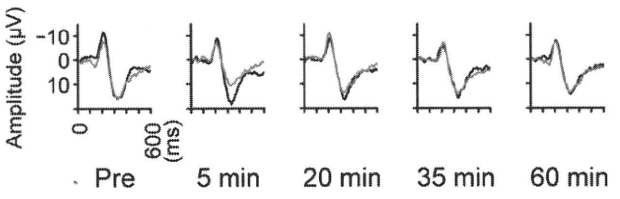

Fig. 2 – Grand averaged waveforms for each run in each session. Recordings in the Control (black) and Smoking (gray) sessions are shown. Each graph corresponds to each run. The horizontal axes indicate the time after stimulus onset.

amplitude ($R=-0.22$, $p=0.03$, Fig. 4b), but not to N2 latency ($R=0.05$, $p=0.62$) or P2 latency ($R=-0.08$, $p=0.41$, respectively).

2.3. VAS scores

The mean VAS score over all the measurements was 5.18±0.13. We found no significant interaction ($F(4, 36)=0.70$, $p=0.53$) or main effect of Session ($F(1, 9)=0.13$, $p=0.73$) or Run ($F(4, 36)=1.9$, $p=0.17$) on the VAS score after a two-way ANOVA (Session and Run). The correlation between PNC and the VAS score was not statistically significant ($R=-0.09$, $p=0.35$, Fig. 4c).

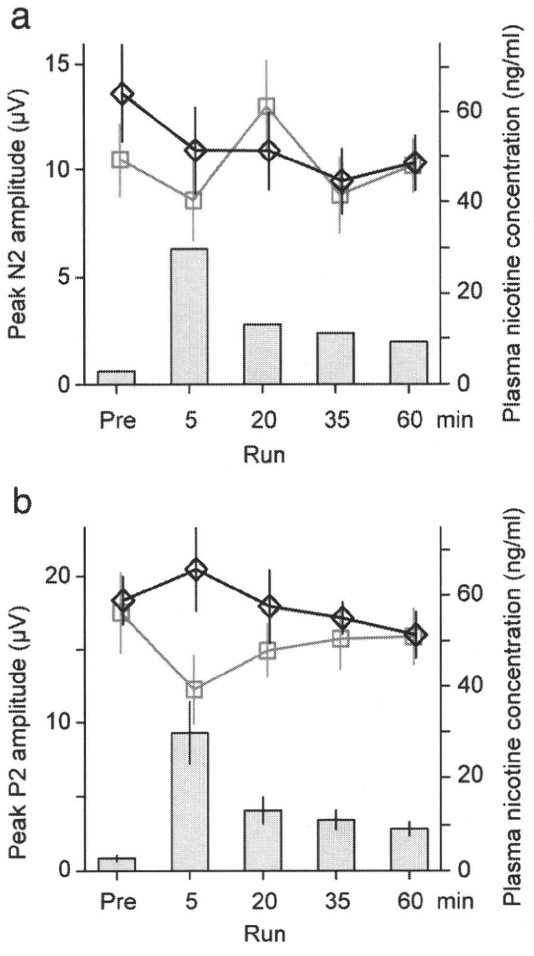

Fig. 3 – Mean±SE of peak N2 and P2 amplitudes, and the plasma nicotine concentration (PNC) for each run. Peak N2 (a) and P2 (b) amplitudes are plotted for the Control (black lines and open diamonds) and Smoking (gray lines and open squares) sessions using the left axes. PNCs are shown with gray bars using the right axes and duplicated in the two graphs. Error bars of PNC are only shown in (b).

2.4. Heart rate (HR)

The mean HR over all the runs and subjects was 63.7±1.0/min. The mean HR over the Control session and over the Smoking session was 59.8±1.3/min and 67.6±1.4/min, respectively. In a two-way repeated-measures ANOVA (Session and Run), we found an interaction of Session×Run ($F(4, 36)=23.5$, $p<0.01$) and the main effects of Session ($F(1, 9)=11.5$, $p<0.01$) and Run ($F(4, 36)=11.5$, $p<0.01$). In a post hoc paired t-test for each run, the HR at 5 min after smoking was significantly larger in the Smoking session than in the Control session ($t(9)=4.9$, $p<0.01$). HR showed a significant positive correlation with PNC ($R=0.39$, $p<0.01$, Fig. 4d).

3. Discussion

We found an effect of smoking to modulate pain related brain activities. The amplitudes of the N2 and P2 components of pain-related potentials were negatively correlated with plasma nicotine concentration. The amplitude of P2 was significantly smaller in the Smoking session. The amplitudes of N2 and P2 components are thought to reflect the intensity of perceived pain (Kakigi et al., 1989; Bromm and Treede, 1991; García-Larrea, 1997). The results of the present study are consistent with the idea that smoking and nicotine have an antinociceptive property.

The results of the present study need a careful interpretation. No placebo was used in the Control session and the psychological effect of smoking could have modulated the results. This point remains to be clarified until a double-blind study is made using denicotinized cigarettes that cannot easily be distinguished from normal cigarettes. The Control session was always conducted after the Smoking session. The order of sessions might have had some impact on the LEPs. However, the difference in the P2 amplitude was significant between sessions in the 5 min run but not in the other runs. The amplitude of N2 was not significantly different between the sessions, but was significantly negatively correlated to the plasma nicotine concentration. These results cannot be fully explained by the order of the sessions or psychological effect of smoking, but suggest that the amplitude of LEP components reflects the plasma nicotine concentration.

Although the P2 amplitude tended to change after smoking as can be seen in Fig. 3b, the difference in the P2 amplitude between the runs within the Smoking session was not statistically significant ($p=0.10$). Change in the P2 amplitude at each time point might have been too small to be detected without taking account of the actual plasma nicotine concentration at each time point.

The tobacco cigarette used in the present study might have been considerably different in the nicotine content or "taste" from cigarettes usually smoked by each subject. Although the subjects reported that the experimental cigarette was

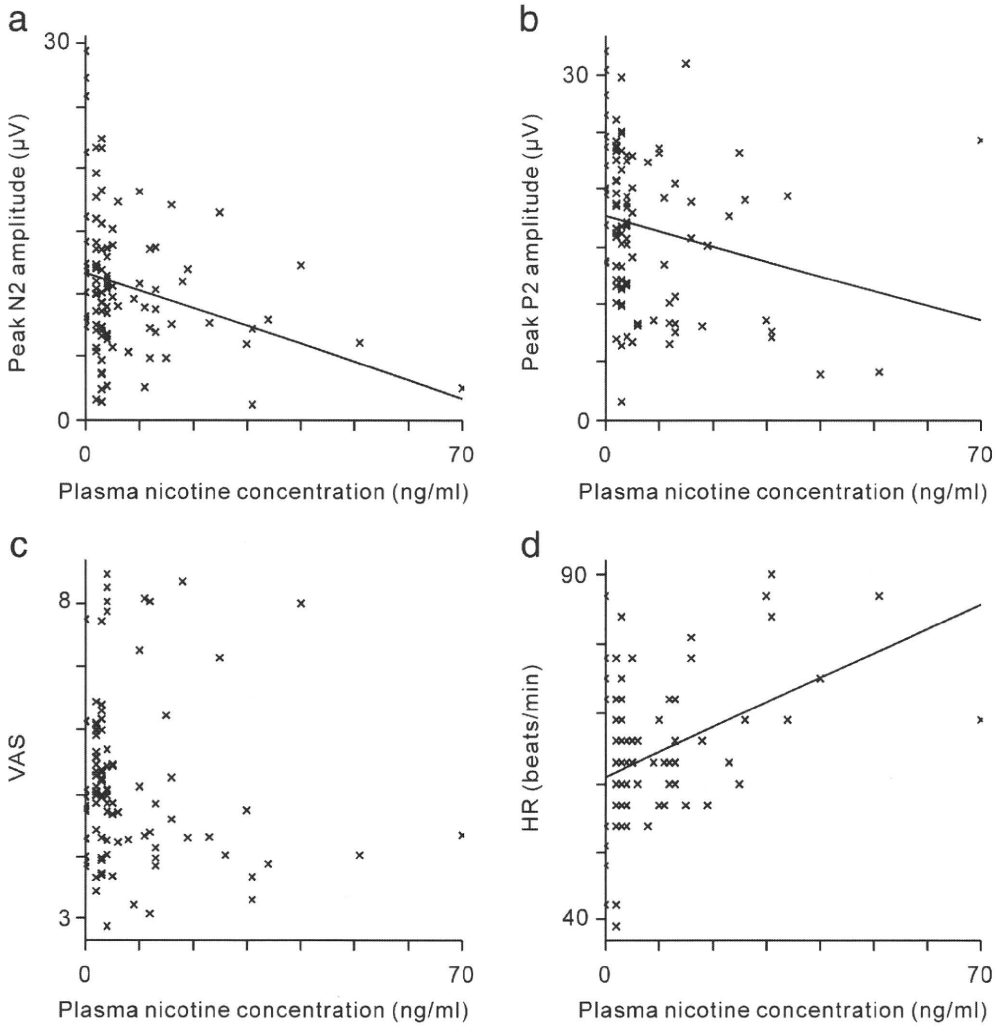

Fig. 4 – Scatter plots of peak N2 (a) and P2 (b) amplitudes, VAS scores (c) and HR (d) versus the plasma nicotine concentration (PNC). Data from both the Smoking and Control sessions are plotted. A fitted linear model is shown where a significant correlation was found (a, b, and d).

acceptable, the difference might have influenced subjects' smoking behavior or emotion and have modulated the effect of smoking and nicotine on LEPs.

For 4 of the 10 subjects, the plasma cotinine concentration was below the limit of detection in the Control session, suggesting that these subjects smoked less regularly than the other subjects. To assess the difference in the effect of smoking and nicotine on LEPs between regular and non-regular smokers, studies on larger number of subjects are needed.

The present study does not directly link smoking or nicotine with brain activities that underlies the processing of intensity or other aspects of pain. However, the most important finding is that the change in the amplitudes of laser-evoked potentials was negatively correlated to the plasma nicotine concentration in contrast to other types of brain activities (Friedman et al., 1974; Woodson et al., 1982; Knott et al., 1999; Houlihan et al., 2001). The mechanism underlying this effect needs to be studied using fMRI or PET, antagonists of nicotine, opioid, or other neurotransmitters. On the other hand, the present study was not designed to distinguish the effect of smoking and nicotine from the effect of recovery from the abstinence. The effect on LEPs could be explained as recovery from a temporary sensitization to pain stimuli as a part of withdrawal symptoms induced by abstinence from smoking, instead of a direct effect of nicotine. To answer this question, studies are needed on non-smokers with administration of nicotine without an exposure to tobacco smoke, for example, using nasal spray or intravenous injection.

We found a difference in the results in the ANOVAs on the amplitudes of N2 and P2. Both the interaction of Session and Run and the main effect of Session were significant on the amplitude of P2 but not on that of N2. In Fig. 3, the difference in the amplitude between the sessions was obvious in the P2 component but not in the N2 component. The N2 and P2 components are thought to reflect the activities of operculoinsular and cingular cortices, respectively (Tarkka and Treede, 1993; Bromm and Chen, 1995; Valeriani et al., 1996; Iannetti et al., 2003; Kakigi et al., 2005). The difference in the results on the N2 and P2 amplitudes might indicate that the modulative effect of smoking is stronger or more lasting on the cingular cortex than on the operculoinsular cortex. Scott et

al. (2007) showed that smoking increased the activity of μ-opioid receptor-mediated neurotransmission in the right anterior cingulate cortex but decreased it in the left amygdala, left ventral basal ganglia, and right thalamus, using positron emission tomography. If the effect of smoking on the LEP amplitudes is mediated by a differential modulation on μ-opioid receptor mediated neurotransmission in these brain areas, the difference in the effect of smoking on the amplitudes of the two LEP components might be consistent.

In contrast with the results in LEPs, we did not find a significant effect of smoking or nicotine to reduce the intensity of subjectively perceived pain. The dose of nicotine by smoking of one cigarette might have not been enough to cause an obvious change in the intensity of perceived pain. Subjective ratings by visual analog scale can considerably fluctuate throughout an experiment and might not be sensitive enough to detect a slight change in perceived pain over time. It might also be possible that smoking does not modulate much the sensory-discriminative aspect of pain, if smoking mainly attenuates the activities in the cingulate cortex but not much in the operculoinsular cortex or other brain areas. Most of human studies that found an antinociceptive effect of smoking and/or nicotine used other techniques to measure the change in subjectively perceived pain. Pomerleau et al. (1984) reported that the smoking of normal cigarettes resulted in a longer pain awareness threshold in a cold pressor test than did the smoking of zero-nicotine cigarettes. Perkins et al. (1994) reported a significant negative effect of the plasma nicotine concentration on the latency of pain detection to a radiant heat stimulus after an administration of nicotine via a nasal spray.

Some previous studies reported that no significant antinociceptive effect was found after smoking or a nicotine dosage in humans. Mueser et al. (1984) did not find any significant effect of smoking on the pain or tolerance threshold, or pain discrimination capacity in experiments with painful electric stimulation. Knott (1990) and Knott and De Lugt (1991) reported that there was no consistent effect of smoking on pain-evoked EEG responses or subjective ratings after painful electrical stimulation in a condition either with or without a warning before the stimulus. In the present study, we used painful laser stimulation and found the effect of smoking to reduce the amplitudes of pain-related evoked potentials. It is difficult to find the cause of the discrepancies in the results of these studies. But one possible cause is that the difference in the method of painful stimulation. Electric stimulation at the surface of the skin activates both Aδ and Aβ fibers, whereas laser stimulation can activate Aδ fibers without activating Aβ fibers. Since the tactile information through Aβ fibers could reach the brain faster than pain information through Aδ fibers, brain processing after an electric stimulus might not be changed by smoking or nicotine. In addition, the dose of nicotine that is not acutely harmful for a human might be too small to elicit a significant antinociceptive effect. The dose of nicotine through tobacco smoking in a usual way might not significantly affect pain-related cognition or behaviors. To obtain more conclusive results, we might need studies using a less toxic nicotine receptor agonist at a higher dose or a more sensitive method to measure subjectively perceived pain.

4. Experimental procedures

4.1. *Subjects*

Ten healthy male volunteers who varied in their smoking habit, aged 23 to 43 (mean 33±6 SD), participated in the study. The mean number of years that the subjects have smoked was 12.4±7.0 years. The mean number of cigarettes they were smoking per day was 13.9±8.0. The subjects were free of any history of psychiatric or neurological disorders, or substance abuse. The study was approved in advance by the Ethics Committee of the National Institute for Physiological Sciences, Okazaki, Japan. Written consent was obtained from all the subjects.

4.2. *Stimulation and recordings*

A Tm:YAG laser (BLM1000S, Carl Baasel Lasertechnik, Starnberg, Germany) was used for noxious stimulation. The laser pulses were 2000 nm in wavelength, 1 ms in duration, and 3 mm in diameter. The laser beam was applied to the dorsum of the right hand between the first and second metacarpal bones. The subjects were instructed to rate the perceived pain sensation after each laser beam on a 10-cm horizontal bar, where the left margin indicated no painful sensation at all, and the right margin the most intense imaginable pain (visual analogue scale, VAS). Before starting each session, laser simulation was tested on each subject to determine the energy of laser to be used in the session and to make the subject accustomed to the stimulation. The laser energy was adjusted to a level at which stimulation produced a VAS score of 5–6. As a result, the same intensity was chosen for both sessions for each subject (mean 157.5±8.4 mJ). The stimulation intensity was kept constant through runs. To avoid tissue damage, the irradiated points were moved slightly for each stimulus.

LEPs were recorded using a scalp electrode placed at Cz referred to the linked earlobes (A1+A2) according to the International 10/20 system. The EEG signals were recorded with a band-pass filter of 0.1–100 Hz at a sampling rate of 1000 Hz, and then digitally filtered with a 50 Hz low-pass filter. The window of analysis was from 100 ms before to 600 ms after the stimulus onset, and the prestimulus period was used as the DC baseline. The impedance of the electrodes was kept below 5 kΩ. A pair of electrodes placed on the supra- and infra-orbit of the right eye was used for the rejection of trials containing artifacts due to blinks. An electrocardiogram was recorded to calculate heart rate, using a pair of disk electrodes placed on each forearm.

4.3. *Procedures*

The experiments were conducted in two sessions, Smoking and Control, with different conditions on separate days. Each session started at 9 AM or 1 PM. The starting time was counterbalanced between subjects and between sessions. The Control session was conducted 5 to 20 (mean 11.2) days after the Smoking session for each subject. The procedures of the experiment were the same for the two sessions, except that subjects smoked a cigarette in the Smoking session but not in

the Control session. For each session, subjects were required to be abstinent from smoking, alcohol, drugs, and caffeine for at least 12 hours before the experiment. Subjects were seated in an armchair in a quiet electrically shielded room, the temperature of which was controlled at 24 to 26 °C. Before the experiment, an indwelling catheter for collecting venous blood samples was placed in the left cephalic vein. There were five runs of recordings at different times: before smoking (Pre), and 5, 20, 35 and 60 minutes after smoking (Fig. 5). Just before the beginning of each run, venous blood was collected for measurements of plasma nicotine and cotinine concentrations, and an electrocardiogram was recorded for 20 s for heart rate calculation. In each run, 12–15 stimuli were applied and LEPs were recorded. The interstimulus interval was varied at random between 14 and 19 s. In the Smoking session, subjects smoked a tobacco cigarette for 5 minutes just after all data were obtained for Pre, and then the remaining four runs followed. The tobacco cigarette of a common brand in Japan with 1.0 mg of nicotine (Japan Tobacco Inc., Tokyo) was used and was reported to be acceptable by all the subjects. The plasma samples were frozen with dry ice and kept at −80 °C until assay. The plasma nicotine and cotinine concentrations were measured using gas chromatography–mass spectrometry. The method was similar to that used previously to analyze urinary cotinine concentrations (Hecht et al., 1999) with the addition of a solid phase extraction step carried out on an MCX column (Water Corporation, Milford, MA). The MCX column was prepared and the sample eluted as described previously (Murphy et al., 2007).

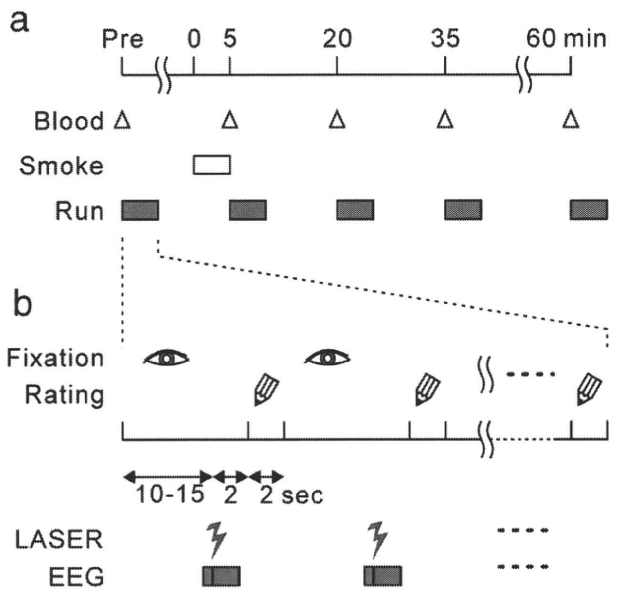

Fig. 5 – Experimental design. (a) Scheme representing the Smoking session. A blood sample was collected just before each run. The procedure for the Control session was the same except for the absence of smoking. (b) At least 12 trials of LEP recording were included in each run. In each trial, the subject fixated on a "fixation" sign on the screen. About 10–15 s after the fixation sign appeared, a laser stimulus was given to the dorsum of the right hand of the subject. EEG was recorded from 100 ms before to 600 ms after the stimulus. After 2 s, a "rating" sign appeared on the screen, and the subject rated the perceived pain on a form. The fixation sign for the next trial appeared in 2 s.

4.4. *Data analysis*

For each run of LEP recordings, 10 artifact-free epochs were averaged after all epochs with eye movements and/or blinks were visually inspected and rejected. When more than 10 artifact-free epochs were found in a run, only the first 10 epochs were averaged and used in further analyses. The mean amplitude of the 100-ms period just before the stimulus was used to adjust the averaged waveform for each run in each session and subject. The peak amplitude and latency of N2 and P2 were calculated using time frames with a latency period of 180–250 ms and 250–360 ms, respectively. The VAS scores were averaged for each run. The effect of smoking was assessed in ANOVAs and post hoc tests using the Control session as a baseline. A two-way (Session and Run) repeated-measures ANOVA was done on each of the amplitude and latency of N2 and P2, averaged VAS scores, and heart rate (HR). The Greenhouse–Geisser epsilon was used in the correction of degrees of freedom when appropriate. Post hoc tests have been operated using paired *t*-test with Bonferroni's correction between the sessions for each run when an interaction was found in the ANOVAs. Effect of Run was also assessed with a one-way repeated-measures ANOVA within the smoking session. Pearson's correlation coefficient was calculated between the plasma nicotine concentration (PNC) and each of the amplitudes and latencies of N2 and P2, VAS score, and HR, and was tested using *t*-test with Fisher transformation. Statistical analyses were carried out with SPSS 15.0J.

Acknowledgments

The nicotine/cotinine assays were done by Dr. Linda von Weymarn and the Analytical Biochemistry Core at the Masonic Center, University of Minnesota Minneapolis, MN 55455, and supported by National Institute on Drug Abuse Grant DA 018974-01A2. The authors would like to thank Prof. Kenneth L Casey for helpful suggestions. This work was supported by a grant from the Smoking Research Foundation, Japan. The entire study has been conducted in a completely scientifically objective fashion. The authors caution that these research findings should not be interpreted as indicating tobacco smoking produces clinically significant analgesia. Instead, the findings support basic scientific studies on the actions of nicotine in the brain.

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The Journal of Pain, Vol 11, No 7 (July), 2010: pp 621-627
Available online at www.sciencedirect.com

Assessing Aδ Fiber Function With Lidocaine Using Intraepidermal Electrical Stimulation

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Abstract: The functions of small fibers can be impaired in peripheral neuropathies, and screening tests for clinical use are required. To verify whether intraepidermal stimulation (IES) is useful for assessing the functions of Aδ fibers in the superficial layer, we investigated sensory thresholds and evoked cortical responses in healthy volunteers before and after a transdermal administration of lidocaine. Pain and tactile thresholds were studied using IES and transcutaneous electrical stimulation (TS), respectively, in 10 healthy volunteers before, and 1 hour, 3 hours, and 5 hours after a local anesthesia with lidocaine. Cortical potentials evoked with IES and TS were also studied in 12 healthy volunteers before and 5 hours after the anesthesia. Although the local anesthesia had no effect on the evoked potentials or the tactile threshold for TS, it markedly increased the pain threshold and almost abolished the evoked potentials for IES. These results suggest that IES is a sensitive tool for detecting functional changes of cutaneous Aδ fibers.

***Perspective:** Compared with other methods of stimulation used to investigate Aδ fiber function, our method is easy to apply and less invasive and can stimulate any site of the body. Therefore, it should be useful as a screening test for patients with neuropathy.*

***Key words:** Aδ fibers, evoked potentials, intraepidermal electrical stimulation, neuropathy, pain.*

The impairment of small fibers can occur in various peripheral neuropathies.[13] Diabetes mellitus is the most common cause, with small fiber involvement beginning in distal parts of the limbs.[23] For an early diagnosis and treatment, screening tests suitable for clinical use are required. Researchers studying small fiber function in patients with peripheral neuropathies have applied various techniques including laser stimulation,[11] a cutaneous silent period,[17] a cooling detection threshold,[29] and warm and heat pain thresholds.[21,25]

Recently, intraepidermal nerve fiber density (IENFD) has been considered a reliable tool for diagnosing conditions affecting small fibers.[19-21,25-27] Shun et al[25] showed that IENFD was much lower in diabetic patients than matched control subjects, with IENFD per millimeter being about 1.8 and 9.4, respectively. Detecting these morphological changes with an electrophysiological test would be of great help for clinical diagnosis.

We have developed a method of electrically stimulating the epidermal area for the selective activation of Aδ fibers.[5] The method is easy to use and can be applied to various cutaneous sites. In the present study, we investigated whether this method is suitable for evaluating the functions of Aδ fibers located in the superficial layer of the skin by measuring pain threshold and evoked potentials before and after the transdermal application of lidocaine in healthy volunteers. We considered the effects of epidermal stimulation after the cutaneous application of lidocaine to mimic the early stages of diabetic neuropathy with small fiber involvement beginning in distal parts of the limbs. Because our method targets Aδ nociceptors or their fibers in the superficial layer of the skin, the results of the epidermal stimulation would be sensitive to the transdermal application of lidocaine, whose effects are stronger for the superficial layers than for the deeper layers.[3]

Received May 1, 2009; Revised July 9, 2009; Accepted October 2, 2009.
Supported by grants from the Ministry of Education, Sports, Sciences, and Technology and from the Ministry of Health, Labour, and Welfare, Japan.
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1526-5900/$36.00

doi:10.1016/j.jpain.2009.10.001

Methods

Subjects

The experiments were performed on 12 healthy volunteers (3 women and 9 men; age, 25 to 43 years). Ten volunteers participated in Experiment 1, all 12 in Experiment 2, and 5 in Experiment 3. The study was approved in advance by the Ethics Committee of the National Institute for Physiological Sciences, Okazaki, Japan, and written consent was obtained from all the subjects.

Stimulation

For nociceptive stimulation, we used a method of intraepidermal electrical stimulation (IES) developed in our laboratory[5] for the selective activation of cutaneous Aδ fibers without the activation of thicker fibers (ie, Aβ fibers). In this study, we used a stainless steel concentric bipolar needle electrode (Nihon Kohden, Tokyo, Japan) for IES.[8] The anode was an outer ring 1.2 mm in diameter and the cathode was an inner needle that protruded 0.1 mm from the outer ring. By pressing the electrode against the skin gently, the needle tip was inserted in the epidermis where nociceptors are located, while the outer ring was attached to the skin surface. The electrical stimulus was a constant current of double pulses (interstimulus interval, 10 ms) lasting 1 ms each. We used double pulses to augment the response. For tactile stimulation, similar cutaneous sites were stimulated using a bipolar felt tip electrode (NM-420S; Nihon Koden), 0.9 mm in diameter with a distance of 23 mm between the anode and cathode (transcutaneous electrical stimulation, TS). The stimulus parameters were the same for IES.

Sensory Threshold and Pain Rating

IES and TS were applied to the dorsum of the hand and foot and the sensory threshold was measured. For IES, we started stimulation with an intensity of 0.01 mA and increased the current in steps of 0.01 mA until the subject felt a pricking sensation, and then decreased it in steps of 0.01 mA to the point where the sensation disappeared. Usually, the pricking sensation disappeared with a decrease of 0.01 mA, but some subjects could feel a similar but weaker sensation at this intensity. Under the pain threshold, no sensations occurred in any subject. Because the threshold was slightly different at each penetration, the measurement was performed at 5 locations, and the mean value was used for the subsequent analysis. The upper limit of the intensity of IES was set at 1.0 mA. The threshold of tactile sensations for TS was measured similarly.

In the threshold measurement experiment for IES, subjects were also asked to score the intensity of perceived sensations on a visual analog scale (VAS), with zero indicating "no pain" and 100 meaning "worst possible pain" at the 5 different locations before the local anesthesia.

Recording of Evoked Potentials

EEG signals were recorded at Cz referenced to linked earlobes (A1-A2) of the International 10-20 system as described previously.[5] We focused on evoked responses recorded at Cz, since in a preliminary study, the maximum response was recorded from the Cz derivation, similar to our previous report.[10] The impedance of the electrodes was kept below 5 kΩ. The EEG signals were recorded with a bandpass filter of 0.1 to 100 Hz at a sampling rate of 2000 Hz. The window of analysis was from 100 ms before to 600 ms after the stimulus onset. The 100-ms period before the stimulus was used as the DC baseline. For each stimulus (TS and IES), at least 10 artifact-free responses were collected and averaged.

Local Anesthesia

To mimic the impairment of cutaneous Aδ fibers in the superficial layer, a lidocaine tape (Penles; NittouDenkou, Tokyo, Japan) was used. The tape was 30.5 mm × 50.0 mm and contained 18 mg of lidocaine. It was chosen because it could be used less invasively than injections.

Experimental Procedures

Experiment 1 (Threshold Measurements)

Effects of lidocaine on the sensory threshold of IES-evoked pain sensations and TS-evoked tactile sensations were examined by varying the period of application. If the IES method was sensitive enough to detect change of function of Aδ fibers, the sensory threshold of IES-evoked pain sensations would increase with an increase in the period of lidocaine application. IES and TS were applied to the dorsum of both hands and both feet. After the threshold was measured (control), the lidocaine tape was applied to the left hand and left foot. Changes in the sensory threshold were examined 1 hour, 3 hours, and 5 hours after the local anesthesia. Therefore, there were 4 runs. At each run, the tape was removed to stimulate and then replaced after the recordings for the next run. The electrode was placed at around the center of the tape (approximately 20 × 40 mm area). Threshold measurements of IES were performed at 5 different points, and the mean value was used for subsequent analyses.

Experiment 2 (EP Measurements)

To obtain objective evidence that the IES method is sensitive enough to detect changes of function of Aδ fibers, we recorded evoked potentials (EPs) after IES and TS before and 5 hour after the lidocaine tape was used. IES and TS were applied to the dorsum of both hands. After the control response was recorded, the lidocaine tape was applied to the left hand for 5 hours, and EPs were recorded again. The intensity of TS was 1.5 times the tactile threshold at each time point. Clear tactile sensations were elicited without any painful sensations using these parameters. The intensity for IES in the control session was 1.5 times the pain threshold. Because none of the subjects felt sensations elicited by IES after the local application of lidocaine in the intensity range (0 to

Table 1. Evoked Potential Values and Thresholds With Each Stimulus Condition (TS and IES) Before and After a 5-Hour Application of Local Anesthesia

		N2 Latency (ms)	P2 Latency (ms)	N2-P2 Amplitude (μV)	Threshold (mA)
Left hand (IES)	Before	204.7 ± 17.2	299.7 ± 28.7	23.9 ± 9.8	0.11 ± 0.04
	5-h application	—	—	—	*
Right hand (IES)	Before	200.4 ± 14.1	310.6 ± 37.7	22.5 ± 7.3	0.13 ± 0.03
	5-h application	202.5 ± 22.1	306.2 ± 31.1	20.8 ± 7.1	0.12 ± 0.03
Left hand (TS)	Before	150.0 ± 10.3	225.9 ± 23.9	17.3 ± 4.6	0.75 ± 0.24
	5-h application	147.9 ± 14.2	218.0 ± 25.7	15.7 ± 5.8	0.81 ± 0.22†
Right hand (TS)	Before	147.3 ± 8.2	224.7 ± 26.7	15.3 ± 3.5	0.75 ± 0.2
	5-h application	150.7 ± 7.4	225.8 ± 24.9	14.8 ± 5.9	0.81 ± 0.25†

Abbreviations: IES, intraepidermal stimulation; TS, transcutaneous electrical stimulation.
*Only 1 subject perceived a tactile sensation at 0.65 mA.
†When changes in TS threshold between the before and 5-hour runs were calculated in each subject and compared between the left and right hands, there was no significant difference ($P = .47$, paired t test).

1.0 mA), we used the stimulus intensity of the control session in the 5-hour session.

Experiment 3 (IES-Evoked Potential Measurements with Time)

We examined the time course of the effects of lidocaine on IES-induced EPs to know whether there is a gradual change that would reflect the number of fibers activated under the pharmacological effect of lidocaine or more importantly, the gradual loss of fibers in peripheral neuropathy. IES was applied to the dorsum of both hands. After the control response was recorded, the lidocaine tape was applied to the left hand (affected side). Changes in the EPs evoked with IES were examined after a 1-hour, 3-hour, and 5-hour application of the local anesthesia. We used the same stimulus intensity (1.5 times the pain threshold for the control session) in all sessions.

Analysis

In Experiment 1, effects of the local anesthesia on the tactile threshold of TS were evaluated statistically using a 1-way repeated-measures analysis of variance (ANOVA). In Experiment 2, IES and TS elicited a similar negative-positive sequence (N2-P2) at different latencies. The peak latency of N2 and P2 was determined during a latency period of 120 to 180 ms and 180 to 280 ms for TS and 170 to 250 ms and 250 to 350 ms for IES, respectively. The local anesthetic effects on the latency and amplitude of each component were assessed with a 2-way (local anesthesia × run) repeated-measures ANOVA. In Experiment 3, the local anesthetic effects on the latency and amplitude of each component were assessed with a paired t test at each time point.

Results

Experiment 1

IES produced a pin-prick sensation, and TS, a clear tactile sensation (tapping or throbbing) before the local anesthesia. The mean pain rating for the control condition (before) at the left hand and foot was 37.5 ± 4.9 and 31.8 ± 7.2, respectively.

The mean tactile threshold for TS in each run (before, 1 hour, 3 hours, and 5 hours) was 0.75, 0.7, 0.76 ,and 0.81 mA, respectively, for the hand and 0.8, 0.9, 0.92, and 0.92 mA for the foot. The tactile threshold did not differ significantly among the 4 runs at each site (ANOVA, $P = .31$ for the hand, $P = .46$ for the foot).

The 1-hour application of lidocaine did not affect the pain threshold for IES for the hand (before, 0.11 ± 0.03 mA; after, 0.11 ± 0.02 mA). Among the 30 measurements (5 measurements × 6 subjects) in the third run (3 hours), however, no sensation could be elicited within the intensity range up to 1.0 mA for 12 measurements. Using the remaining 18 measurements, the mean threshold was 0.41 ± 0.35 mA. In the fourth run (5 hours), 5 of the 6 subjects did not feel a sensation in any measurement. Results of the foot stimulation were similar. The mean pain threshold was 0.14 ± 0.02 mA for the control run, 0.14 ± 0.03 mA for the 1-hour run, and 0.20 ± 0.09 mA for the 3-hour run. In the 5-hour run, IES did not elicit any sensation up to 1.0 mA in 8 of 30 measurements. Using the remaining 22 measurements, the mean threshold was 0.56 ± 0.18 mA. Taken together, the local application of lidocaine clearly increased the pain threshold but did not affect significantly the tactile threshold as expected.

Experiment 2

The peak latency and peak amplitude of evoked potentials and the threshold for each measurement are shown in Table 1. The N2-P2 complex after TS that ranged from about 150 to 230 ms in latency was clearly identified in each subject in the control and 5-hour runs (Fig 1). Results of the 2-way ANOVA (lidocaine × run) showed no significant effect of these 2 factors on the latency and amplitude of the N2-P2 complex. The mean TS-evoked sensation threshold was 0.75 ± 0.24 in the control run and 0.81 ± 0.22 after the 5-hour application of lidocaine for the left hand and 0.75 ± 0.2 and 0.81 ± 0.25, respectively, for the right hand. The mean threshold did not differ significantly between the 2 runs for either hand (paired t test, $P > .05$), and therefore the persistence of the response to TS after the application of lidocaine was not due to a higher intensity of stimulation.

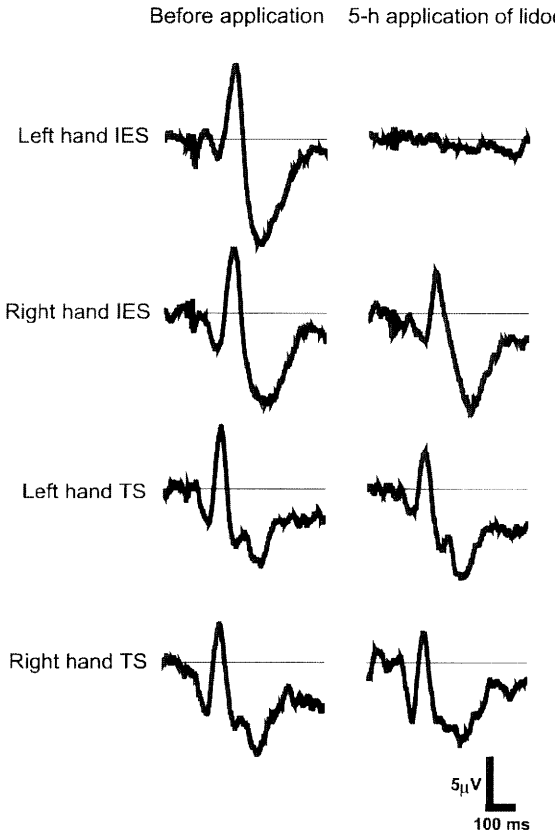

Figure 1. Grand-averaged waveforms of potentials evoked by intraepidermal stimulation (IES) and transcutaneous electrical stimulation (TS) recorded at Cz. Left traces, before lidocaine. Right traces, after a 5-hour application of lidocaine taped to the left hand.

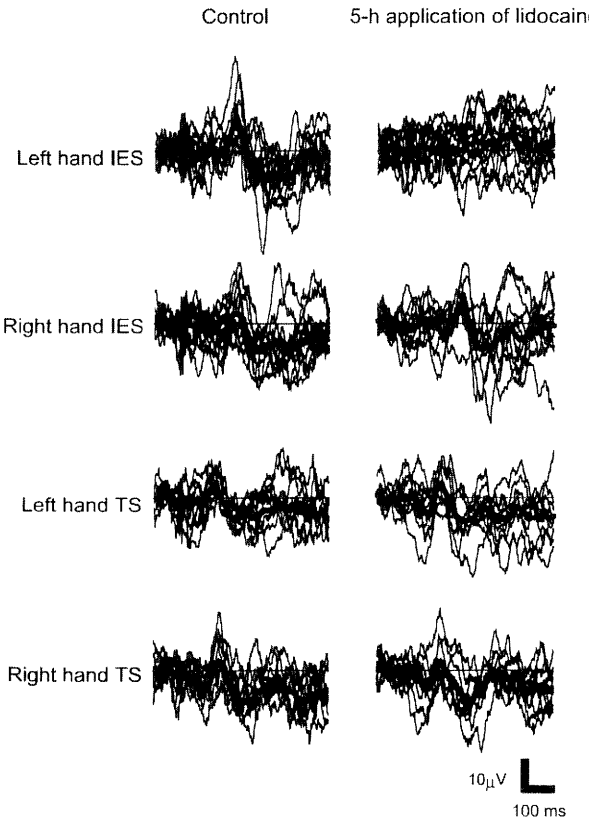

Figure 2. Superimposed waveforms of potentials evoked by intraepidermal stimulation (IES) and transcutaneous electrical stimulation (TS) recorded at Cz in a representative subject. Left traces, before lidocaine. Right traces, after a 5-hour application of lidocaine taped to the left hand.

The N2-P2 complex after IES that ranged from about 200 to 300 ms in latency was clearly identified in each subject for the control condition, whereas stimulation at the same intensity elicited neither a pinprick sensation nor evoked potentials in any subjects after the 5-hour application of lidocaine (Fig 1). The peak latency and peak amplitude of the N2-P2 complex on the control-side (right hand) did not differ between before and 5 hours after the local anesthesia. Fig 2 shows the superimposed waveforms in a representative subject.

Experiment 3

The peak latency and peak amplitude of evoked potentials are listed in Table 2. The 1-hour application of lidocaine did not affect the peak latency or peak amplitude of evoked potentials, whereas after the 3-hour application, 4 of 5 subjects did not feel any sensation at a intensity of 1.5 times the control threshold. After the 5-hour application, none of the 5 subjects felt a sensation. Similarly, EPs were not detectable in 4 of 5 subjects in the 3-hour run and in any of the 5 subjects in the 5-hour run. The grand-averaged waveforms across all the subjects for each run are shown in Fig 3. The peak latency and peak amplitude of the N2-P2 complex on the control side (right hand) did not differ among the 4 (pre, 1-hour, 3-hour, and 5-hour) runs.

Discussion

In the present study, we tested whether IES can be used to evaluate the function of Aδ fibers in the superficial layer of the skin. Results indicated that IES could be a sensitive tool to find impaired Aδ fibers in the superficial layer of the skin and a potential screening test for diabetic neuropathy. The method has several advantages with respect to sensitivity, cost, invasiveness, and convenience. Given the extensive impairment of Aδ fibers in the early stages of neuropathy, affected individuals are expected to have an increased pain threshold with intact tactile sensations. Because this method can be applied to various parts of the body, one can also test whether the degree of impairment differs between distal and proximal areas. Recordings of evoked potentials can also provide objective evidence.

Theoretically, a concentric bipolar electrode can be regarded as a radial assembly of an infinite number of tripolar electrode arrays that can reduce undesired current spread.[16] In the case of our concentric bipolar needle electrode, the passing current is expected to be restricted to the needle tip, where free nerve endings exist without loop pathways extending to deeper layers. In previous studies, we reported that (1) IES elicits a clear pricking sensation without any tactile sensations, (2) the peripheral conduction velocity of signals evoked by IES is about

Table 2. Peak Latency and Amplitude of IES-Evoked Potentials Before and After 1-Hour, 3-Hour, and 5-Hour Applications of Lidocaine

		N2 Latency (ms)	P2 Latency (ms)	N2-P2 Amplitude (μV)
Left hand (lidocaine)	Before	192.8 ± 12.9	307.8 ± 23.0	21.6 ± 8.5
	1-h application	194.4 ± 19.4	312.4 ± 35.2	19.9 ± 6.2
	3-h application	—	—	—
	5-h application	—	—	—
Right hand (control)	Before	197.2 ± 7.3	318.4 ± 32.8	21.6 ± 6.3
	1-h application	200.2 ± 17.9	312.2 ± 42.0	20.3 ± 5.8
	3-h application	195.4 ± 11.2	307.2 ± 38.0	18.3 ± 8.7
	5-h application	204.0 ± 13.2	288.6 ± 44.2	19.0 ± 9.4

Abbreviation: IES, intraepidermal stimulation.

15 m/s, and (3) IES evokes pain-related potentials or magnetic fields similar to those evoked by laser beams.[5-7] These findings indicate that IES can selectively activate Aδ fibers. The present results that the local application of lidocaine affected IES-induced pain sensations but not TS-induced tactile sensations are consistent with this notion. The difference in latency of the N2/P2 component, about 60 ms, between IES and TS can be well explained by the difference in the peripheral and spinal conduction velocities between Aδ and Aβ fibers.[7] Because each conduction velocity is similar between the peripheral nerve and spinal cord,[9,28] a gross calculation reveals that it takes 20 ms to travel 120 cm (from the hand to brain) at a conduction velocity of 60 m/s (Aβ fibers), whereas it takes 80 ms at 15 m/s (Aδ fibers). Although it is well recognized that pain-related EPs are influenced by the subject's attention or other internal states, the present results showed that EPs and the sensory threshold in the control condition (right hand) did not differ between runs (before and after the 5-hour application). Therefore, the influence of such factors was minimal in the present study.

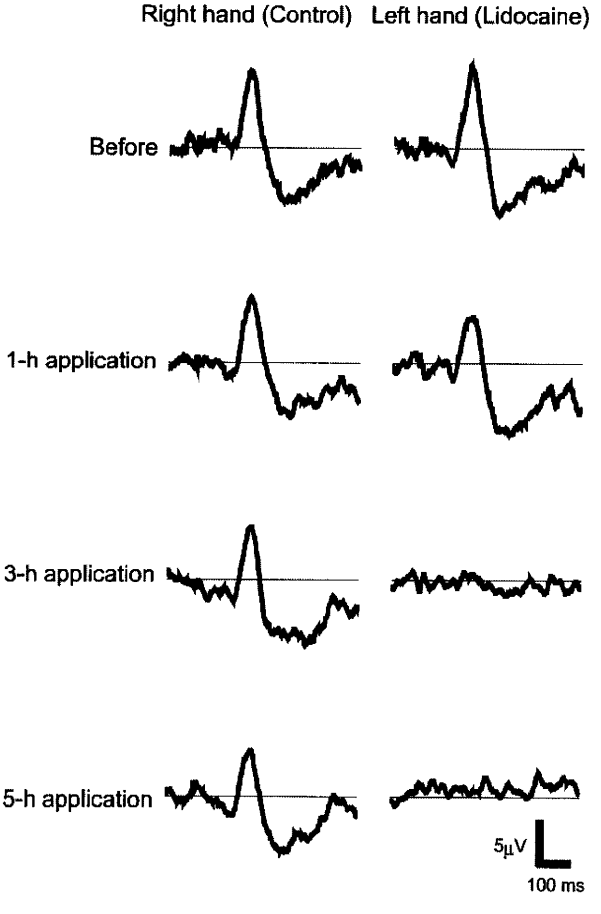

Figure 3. Gradual change of potentials evoked by intraepidermal stimulation (IES) with various application times (1-hour, 3-hour, and 5-hour application).

In a previous study using laser stimulation, small-diameter fibers were selectively blocked by a subcutaneous injection of lidocaine.[22] In the present study, we used the transdermal application of lidocaine for a local anesthesia. Lidocaine would penetrate the stratum corneum and then diffuse into the epidermis and dermis. The diffusion seemed slow, because there was no effect on the pain threshold and EPs after a 1-hour application of the lidocaine tape.

There are several possible explanations for the present results. First, the effect of lidocaine was selective regarding the Aδ fibers. That is, lidocaine was sensitive to the IES-evoked activation of Aδ fibers but not to the TS-evoked activation of Aβ fibers. Second, the effect of lidocaine was stronger for the superficial layer of the skin than deeper layers, and therefore the greater effects of lidocaine on the sensations of pain and EPs induced by IES than those induced by TS might be due to the difference in the depth of each receptor/fiber. Anatomic findings show that nociceptive fiber terminals are located in the epidermis and superficial layer of the dermis,[12,15] whereas the other fibers run more deeply in the dermis.[14] In support of the second view, warm sensations were unaffected by the application of EMLA (Eutectic Mixture of Local Anesthetics) cream despite marked effects on pain sensation, a finding interpreted to mean that C-fiber warm receptors could be located in slightly deeper layers of the skin.[3] Although the results of the present study did not favor one particular explanation over the other, the lidocaine-induced impairment of Aδ fibers with intact Aβ fiber function in either case mimicked the predominant impairment of small fibers in some kinds of neuropathies.

Another possible explanation for the different effects of lidocaine on Aβ and Aδ fibers is that the site of activation, that is, receptors or fibers, differs between TS and IES. Because lidocaine may preferentially act at the site

of receptors (impulse generation) more strongly than at the site of fibers (conduction block),[24] its effects would differ between TS and IES if they activate different sites more strongly. However, the fact that both laser beam–induced pain[3] and IES-induced pain are attenuated by lidocaine implies that lidocaine suppresses the activation of both receptors and fibers, that is, both nerve conduction and impulse generation. Laser beams activate cutaneous nociceptors, whereas IES would bypass the transduction process. Although it is not clear whether the effect of transdermal lidocaine was truly related to a selective effect on small-diameter fibers or related to the location of the receptors in the skin, the present results showed that the IES method could detect the impaired functioning of Aδ fibers in the superficial layer of the skin.

Recently, IENFD has emerged as a reliable tool for diagnosing conditions affecting small fibers,[19-21,25-27] and studies have indicated that changes in IENFD correlate with other measures, for example, warm,[25] cold,[26] and heat pain threshold.[21] If changes in IENFD partly reflect the impairment of Aδ fibers, results of IES pain threshold examinations in patients with peripheral neuropathies would also correlate with changes in IENFD, although the present method cannot identify changes in C-fibers. We recently developed a method using IES for the selective stimulation of cutaneous C-fibers.[18] A combination of the 2 (ie, IES for Aδ and C fibers) might be a good tool for diagnosing small fiber impairment.

In recent European guidelines on neuropathic pain assessment, laser stimulation was recommended as the best neurophysiological tool.[4] In some studies, lasers that can activate Aδ fibers were used to evaluate small-fiber function in diabetic neuropathy,[1,2,11] but their clinical usefulness is limited in that such devices are expensive and hard to control. In contrast, an electrical stimulator is more convenient and less invasive and therefore may be a better alternative for clinical examinations. Further clinical studies, for example, of the correlation of the IES-evoked pain threshold with the clinical manifestations of neuropathies or results of other clinical examinations, are necessary to evaluate the advantages of this method.

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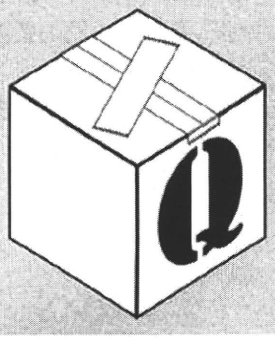

34 「痛覚失認」,「二点識別覚」などの体性感覚性高次脳機能障害について知りたいのですが.

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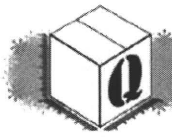
関連キーワード　痛覚失認　二点識別覚　島

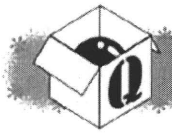
本稿では,「痛覚失認」と「二点識別覚」に分けて解説する.

痛覚失認



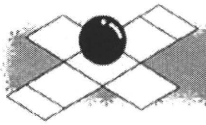
知っておきたい基本知識

痛覚失認（asymbolia for pain）とは, 侵害刺激の性状や部位を認識できるにもかかわらず, 痛み特有の不快な情動体験やそれにともなう行動が欠如する状態を指す. 侵害刺激の処理は古くから弁別面（部位, 強度, 性状）と情動面に分けられてきた. 後者に関連する不快な痛み体験は, しばしばわれわれを悩ませる症状となりうるが, 一方, 生存の観点からすれば非常に重要な機能である. 侵害刺激を認識しても, それから逃れたいという強い情動がなければ, 侵害受容系は意味をなさなくなる. 痛覚失認は, 痛みの情動-動機づけ側面が選択的に失われた状態であるといえる.



少し詳しく

1928年に痛覚失認を初めて報告したSchilderとStengelの症例の剖検所見では, 左半球縁上回付近に大きな梗塞があり, 角回や島にも障害が認められた. その後, 痛覚失認の責任部位候補として第二次体性感覚野（SII）, 島, 下頭頂葉などが報告された. Berthierら[1]は, 痛覚失認の患者6名の臨床症状とCT所見を詳細に検討し, 侵害刺激（針および熱刺激）の認知は保たれているにもかかわらず, 逃避反応あるいは適切な情動反応が欠如すること, 言葉やジェスチャーによる威嚇に対しても無反応であること, 病変が島を含むこと, などが共通点であることを見いだし, 痛覚失認の責任部位が島であり, 感覚野と辺縁系の連絡遮断によるものであろうと結論した. 島近傍に位置するSIIの関連が問題になるが, CT検査ではSIIが保たれている症例にも痛覚失認を生じていること, 視覚や聴覚による威嚇への無反応はSIIの病変では説明できないこと, などからSIIの関与は否定的であるとした.



じっくり学ぶ

侵害情報処理の2つの経路は, 侵害情報を上位中枢へ伝える脊髄後角の二次細胞のレベルで始まる[2]. すなわち, 侵害受容線維のみから入力を受ける特異的侵害受容細胞（nociceptive specific：NS）と, 触覚刺激から侵害刺激に至る種々の強さの刺激に対して段階的に発火を増加させる広域作動細胞（wide dynamic range：WDR）の2種類がある. 両者は脊髄視床路（spinothalamic tract：STT）を上行し視床に投射するが異なる伝導路を介して投射しており, 投射部位も異なる.

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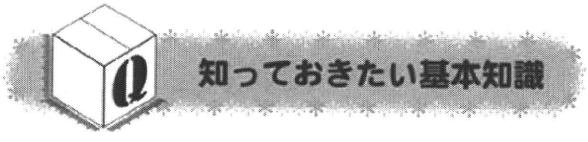
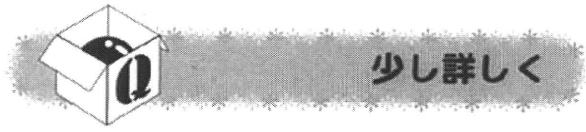
それぞれの視床核は異なる皮質領野へ投射するのであり，侵害情報処理は明らかに異なる2つの処理経路を持つことになる．おおむね，WDR細胞は脊髄後角第V層にあり，その信号は腹側STTを上行し，視床VP核を経て第一次体性感覚野（SI）へ伝えられる．この経路の細胞は受容野が小さく明瞭なsomatotopyを示し，刺激強度を反映して発火を増す．したがって侵害情報の弁別的側面に関与すると考えられる．一方NS細胞は脊髄後角第I層にあり外側STTを上行して視床のVMpoやMdvcに投射し，島へ連絡する．島の細胞は受容野が大きく，内臓，味覚あるいは圧受容器などから同時入力を受けることが多い．したがって島は侵害情報を含む各種刺激にともなう内部環境の変化を統合する機能を持つと考えられる．解剖学的には，島は感覚野と辺縁系の中間に位置し，この経路は内部環境変化にともなう情動-動機づけ側面に関与していると考えられる．痛覚失認は後者の経路が遮断された状態であると思われる．島の障害による痛覚失認の患者が，脅威となるような視覚，聴覚刺激にも反応しない点は，島が幅広い警告信号統合の場であることを示唆する．理論的には前者の経路のみが遮断された場合には，侵害情報の弁別ができないにもかかわらず情動-逃避行動の側面は保たれる，という状態が生じると考えられる．実際に，中心後回（SI）を障害された患者が，侵害刺激（レーザー）を認知できないにもかかわらず不快な情動や回避行動を示した，とする報告[3]がある．

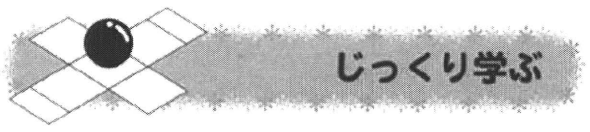
二点識別覚



二点識別覚閾値は体性感覚における空間的，時間的識別能を示すものであり，臨床的検査方法として広く用いられている．皮膚に同時に二点与えられた感覚情報が一点か二点か（空間的二点識別），あるいは同じ部位に短い時間間隔で与えられた2回の刺激を1回と感じるか2回と感じるか（時間的二点識別）という認知は，おもに皮膚感覚受容器の感覚受容野，中枢神経系内の抑制機構によって規定される．また，大脳皮質内における認知の過程も最終的な判断に至るまでに，重要な役割を担っていると考えられる．



最近の神経イメージング手法を用いた研究により，空間的な二点識別には，SIとSIIで情報処理された後，inferior parietal lobule（IPL），特に左半球のIPLが重要な役割をはたすことが報告されている．時間的な二点識別では，pre-supplementary motor areaが重要な役割を担っていると報告されている．したがって，空間的な二点識別と時間的な二点識別ではその識別に関わる神経基盤が異なっている可能性が考えられる．臨床での研究において，IPLおよび周辺のsupramarginal gyrus（SMG）が損傷すると，触覚が消失し識別覚に影響を与えるとされている．また，刺激の鋭さなどを識別する際には体性感覚野の3b野や1野，2野からの情報を最終的にIPLおよびSMGが受けて処理するとされており，IPLは体性感覚の識別において高次な役割を果たしていると考えられる．



二点識別覚検査は臨床的に頻繁に行われる検査の一つであるが，その情報処理メカニズムに関しては実は今までほとんど明らかにされていない．臨床においては，二点識別閾値は変動することがよく知られており，同一個人においても施行ごとに変動が認められる．同じ刺激条件下でも一点と二点に認知が分かれるために，threshold（閾値）はその中間値が採用されることもある．

この認知の変動の原因として2つの要因が考えられる．第一に，刺激そのものの変動が認知に影

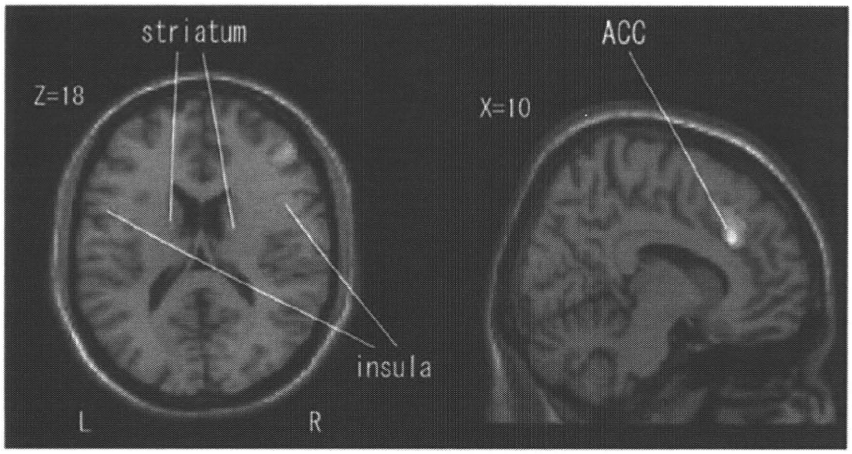


図1　二点識別課題（TPD）とコントロールと比較したときに有意に活動した皮質下部位を表した図

（Akatsuka K, et al：Neuroimage 40(2)：852-858, 2008[5]より引用）

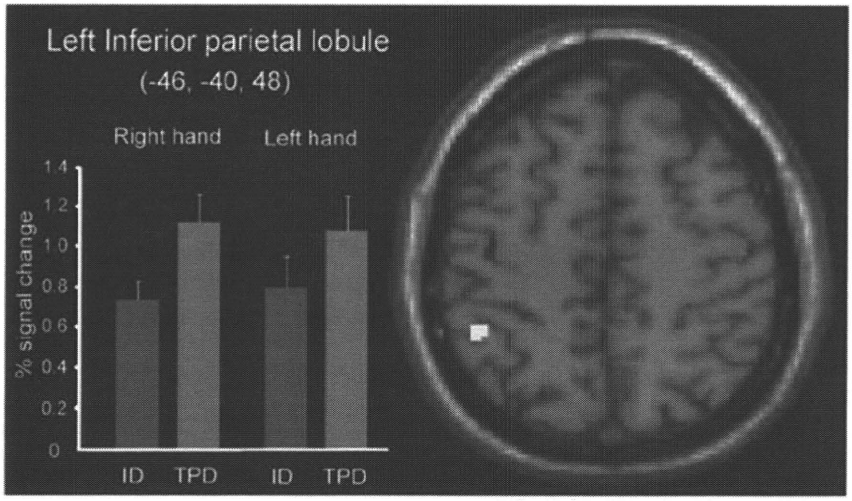


図2　二点識別課題と強度識別課題を直接比較した際に，二点識別課題時に有意に活動した部位を表した図

（Akatsuka K, et al：Neuroimage 40(2)：852-858, 2008[5]より引用）

響を及ぼす可能性が考えられる．日常において二点識別感覚試験はコンパスを用いて行われることが多く，プローブの接触の強度や深さが変わることによって感覚皮質に到達する神経活動そのものが変化してしまう可能性がある．厳密に言えば，2点の刺激はまったく同じタイミングで，しかも同じ強さで与えられなければならないが，臨床現場ではそれは不可能に近い．第二に，先行する刺激がそれに続く刺激の認知に影響を及ぼす可能性が考えられた．つまり，先行刺激と比較して与えられた刺激が長い場合，より二点と判断しやすく，逆に先行刺激と比較して短い場合により一点と判断しやすい可能性がある．

このような問題点を解決するために，私達は，コンピューターで制御できる電気刺激を用いた新しい二点識別検査用機器を開発した[4]．これによって，末梢神経活動の変動が認知に影響を及ぼす可能性を否定することができた．

脳波および脳磁図を用いた電気生理学的検査法では，二点識別時の脳活動を，認知機能に関連が深い P300 反応とミスマッチ陰性電位（mismatch negativity）を計測した．すると，認知初期には，SⅠとSⅡが主として活動して情報処理することが明らかになった．

機能的 MRI を用いた検査では，体性感覚刺激として，4種類の電気刺激（強い一点刺激・強い二点刺激・弱い一点刺激・弱い二点刺激）を用い，二点識別課題と強度識別課題時の脳活動部位を計測した．二点識別課題とコントロールを比較すると，IPL，anterior cingulate cortex（ACC），

pre-frontal gyrus (PFG), inferior frontal gyrus (IFG), left S I, anterior insula, striatum, それに the anterior lobe of the cerebellar vermis (ALV) が有意に活動していた. 強度識別課題とコントロールを比較した場合にも同様な部位が活動していた (**図 1**). 二点識別課題と強度識別課題を直接比較すると, 左の IPL が二点識別課題時に有意に活動していた (**図 2**). この結果は, ヒトが与えられた刺激を一点か二点かを識別するときには IPL が重要な役割を担っていることを示唆するものである.

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痛みと痒みの脳内認知機構の解明

柿木隆介[*1, 2]，望月秀紀[*1, 3]

Aδ線維を上行するfirst painとC線維を上行するsecond pain共に，刺激対側の第1次体性感覚野，両側半球の第2次体性感覚野，島，帯状回，扁桃体に活動が見られるが，島と帯状回の一部にはsecond painに特異的に反応する部位が発見された。second painが，より情動に関係する事を示唆する所見と考えられる。また，痛そうな写真を見て「痛い」と心で思う時には，実際に痛みが与えられた時と同様の部位が活動し，「心の痛み」に合致する所見であった。また，瞑想に入ると痛みを感じないヨガの達人では，痛み刺激に対して通常の部位の活動が見られず，中脳被蓋部に活動が見られ，下行性抑制系の亢進が想定された。近年開発した通電型の痒み誘起位置を用いて，痒みの脳内認知機構を解析したところ，痛み刺激時と類似しているが，楔前部（precuneus）に痒み独自の活動が見られた。

Recently, both electrophysiological studies such as magnetoencephalography (MEG) and hemodynamic studies such as functional magnetic resonance imaging (fMRI) are intensively being used to elucidate underlying mechanisms of human pain and itch perception. MEG following Aδ (first pain) and C fiber stimulation (second pain) were similar except for a longer latency for the latter. At first, primary somatosensory cortex (SI) contralateral to the stimulation is activated and then secondary somatosensory cortex (SII), insula, amygdala and anterior cingulate cortex (ACC) in the bilateral hemispheres are activated sequentially. As for findings using fMRI, the stimulation of both C and Aδ fibers activated the bilateral thalamus, bilateral SII, right (ipsilateral) middle insula, and bilateral Brodmann's area (BA) 24/32, with the majority of activity found in the posterior portion of the ACC. However, magnitude of activity in the BA32/8/6, including ACC and pre-supplementary motor area (pre-SMA), and the bilateral anterior insula was significantly stronger following the stimulation of C nociceptors than Aδ nociceptors. Findings following itch stimulation were similar to those following pain stimulation, but the precuneus may be itch selective brain region. This unique finding was confirmed by both MEG and fMRI studies.

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Keywords: Pain, Itch, MEG, 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り，従来から行われてきた脳波（EEG）に加え，ポジトロン断層撮影（PET），機能的磁気共鳴画像（fMRI）及び脳磁図（MEG）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，痛みと痒みに関連する脳活動に分けて，脳磁図とfMRIを用いた著者らの最近の研究成果を紹介したい。

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痛みの脳内認知機構

2.1 脳磁図を用いた研究

先ず，Aδ線維を上行する first pain についてまとめる。最近著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（Epidermal stimulation，ES 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]。ES 法は，針の部分が約 0.2mm の押しピン型電極を用いて表皮内を電気刺激（0.5ms，0.1-0.3mA）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無いこと，電気刺激であるので time-lock が非常に良いこと，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。

手背刺激により約 100 ミリ秒を頂点とする微弱な活動が SI 領域に認められる（図 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 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）が見られる。従って視床－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。従って 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。

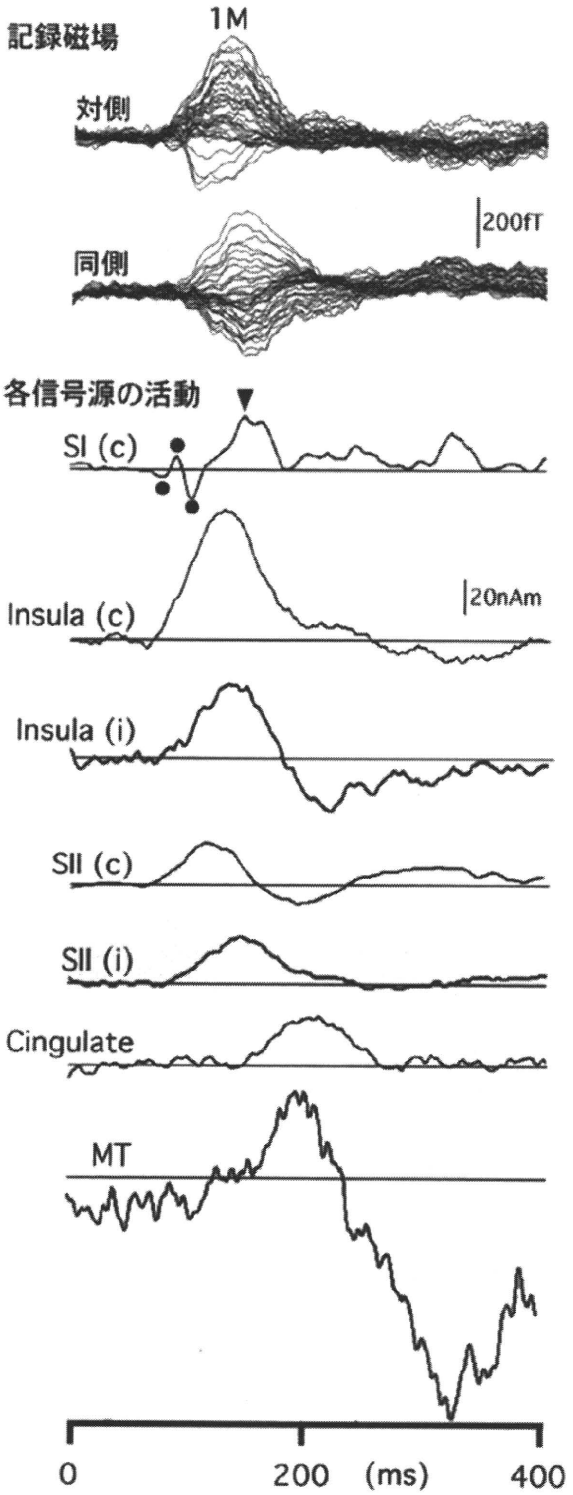

図 1 Aδ 線維を上行する信号による脳磁図反応（SI，SII，島，前部帯状回および内側部側頭葉の活動）
上段二つのトレースが記録磁場波形，下段 7 つのトレースが各信号源の活動時間経過を示す。c：刺激対側半球，i：刺激同側半球，MT：前内側部側頭葉。（文献 3）より引用）

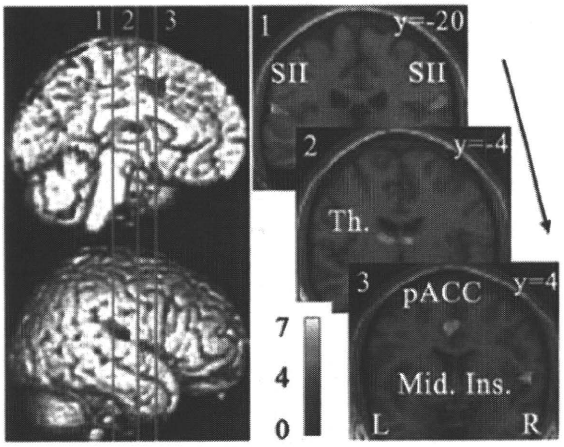

図2 Aδ線維刺激とC線維刺激に共通して有意差を示した部位
左の図の3本の垂直線は各々の冠状断面を示す。Th.= 視床，SII= 第2次体性感覚野，Ins. = 島，pACC= 前帯状回の後方部。Mid. Ins.= 島中央部。（文献8）より引用）。

刺激後200-300ミリ秒の脳活動を解析すると，前部帯状回と前内側部側頭葉（MT，扁桃体，海馬を含む）に活動が推定される（**図1**）。前内側部側頭葉の活動は島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，我々は，視床－島－前部帯状回及び前内側部側頭葉，の経路を推定している。視床－SI－SIIの経路が刺激のdiscriminativeな側面（刺激の部位，強さ，種類）に関わり，視床－島－前部帯状回及び前内側部側頭葉，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二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あげられる。最近私達は，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0.1mmで，プレート中央部の25mm四方の部分に，1mm毎に縦横26列の小さな穴（直径0.4mm，面積0.125mm^2）を穿ったものである。これを皮膚上にお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1-2m/秒であり，脊髄伝導速度は約1-4m/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，前内側部側頭葉（MT）の活動が見られた。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，second pain，すなわち内臓痛やガン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。

2.2 fMRIを用いた研究

記述した刺激方法を用いて，Aδ線維とC線維を刺激し，事象関連fMRIを記録した[8]。驚くべき事に，C線維刺激による場合の方が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（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（**図2**）。次に2種類の刺激間に有意な差が見られた部位を解析したところ，右側半球のBrodmannの24/32/8野（aACCの背側と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（**図3**）。second pain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，second pain認知がfirst pain認知よりも情動に関係が強い事を示唆している。

最近，私達は，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」を見ただけでも，pACCと島が活動する事を明らかにした[9]。これは「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事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

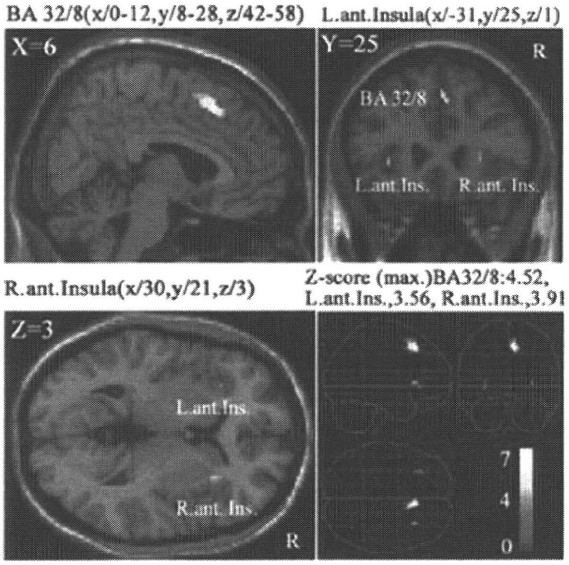

図3　C線維刺激による活動がAδ線維刺激による活動より有意に大きかった部位
両側の島全部，帯状回前部とpre-SMAに有意差が見られた。（文献8）より引用）。

み刺激を与えても，視床，SII，島，帯状回の活動は見られず，前頭葉，頭頂葉，中脳に活動が見られた[10]。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，瞑想中は何らかの機序により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。

2.3 その他の最近の研究

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 & Wall[11]は脊髄を責任部位としているが，我々は脳磁図を用いた詳細な研究により，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pathway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り，ヒトのA-delta線維を上行する痛覚信号を伝導する脊髄視床路のpathwayには少なくとも2種類があり，伝導速度は約17m/秒のものと，約10m/秒のものがある事を証明した。前者は第1次体性感覚野に到達し，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事がわかり，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inferior parietal lobule（BA 40）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，ヒトでは未だ証明されていなかった。我々は，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した。すると，喫煙（ニコチン）はAδ線維を上行するfirst pain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second pain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（ES電極）を用いた実験に関しては既述したが，最近我々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。

痒みの脳内認知機構

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することができる。しかし，時間分解能が低いため，同定されたそれらの脳部位がどのような機能的なつながりをもっているのか，すなわち，