast or first pain and nonmyelinated C-fibers that give rise to a slow or second, duller, more diffuse type of pain. Noxious information is transmitted through the Aδ- and C-fibers to the superficial dorsal horn, especially the substantia gelatinosa (SG) neurons [26,46,55], as recently reviewed [9]. Descending pathways from the midbrain and brainstem exert an inhibitory effect on dorsal horn transmission, which is subject to various modulatory influences [14]. It has been well established that the descending noradrenergic and serotonergic pathways modulate nociceptive transmission in the spinal dorsal horn (see reviews [32,40,54]). Compared with noradrenaline (NA) and serotonin (5-HT), little is known about the roles of the descending dopaminergic pathway. The periventricular, posterior region (A11) of the hypothalamus is the principle source of descending dopaminergic pathways [10,42,47]. Focal electrical stimulation in the region of the A11 area suppresses the nociceptive responses of neurons in the spinal dorsal horn [12]. These findings strongly suggest that the descending dopaminergic pathway plays an important role in the process of antinociception in the spinal cord. In addition, behavioral studies demonstrate that intrathecal administration of DA induced thermal antinociceptive effects through D2-like receptors when assessed by the tail flick test [3,22]. Whole-cell patch-clamp recordings reveal that DA produces

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hyperpolarization and an outward current in a proportion of SG neurons in transverse spinal cord slices [48]. However, it has not been determined whether the excitatory postsynaptic currents (EPSCs) that are inhibited by DA in slice preparations are responsible for pain transmission. Nor have the effects of DA on EPSCs been examined with whole-cell patch-clamp recordings *in vivo*. Even though some SG neurons have been shown to spread their dendrites more than 500 μm rostrocaudally [45], the thicknesses of the transverse spinal cord slices are usually less than 500 μm [55]. Therefore, it is possible that the dendrites of the SG neurons are transected and/or injured in the slice preparations, making it difficult to evaluate the overall effect of DA on the SG neurons. Thus, we sought to investigate the effects of DA on synaptic responses to noxious stimuli and analyze the synaptic actions of DA on SG neurons *in vivo*.

2. Methods

All the experimental procedures involving the use of animals were approved by the Ethics Committee on Animal Experiments, Wakayama Medical University and were in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines.

2.1. Preparation

The methods used for the *in vivo* patch-clamp recording were similar to those described previously [13,36,44]. Male Sprague–Dawley rats (5–7 weeks of age, 150–250 g) were anesthetized with urethane (1.2–1.5 g kg^{-1}, intraperitoneal). Artificial ventilation of the pneumothorax was not performed as the rats could be maintained in good condition without artificial ventilation by supplying oxygen through a nose cone (Fig. 1) [44]. If a withdrawal reflex appeared, then a supplemental dose of urethane was given during surgery and the data collection period. A heating pad was placed beneath the rat to maintain its body temperature. The lumbar spinal cord was exposed at the level from L3 to L5 by a thoracolumbar laminectomy at the level from Th12 to L2 and the rat was placed in a stereotaxic apparatus (Model STS-B & SR-5R-HT, Narishige, Tokyo, Japan). Under a binocular microscope with 8× to 40× magnification, the dura was cut and removed. Then, the dorsal root that enters the spinal cord above the level of recording sites was gently shifted bilaterally, using a small glass retractor, to expose Lissauer's tract so that a recording electrode could be advanced into the SG from the surface of the spinal cord. The pia-arachnoid membrane was removed using microforceps to make a window large enough to allow the patch electrode to enter the spinal cord. The surface of the spinal cord was irrigated with 95% O_2 5% CO_2-equilibrated Krebs solution (10–15 ml min^{-1}) (mM: NaCl 117, KCl 3.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, glucose 11, and $NaHCO_3$ 25) through glass pipettes at 36.5 ± 0.5 °C. At the end of the experiments, the rats were given an overdose of urethane and then killed by exsanguination.

2.2. Patch-clamp recordings

The patch electrodes were pulled from thin-walled borosilicate glass capillaries (o.d. 1.5 mm) using a p-97 puller (Sutter Instrument, Novato, CA, USA), and were filled with a patch-pipette solution composed of the following (mM): potassium gluconate 135, KCl 5, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, ATP-Mg 5 and Hepes-KOH 5; pH 7.2. When necessary, guanosine-5′-*O*-(2-thiodiphosphate) (GDP-β-S) was added at a concentration of 2 mM to the patch-pipette solution. The electrode with a resistance of 8–12 MΩ was advanced at an angle of 30–45 degrees into the SG through the window in the pia-arachnoid membrane using a micromanipulator

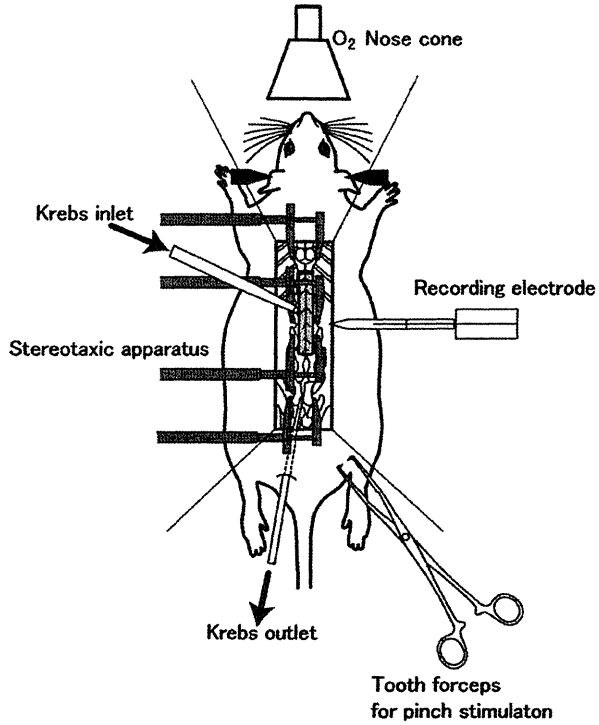

Fig. 1. Schematic diagrams of *in vivo* rat preparation. Whole-cell patch-clamp recordings were done while supplying oxygen to a urethane-anesthetized rat through a nose cone. The lumbar spinal cord was exposed at the level from L3 to L5 by a thoraco-lumbar laminectomy at the level from Th12 to L2, then the rat was placed and fixed in a stereotaxic apparatus. The surface of the spinal cord was irrigated with 95% O_2 5% CO_2-equilibrated Krebs solution from inlet to outlet. Drugs were dissolved in Krebs solution and applied by perfusion. The noxious and innocuous mechanical stimuli were applied to the receptive field of the ipsilateral hindlimb with toothed forceps and air puffs.

(Model MWS-32S, Narishige, Tokyo, Japan). A giga-ohm seal (resistance of at least 10 GΩ) was then formed with neurons at a depth of 30–150 μm. Membrane potentials were held at −70 mV in voltage-clamp mode. After making a giga-ohm seal, the membrane patch was ruptured by a brief period of more negative pressure, thus resulting in a whole cell configuration. Signals were collected using an Axopatch 200B amplifier in conjunction with a Digidata 1440A A/D converter (Molecular Devices, Sunnyvale, CA, USA) and stored on a personal computer using pCLAMP 10 data acquisition program (Molecular Devices, Sunnyvale, CA, USA). They were analyzed using Mini Analysis 6.0 software (Synaptosoft, Fort Lee, NJ, USA) and pCLAMP 10 data acquisition program.

2.3. Stimulation protocols

The noxious and innocuous mechanical stimuli were applied to the receptive field of the ipsilateral hindlimb using toothed forceps or air puffs (Pressure system IIe, Toohey Company, Fairfield, NJ, USA), respectively. To keep a fixed strength noxious stimulation, the toothed forceps was clamped during skin pinching.

2.4. Electrical stimulation of A11

The methods used for electrical stimulation of the brain during *in vivo* patch-clamp recording on SG neurons were similar to those described previously [23]. A burr hole was made in the skull, and the 26 gauge (0.46 mm outer diameter (OD)) guide cannula was inserted 2 mm above the A11 and fixed to the skull by dental cement. A concentric bipolar stimulating electrode (0.2 mm OD; model IMB-9002; Inter Medical Company, Nagoya, Japan) was inserted

through this guide cannula. The stimulating sites were aimed at A11 (stereotaxic coordinates: 3.0–3.5 mm posterior to the bregma and 0.5 mm lateral, 8.0 mm ventral to the dura) [37]. The stimulating electrode was equipped with stoppers to extend 2 mm beyond the tip of the guide cannula. The electrical stimulation was performed with rectangular pulses (duration, 100 μs; intensity, 100 μA; frequency, 10 Hz).

2.5. Drug application

Drugs were dissolved in Krebs solution and applied by perfusion via a three-way stopcock without any change in the perfusion rate or the temperature. The time necessary for the solution to flow from the stopcock to the surface of the spinal cord was approximately 10 s. The drugs used in this study were DA, SKF38393, sulpiride, tetrodotoxin (TTX) (Wako, Osaka, Japan), quinpirole, barium chloride dihydrate, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and GDP-β-S (Sigma, St Louis, MO, USA). Sulpiride and CNQX were first dissolved in dimethyl sulfoxide (DMSO) at 1000 times the concentrations to be used. DA, TTX, barium chloride dihydrate, SKF38393 and quinpirole were first dissolved in distilled water at 1000 times the concentrations to be used. These drugs were diluted to the final concentration in Krebs solution immediately before use.

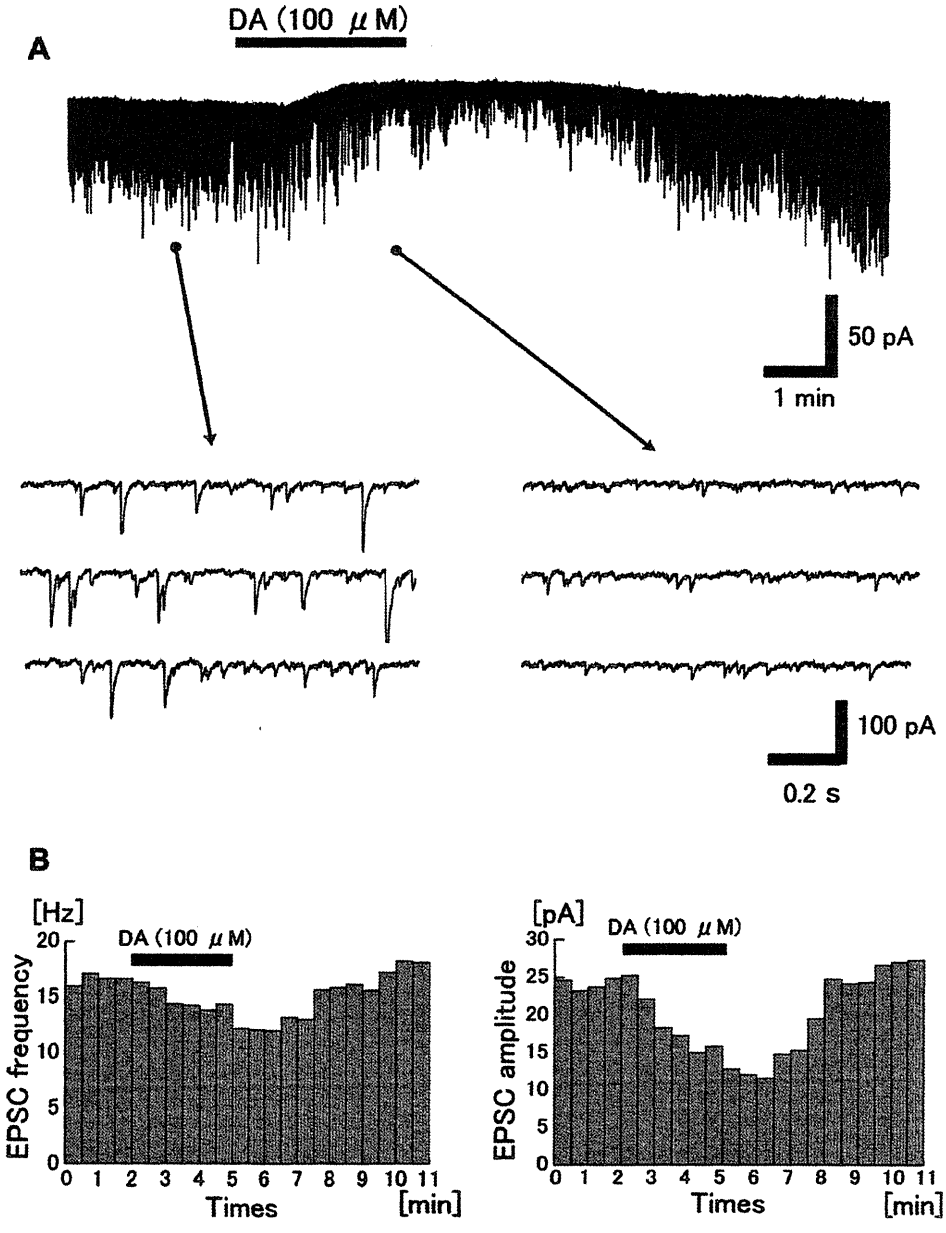

Fig. 2. DA induces an outward current and decreases the frequency and amplitude of EPSCs in SG neurons. (A) In voltage-clamp mode (V_H = −70 mV), bath application of DA (100 μM) produced an outward current in 155 out of 219 (70.8%) neurons recorded. The neuronal activity in this figure shows a typical response to DA. In this and subsequent figures, a bar shown above the recording indicates the period during which drugs were superfused. The three traces, shown in an expanded scale in time, indicate that the frequency and amplitude of EPSCs are clearly reduced during DA perfusion compared with those of the controls. (B) The frequency and amplitude of EPSCs following the application of DA (100 μM), plotted against time. Each bar indicates data calculated from the EPSCs measured for 30 s. (Same neuron as in Fig. A).

2.6. Statistical analysis

All numerical data were expressed as the mean ± S.E.M. Statistical significance was determined as $P < 0.05$ using Student's t test and Student's paired t test. In electrophysiological data, n refers to the number of neurons studied. The membrane potentials were not corrected for the liquid junction potential between the Krebs and patch-pipette solutions.

3. Results

An animal preparation could be maintained in a stable condition for over 10 h, which was comparable to that maintained in previous experiments using an artificial ventilator. Whole-cell patch-clamp recordings were made from 272 SG neurons. All neurons studied had membrane potentials more negative than −50 mV. The membrane potentials were −61.7 ± 0.5 mV ($n = 166$). All SG neurons tested exhibited EPSCs at a holding potential (V_H) of −70 mV where no inhibitory postsynaptic currents (IPSCs) were observed, because the reversal potential for IPSCs was near −70 mV [57].

3.1. Pre- and postsynaptic DA actions in SG neurons

In the voltage-clamp mode, perfusion with DA (100 μM) induced a clear outward current in 155 out of 219 (70.8%) neurons recorded (Fig. 2A). The average amplitude of the DA-induced outward currents was 19.5 ± 1.6 pA ($n = 64$). In 19 of 219 neurons tested, an inward current was observed (data not shown). When DA was applied repeatedly at 5-min intervals, it produced similar responses with similar amplitudes (Fig. 3A). Furthermore, an outward current did not show any attenuation during the 10 min continuous application of DA (Fig. 3B). During the simultaneous application of TTX (1 μM), a Na^+ channel blocker, DA (100 μM) also induced outward currents (13.3 ± 2.3 pA; $n = 10$) (Fig. 3C). All of the neurons examined exhibited miniature EPSCs (mEPSCs) that were completely blocked by a non-NMDA receptor antagonist, CNQX (10 μM), and in its presence DA generated an outward current ($n = 5$) (Fig. 3D). DA sometimes decreased the frequency and amplitude of EPSCs (65.2 ± 6.3% of the control frequency and 75.1 ± 3.2% of the control amplitude; $n = 19$, Fig. 2B). The frequency and amplitude of EPSCs were suppressed by the application of TTX (73.1 ± 10.0% of the control frequency and 74.9 ± 5.6% of control amplitude; $n = 10$) within 30 s, suggesting that a substantial amount of EPSCs initiated by action potentials from interneurons or primary afferents were included under these *in vivo* conditions. Moreover, in the presence of TTX, DA significantly decreased the frequency of mEPSCs compared with that of TTX alone (73.6 ± 9.4%; $n = 10$, $P < 0.05$).

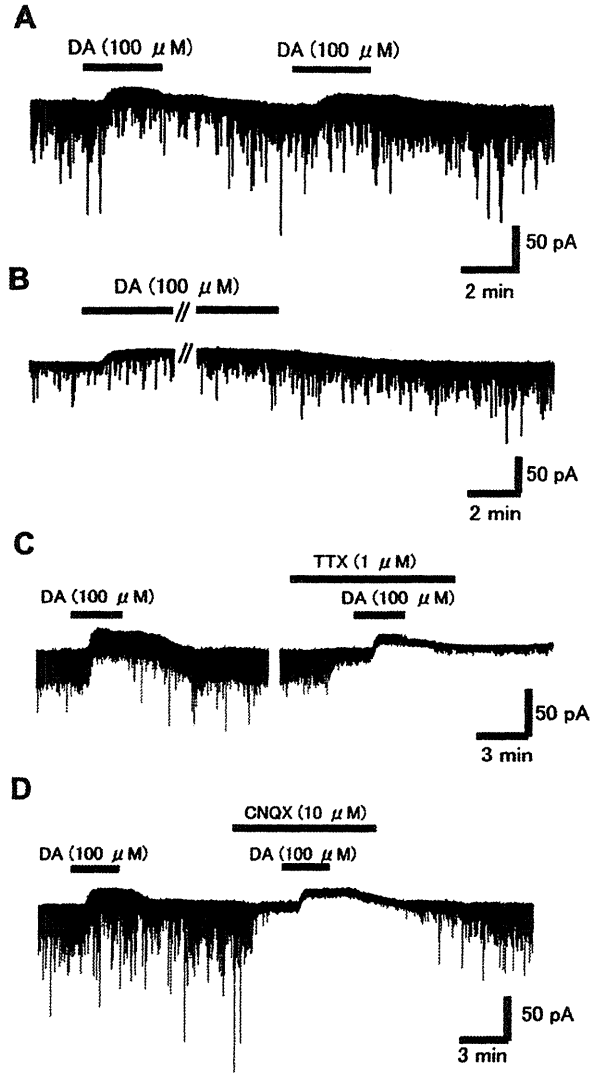

Fig. 3. Characterization of the DA-induced outward currents. (A) When DA (100 μM) was repeatedly applied at 5-min intervals, it produced similar outward currents. (B) DA (100 μM) application for 10 min induced an outward current without desensitization. (C) During the simultaneous application of TTX (1 μM), DA (100 μM) also induced outward currents (13.3 ± 2.3 pA; $n = 10$). (D) In the presence of CNQX (10 μM), DA (100 μM) induced an outward current without any decrease in the amplitude.

3.2. Pharmacological analysis of the DA-induced responses

Bath application of the K^+ channel blocker Ba^{2+} (1 mM) alone induced a small inward current (Fig. 4A), which seems to be due to inhibition of K^+ channels [55]. The average of the DA-induced outward currents in the presence of Ba^{2+} was 6.3 ± 1.8 pA ($n = 8$) and it significantly decreased to 34.7 ± 7.8% of the controls (18.9 ± 3.3 pA; $n = 8$, $P < 0.05$) (Fig. 4A). To examine the involvement of G-proteins in the DA-induced outward current, GDP-β-S (2 mM), a non-hydrolysable analogue of GDP that competitively inhibits G-proteins, was added to the pipette solution. When DA (100 μM) was applied just after establishing the whole-cell configuration with pipettes containing potassium gluconate and GDP-β-S, an outward current was clearly observed (19.4 ± 2.5 pA; $n = 8$). When DA was again applied 30 min later, it was significantly suppressed (4.3 ± 1.6 pA, 22.3 ± 8.9% of the control amplitude; $n = 8$, $P < 0.01$) (Fig. 4B). These findings suggested that the DA-induced outward current was mediated by K^+ channels through activation of G-proteins.

3.3. Effects of DA receptor agonists and antagonists

We further examined which subtype of DA receptors is involved in the DA-induced outward currents by using DA receptor agonists and antagonists. SKF38393 (100 μM), a D1-like receptor agonist, induces little or no outward current (1.9 ± 0.6 pA; $n = 9$) (Fig. 5B), while quinpirole (100 μM), a D2-like receptor agonist, produced a clear outward current (15.7 ± 2.0 pA; $n = 9$) (Fig. 5A). The SKF38393-induced outward current was significantly lower than either the DA- or quinpirole-induced outward current ($P < 0.01$). There was no significant difference between DA-induced outward current and that of quinpirole ($P > 0.05$) (Fig. 5D). When a D2-like receptor antagonist, sulpiride (30 μM), was administrated 5 min prior to the DA application, the average amplitude of the

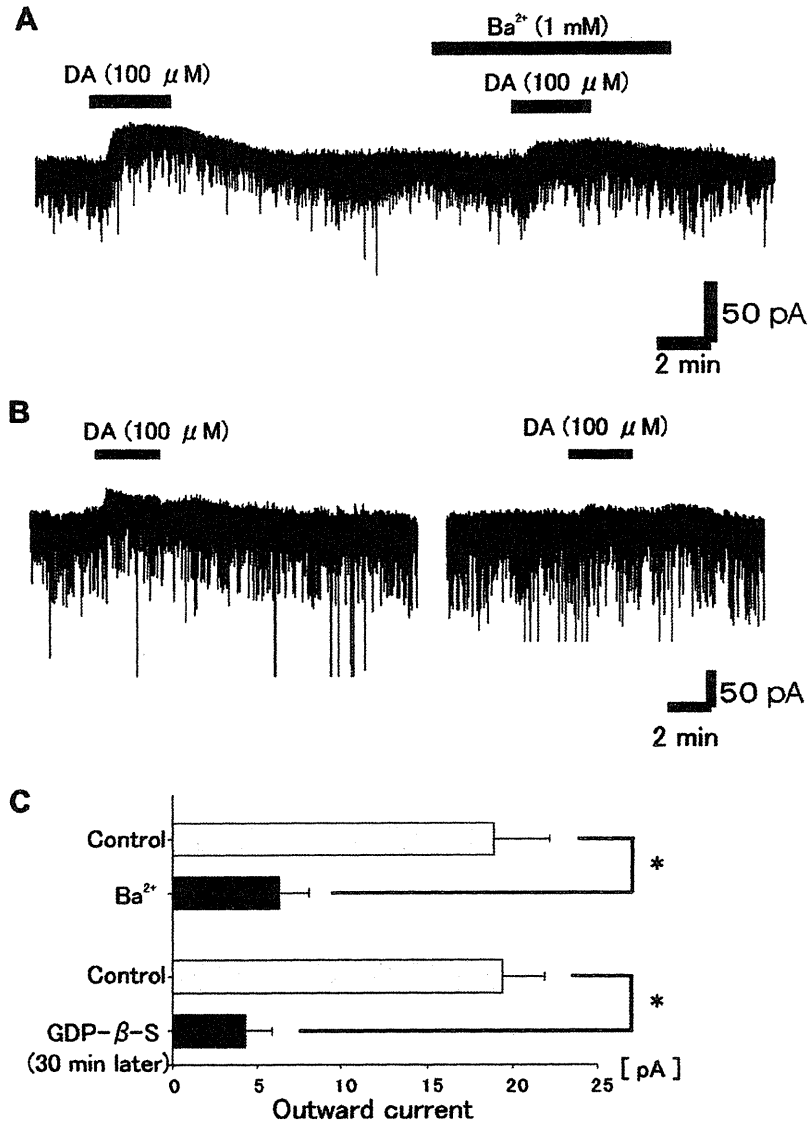

Fig. 4. Inhibition of DA-induced outward current by Ba^{2+} and GDP-β-S. (A) DA (100 μM) was administrated in the absence and presence of Ba^{2+} (1 mM). The outward current was significantly reduced in the presence of Ba^{2+} (34.7 ± 7.8% of the controls; $n = 8$; $P < 0.05$). (B) An outward current was recorded with potassium gluconate pipette solution containing GDP-β-S. DA produced an outward current just after establishing whole-cell recording, but it was markedly attenuated when DA was again applied 30 min later (22.3 ± 8.9% of control amplitude; $n = 8$; $P < 0.05$). (C) Summary of DA-induced outward current in the presence of Ba^{2+} or intracellular injection of GDP-β-S, relative to the control. In this and subsequent figures, horizontal lines accompanied by bars indicate S.E.M.; statistical significance ($P < 0.05$) between data shown by bars is indicated by an asterisk (*); n.s.: not significant.

DA-induced outward currents was 5.4 ± 1.9 pA, $n = 6$ (Fig. 5C), 34.1 ± 10.0% of that in the absence of sulpiride ($P < 0.05$).

3.4. Effects of DA on the responses to noxious stimuli

All 33 SG neurons examined responded to noxious (pinch) or innocuous (air puff) mechanical stimuli. In the voltage-clamp mode, either pinch or air stimuli applied to the ipsilateral hindlimb elicited a barrage of EPSCs; these disappeared within 1 s after the stimuli were terminated (Fig. 6A and B). The limb point most sensitive to stimulation was different for each cell tested. Stimulating the contralateral hindlimb did not elicit any synaptic responses (data not shown). The peak amplitudes were not determined, because multiple summations resulting from the high-frequency bursting of EPSCs made it difficult to obtain an accurate estimation. So, we investigated the change of frequency and area surrounded by the baseline and border of EPSCs (Fig. 7A). It was considered significant if EPSCs decreased or increased by more than 10%. The pinch stimulus initially produced large and summated EPSCs that were followed by a barrage of EPSCs, and again large EPSCs at the end of the stimulus (Fig. 6A). When DA (100 μM) was applied to the surface of the spinal cord, the area of the evoked EPSCs decreased in 4 of 6 neurons tested (74.6 ± 7.5% of the control; $n = 6$, $P < 0.05$) (Fig. 7B), although the amplitudes of the large EPSCs at the beginning and end of stimulation were not affected (Fig. 6A). Similarly, the frequency of EPSCs decreased in 4 of 6 neurons tested (78.0 ± 8.0% of the control; $n = 6$, $P < 0.05$) (Fig. 7B). The barrage of EPSCs induced by pinch again appeared after the washing-out of DA. The area of the evoked EPSCs by innocuous (air puff) mechanical stimuli decreased as much as with the pinch stimuli (Fig. 7B). The area of the evoked EPSCs after an air puff decreased in 14 of 18 neurons tested (70.9 ± 11.0% of the control; $n = 18$, $P < 0.01$) and the frequency of EPSCs after an air puff decreased in 11 of 18 neurons tested (80.6 ± 6.3% of the control; $n = 18$, $P < 0.01$)

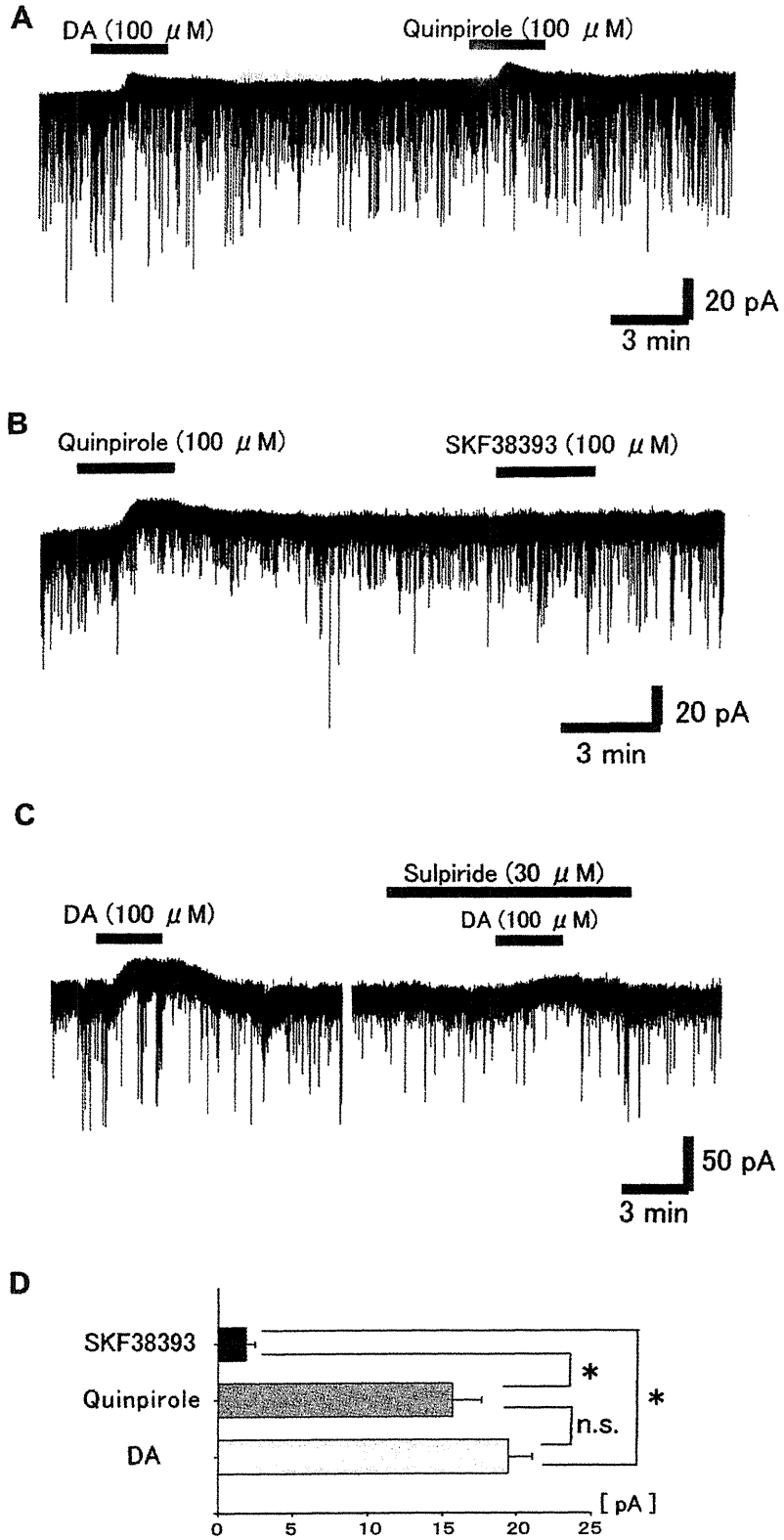

Fig. 5. Effect of DA receptor agonists and antagonists on the DA-induced outward currents. (A) Quinpirole (100 μM), a D2-like receptor agonist, mimicked the DA-induced outward current. (B) SKF 38393 (100 μM), a D1-like receptor agonist, induced no outward current, whereas quinpirole (100 μM) induced an outward current in the same neuron. (C) In the presence of a D2-like receptor antagonist, sulpiride (30 μM), a DA-induced outward current was markedly attenuated (34.1 ± 10.0% of that in the absence of sulpiride; $n = 6$; $P < 0.05$). (D) An SKF38393-induced outward current was zero or very low (1.9 ± 1.7 pA; $n = 9$). In contrast, quinpirole-induced outward current amplitude was 15.7 ± 3.9 pA ($n = 9$) which was similar to DA-induced outward currents (19.5 ± 12.4 pA; $n = 64$). The SKF38393-induced outward current was significantly lower than a DA- or quinpirole-induced outward current ($P < 0.01$). There was no significant difference between a DA-induced outward current and that of quinpirole.