cultures,[15] and the possibility that negative affectivity might be a third (confounding) variable that explains the significant associations found between alexithymia and measures of patient functioning[7] beyond the cultural differences.

To date, no other researchers have published findings regarding the associations between alexithymia and pain catastrophizing when controlling for negative affect (eg, measures of depression and anxiety) in patients with chronic pain. Our finding that anxiety (but not depression) made a unique contribution to the prediction of catastrophizing raises the possibility that anxiety plays a more important role than depression in the associations between negative affect and alexithymia. Future researchers should examine this possibility further by including measures of both depression and anxiety when examining the role that negative affect plays in contributing to (or being influenced by) alexithymia.

Although catastrophizing is reported to have the function of distraction,[65] previous studies reported that alexithymic students used significantly more distraction than nonalexithymic students.[66] In addition, alexithymic individuals have problems in interpersonal relationships,[33] and report reduced social support.[67] It is possible that catastrophizing plays a maladaptive role in eliciting social support in alexithymic patients with chronic pain.

Because Japanese people place such a high priority on harmony,[19] they may be less likely to express their negative feelings and intents directly, relative to individuals from western cultures. This may make it more difficult for alexithymic individuals in Japan to obtain support from other people and result in greater anxiety. If this is the case, and considering the social context of pain catastrophizing,[39] it might be possible that catastrophizing may tend to work more as a strategy to get support from others and reduce anxiety among alexithymic individuals. Future research is needed to determine whether our findings replicate in other Japanese samples and other samples from different cultures.

A competing hypothesis to viewing catastrophizing as a coping strategy used by alexithymic individuals with chronic pain is that alexithymia is a form of "emotional constriction" that contributes to experiential and emotional avoidance.[27] In stressful situations, people with more alexithymia may be less able to simply be aware of or even just "feel" the unpleasant emotions associated with the stressful situation, but cope by trying to "think" cognitively about the event.

The possibility that time spent being aware of one's emotions is adaptive—or at least leads to an attenuation of perceived stress—is supported by a recent neuroimaging study in which healthy patients were asked to self-reflect (a cognitive task) or be aware of their emotions and feelings.[68] These investigators found less activity in the amygdala (thought to be associated with emotional arousal) during the emotional awareness condition than during the cognitive condition.[68] Depending on the extent to which persons with alexithymia are unable to be aware of their emotions, they may experience more arousal, as they may be unable to use effective strategies (ie, reappraisal and affect labeling) for processing negative emotions.[69,70] As emotional arousal escalates, this could contribute to an increase in cognitions and thoughts that are linked to negative emotions, that is, catastrophizing cognitions. This hypothesis is consistent with the findings from the current study and with the findings from other studies, showing that alexithymia is associated with measures reflecting catastrophizing, distress, and negative affectivity.[7,16] With research showing that depressed individuals with chronic pain exhibit significantly more negative automatic thoughts than nondepressed individuals with chronic pain or healthy controls,[71] it is possible that the greater negative affect reported by individuals with alexithymia may be due to a limited ability to process emotions, which could lead to increased catastrophizing, and ultimately contribute to emotional distress.[39] Consistent with this possibility, in our sample, the alexithymic group showed significantly higher scores on the measures of pain catastrophizing and anxiety, and a nonsignificant trend to be positively associated with depression. These findings are also consistent with past findings suggesting an association between alexithymia and negative affect.[5,7]

The limitations of this study include the exclusive use of self-report measures, which may enhance the strength of associations found among the variables because of shared method variance. Although self-report measures are appropriate when assessing subjective domains such as alexithymia, pain, and affect, observational measures could be used to assess physical functioning. Future research should include observational measures of patient functioning, when possible. A second limitation is that the sample came from patients seen at a medical clinic that tends to treat patients presenting with more severe pain problems, which may affect the distribution of our data and it may be a confounding factor for our results. Therefore, the extent to which the findings generalize to other Japanese patients—especially perhaps other Japanese patients with less severe pain problems—is not clear. Research using samples from other pain clinics is needed to help determine the generalizability of the current findings. A third limitation is that we excluded both TAS-DDF and TAS-EOT scales from analyses because of their low internal consistency in our sample. Previous findings suggest that the TAS-DIF scale tends to be more strongly associated with pain outcomes than the TAS-DDF and the TAS-EOT scales, and so excluding the latter scales from our analyses may not have excluded the most important (to pain) alexithymia domain from our analyses.[6,7] Still, it would be useful to determine the associations between the difficulty describing feelings and externally oriented thinking domains of alexithymia and pain-related outcomes in other Japanese samples of patients to better understand their potential role.

Finally, because the data collected in this study were cross-sectional, it is not possible to draw conclusions about causal associations among the study variables. Using these data, we cannot determine, for example, whether alexithymia contributes to negative outcomes (by its effects on negative affect) or whether negative affect influences alexithymia and the other outcomes. Both possibilities remain viable. Research is needed to determine whether a treatment that reduces alexithymia results in subsequent improvements in catastrophizing and emotional functioning. The findings do suggest, however, that such research is warranted.

Despite the study's limitations, the findings confirm the cross-cultural importance of alexithymia as being associated with pain interference and indicate that, at least in our sample of Japanese patients with chronic pain, alexithymia is also significantly associated with depression, anxiety, and catastrophizing. Longitudinal and in particular experimental research in which alexithymia is altered in 1 group but not in another is needed to help tease out the

relative influence of the variables examined in this study on the other variables.

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

Ken Takahashi[a], Toru Taguchi[a], Satoshi Tanaka[b], Norihiro Sadato[b], Yunhai Qiu[c], Ryusuke Kakigi[c], Kazue Mizumura[a,*]

[a] Research Institute of Environmental Medicine, Nagoya University, Japan
[b] Division of Cerebral Integration, National Institute of Physiological Science, Japan
[c] Department of Integrative Physiology, National Institute of Physiological Science, Japan

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ABSTRACT

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

1. Introduction

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

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

* Corresponding author at: Department of Neural Regulation, Research Institute of Environmental Medicine, Nagoya University, Nagoya 464-8601, Japan.
Tel.: +81 52 789 3864; fax: +81 52 789 3889.
E-mail address: mizu@riem.nagoya-u.ac.jp (K. Mizumura).

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

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

2. Materials and methods

2.1. Subjects

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

2.2. Stimulus

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

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

Table 1
Stimulus intensity for the muscle and cutaneous stimuli.

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

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

2.3. Imaging procedure

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

2.4. Image processing and analyses

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

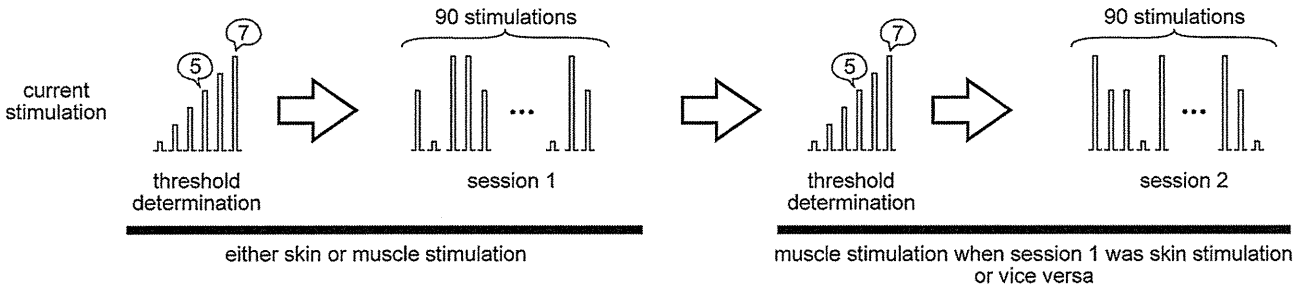

Fig. 1. Schematic diagram of stimulus application. Electric current stimulation was applied to the left leg for each subject. Subjects received two fMRI scanning sessions (skin and muscle stimulations. The order was randomized). Before each session, determination of pain threshold was carried out. Balloons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

Table 2
Predefined contrasts for fMRI analysis.

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

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

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

3. Results

3.1. Pain perception

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

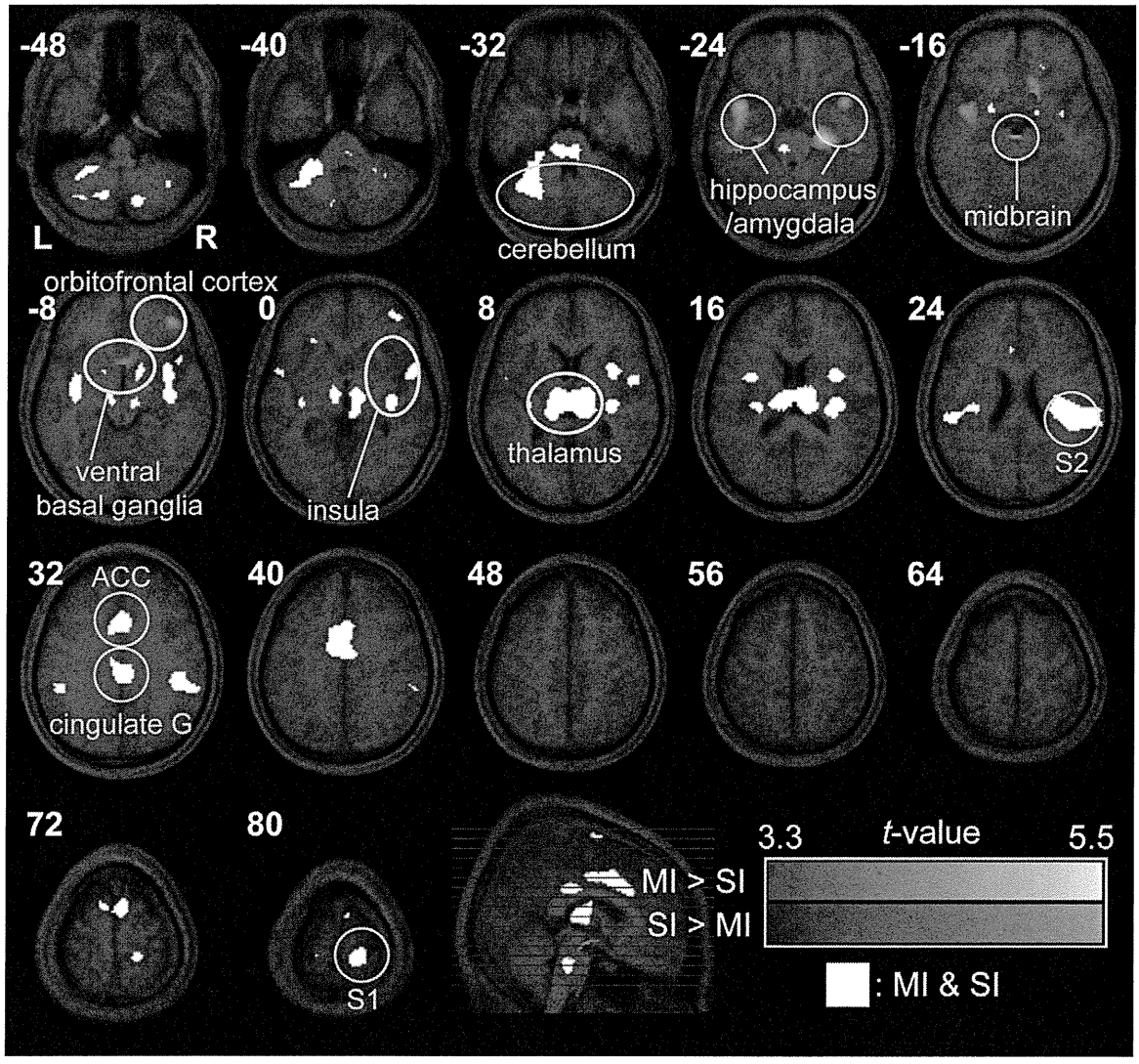

Fig. 2. Sequential brain maps of t-scores representing brain activities in response to electrical painful stimuli. Color scales indicate signal intensity increases during painful stimuli. *Red-yellow*: specific response to painful muscle stimulation; *Blue-green*: specific response to painful skin stimulation.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

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

3.2. Response to painful muscle stimulation

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

Table 3
Brain regions responding to painful electric stimuli.

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

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

* False discovery rate corrected $p < 0.05$.

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

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

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

3.3. Response to painful skin stimulation

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

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

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

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

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

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

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

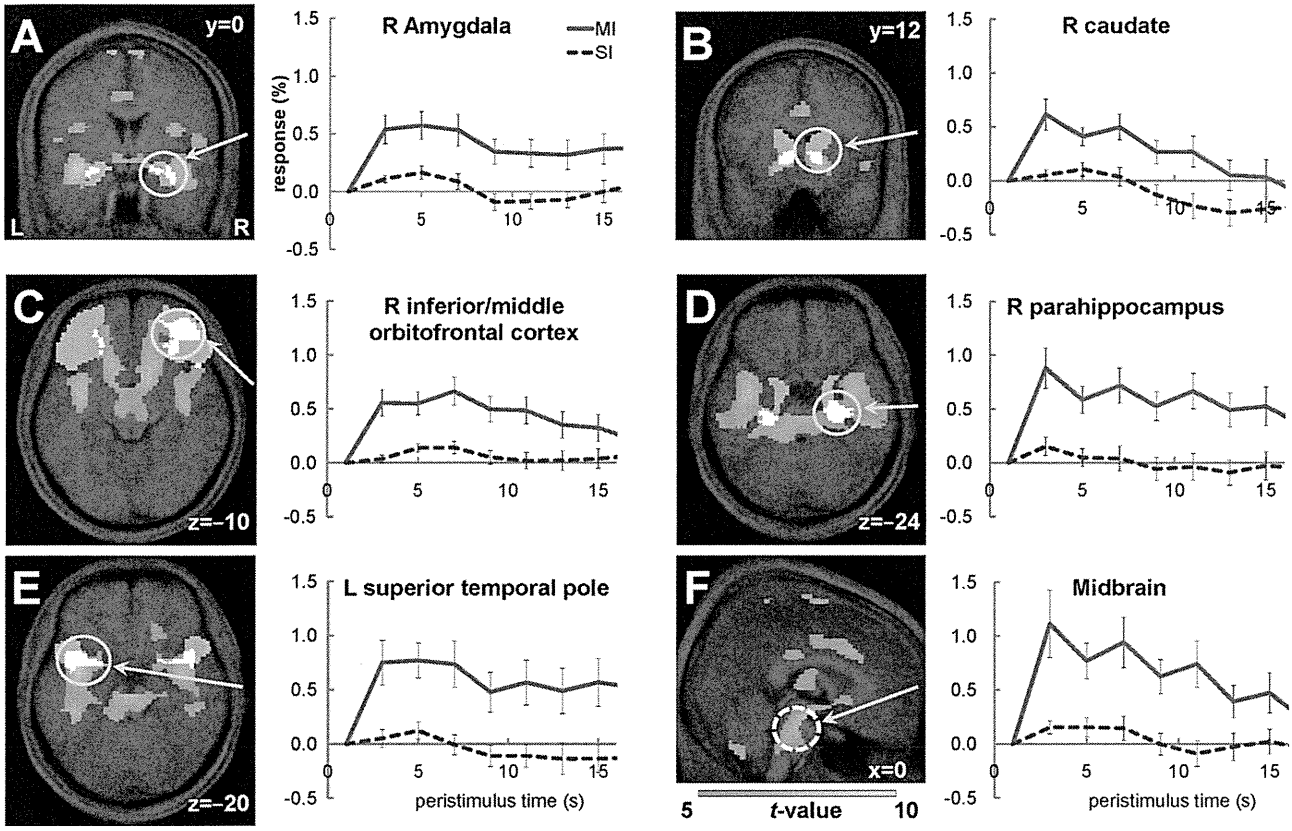

Fig. 3. Activation maps and peristimulus time–response curves at the brain regions that showed greater activities for painful muscle stimulation than for painful skin stimulation. (A) amygdala, (B) caudate, (C) inferior and middle orbitofrontal cortex, (D) parahippocampus, (E) superior temporal pole,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

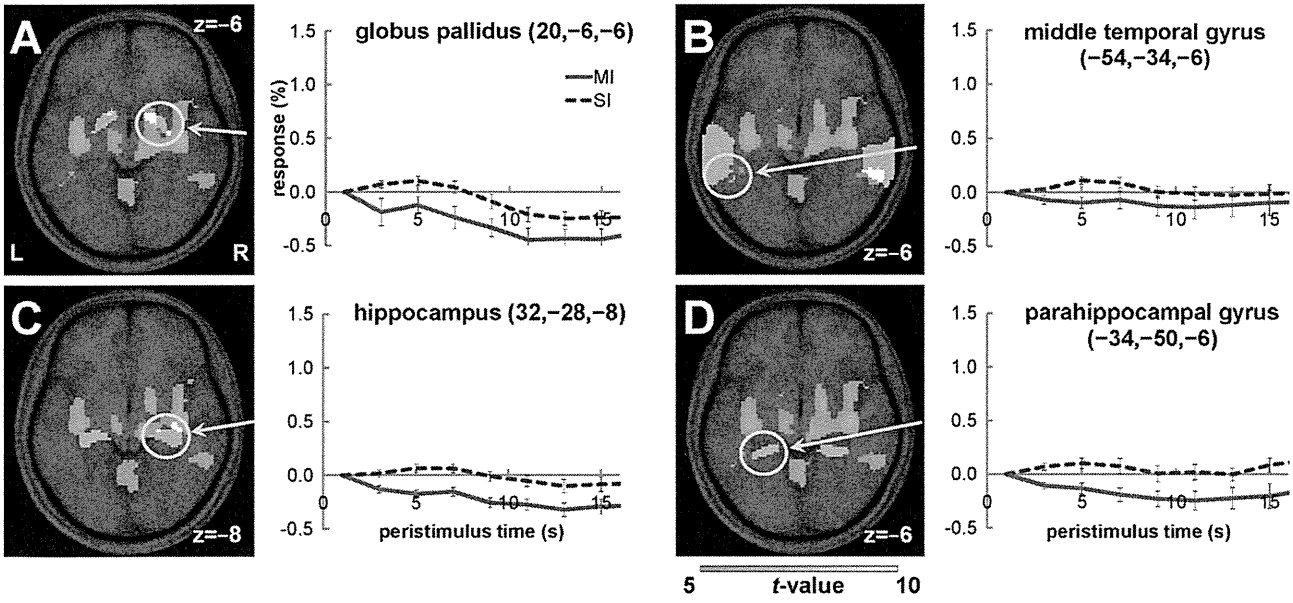

Fig. 4. Peristimulus time–response curves at brain regions that showed greater response to painful skin stimulation than to muscle stimulation. (A) globus pallidus, (B) middle temporal gyrus, (C) hippocampus, and (D) parahippocampus. These anatomical regions are indicated by blue regions in the activation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

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

4. Discussion

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

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

4.1. Midbrain

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

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

4.2. Amygdala/hippocampal regions

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

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

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

4.3. Orbitofrontal cortex

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

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

4.4. Ventral Basal ganglia

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

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

4.5. Superior temporal pole

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

4.6. Brain regions preferentially respond to skin pain

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

4.7. Limitation of the present study

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

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

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Treadmill running and static stretching improve long-lasting hyperalgesia, joint limitation, and muscle atrophy induced by cast immobilization in rats

Atsuko Morimoto[a,b,c,1], Handriadi Winaga[b,d,1], Hiroki Sakurai[b,c], Mika Ohmichi[c,e], Takahiko Yoshimoto[b,c], Yusuke Ohmichi[b,c,e], Takuya Matsui[a], Takahiro Ushida[b], Tadashi Okada[a], Jun Sato[b,c,*,1]

[a] *Department of Physiology, Aichi Medical University, Aichi 480-1195, Japan*
[b] *Multidisciplinary Pain Center, Aichi Medical University, Aichi 480-1195, Japan*
[c] *Research Institute of Environmental Medicine, Nagoya University, Aichi 464-8601, Japan*
[d] *Department of Orthopedics and Traumatology, Hasanuddin University, Makassar 90245, Indonesia*
[e] *Department of Anatomy, Aichi Medical University, Aichi 480-1195, Japan*

HIGHLIGHTS

- Two-week cast immobilization induced chronic hyperalgesia in the hindlimb of rats.
- The cast immobilization also induced joint limitation and calf muscle atrophy.
- Treadmill running and static stretching after cast removal counteracted these changes.

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ABSTRACT

The effects of exercise on chronic pain induced by immobilization are incompletely understood. The purpose of this study was to investigate whether 30 min of treadmill running (TR; active exercise) and 10 min of static stretching (SS; passive exercise) of the immobilized hindlimb reduce widespread chronic pain, joint limitation, and hindlimb muscle atrophy induced by cast immobilization in rats. One hindlimb of Sprague Dawley (SD) rats was immobilized for 2 weeks with a cast, and remobilization was conducted for 7 weeks. MRI study showed that cast immobilization had induced inflammatory changes in the immobilized hindlimb, beginning as early as 2 h after cast removal; these changes continued for 2–3 days. Mechanical hyperalgesia in the calf and hindpaw developed as early as 2 h after cast removal and continued for 7 weeks. TR and SS were initiated 3 days after cast removal and were continued 3 times per week for 2 weeks. Both forms of exercise significantly inhibited mechanical hyperalgesia in the calf and hindpaw in immobilized rats. Range-of-motion limitations in the knee and ankle joints and calf muscle atrophy after cast removal were also decreased by both TR and SS. This study is the first to demonstrate the beneficial effect of TR and SS on widespread chronic pain, joint limitation, and muscle atrophy in a cast-immobilized rat model.

1. Introduction

It has been postulated that physical immobilization is one of the main contributors to long-lasting pain after trauma or surgery in an extremity [6,20]. However, the mechanisms of sustained immobilization-induced chronic pain remain poorly understood. We have demonstrated that rats exposed to 2-week cast immobilization of 1 hindlimb exhibit local inflammatory changes and spontaneous pain in the immobilized hindlimb as well as long-lasting mechanical hyperalgesia in both the immobilized and contralateral hindlimbs (chronic post-cast pain; CPCP) [14]. In another study, we demonstrated that cast immobilization-induced ischemia/reperfusion injury with production of oxygen free radicals in the immobilized hindlimb leads to astrocyte activation in the bilateral spinal dorsal horn and, as a result, widespread hyperalgesia in rats with CPCP [13].

Exercise therapy is an effective treatment and the most widely used type of conservative treatment for various chronic pain in humans [7,8,21]. Exercise therapy includes two modalities: active

* Corresponding author at: Futuristic Environmental Simulation Center, Research Institute of Environmental Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan. Tel.: +81 52 789 3895; fax: +81 52 789 3999.
E-mail address: jun@riem.nagoya-u.ac.jp (J. Sato).
[1] These authors contributed equally to this work.

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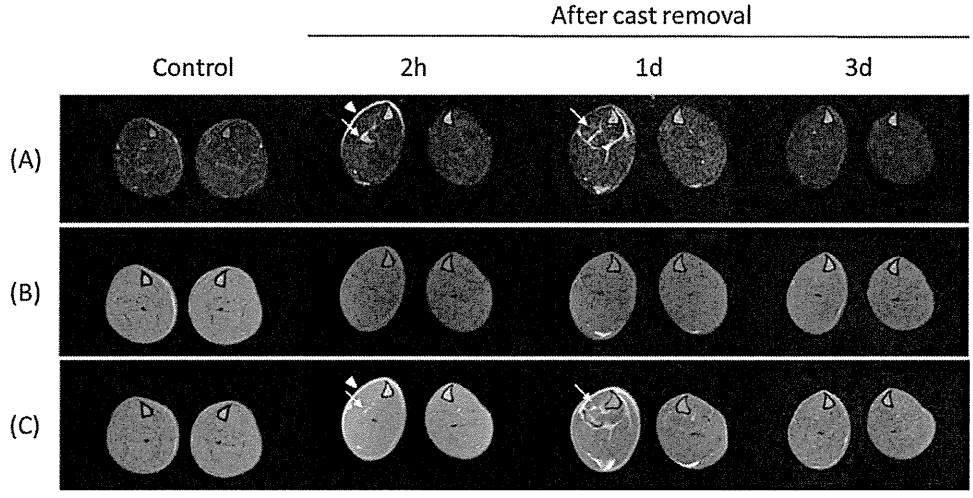

Fig. 1. Changes in hindlimb MRI images after cast removal: T2WI (A), T1WI (B), and T1WI with gadolinium enhancement (C).

(i.e., aerobic, muscle strengthening, and stabilizing exercises) and passive (i.e., stretching, manual therapy, and mobilization). Recent animal studies have reported that active aerobic exercise decreases neuropathic pain [3,4,9,19] and musculoskeletal pain after muscle injury [1]. It has also been suggested that changes in central nervous system neurotransmitters and neuromodulators, such as endogenous opioids, are involved in the mechanism of exercise effects [11]. However, no concrete evidence exists for the effects of exercise on hyperalgesia after physical immobilization. The present study, therefore, aims to investigate the effects of treadmill running as active exercise on long-lasting mechanical hyperalgesia in CPCP rats. Passive exercise is also used to improve joint range of motion and muscle flexibility and to relieve musculoskeletal pain in humans. Thus, the second purpose of this study is to test effects of static stretching (i.e., passive exercise) on long-lasting mechanical hyperalgesia in rats with CPCP.

Parts of this study were presented in abstract form at the 14th World Congress of Pain, Milan, 2012 [23].

2. Materials and methods

2.1. Animals

All experiments were conducted with the approval of the Animal Care Committee of Aichi Medical University and in accordance with the guidelines of the International Association for the Study of Pain for pain research in animals. Male Sprague Dawley rats (300–360 g, Japan SLC, Japan) were housed in standard transparent plastic cages (2–3 per cage) lined with sawdust under a 12/12-h light/dark cycle with free access to food and water. We attest that all efforts were made to minimize both the number of animals used and their suffering.

2.2. Hindlimb cast immobilization

CPCP was generated through 2-week hindlimb cast immobilization, in accordance with findings of our recent study [14]. In brief, a plaster cast was applied from the trunk to the middle of left hindpaw under anesthesia with pentobarbital sodium (40 mg/kg, i.p.). If any signs of circulation impairment (e.g., congestion, ischemia, or pressure ulcer) in the immobilized hindlimb or severe damage to the cast were observed during the 2-week immobility period, the plaster cast was replaced (6 of 31 rats). If any problems were observed in the replaced cast, the rat was excluded from further investigation (4 of 31 rats).

2.3. Exercise

All rats were trained on the treadmill for 3 days before cast immobilization. Three days after cast removal, rats were randomly assigned to three experimental groups: (1) CPCP without exercise (CPCP, $n=10$); (2) CPCP with treadmill running (CPCP+TR, $n=9$); and (3) CPCP with static stretching (CPCP+SS, $n=8$). The rats in the CPCP+TR group were made to run on a motor-driven treadmill (Modular Treadmill, Columbus Instruments, USA) for 30 min. The exercise intensity consisted of running at a speed of 12 m/min, a speed at which all CPCP rats were capable of running in this experiment. The rats in the CPCP+SS group had their muscles stretched for 10 min. For the stretching procedure, the animals were anesthetized with 1% isoflurane, and 1 hindlimb was suspended longitudinally for 10 min by a custom-built stretch apparatus with a pulley system suspending a 25-g load in order to simulate a clinical situation, as described by Ikeda et al. [10]. The TR and SS exercises began 3 days after cast removal and continued 3 times per week for 2 weeks ("trial" = 6 times total).

2.4. Local tissue changes, joint limitation, and muscle atrophy

To test the effect of cast immobilization in the calf skin and muscle tissues, MRI examination was performed using a 1.5-Tesla small animal scanner (MRImini SA 1508; DS Pharma Biomedical, Japan). Each rat was anesthetized with 1% isoflurane and positioned prone in the body coil. The MRI protocol included 3 scans: (1) axial T1-weighted imaging (T1WI) obtained with the spin-echo sequence with a repetition time (TR) of 500 ms and echo time (TE) of 9 ms; (2) axial T2-weighted imaging (T2WI) obtained with the spin-echo sequence with a TR of 2000 ms and TE of 69 ms; and (3) axial T1WI with gadolinium enhancement. Gadolinium (Bayer Schering Pharma, Japan) was dissolved in saline and a volume of 0.1 mL administered intravenously (0.2 mL/kg) in the tail. All images were obtained with a 256 × 128 matrix, 20-mm × 40-mm field of view, 4 averages, 10 coronal slices, 2-mm slice thickness, and 0.4-mm interslice gap.

The range of motion (ROM) of the immobilized knee and ankle joints was measured using a custom protractor (AJ124; Raymay,

Japan). Each rat was anesthetized with 1% isoflurane and positioned in a homemade locking device. Passive joint ROM was measured in degrees, while the hindlimb was suspended longitudinally with 10 g of traction.

To investigate calf muscle atrophy, each rat was restrained with a sock from the head to the pelvis and placed in supine position. Muscle atrophy was evaluated by measuring calf width with a manual caliper. The caliper was touched lightly to the thickest area of the calf while holding the hindpaw in a maximally extended position.

2.5. Mechanical hyperalgesia

The nociceptive threshold in the calf skin was determined as the response to probing of the calf skin with a series of calibrated homemade von Frey filaments (VFFs; diameter, 0.5 mm) applied perpendicularly to the medial surface of the skin over the calf muscle belly. To evaluate mechanical pain thresholds in the hindpaw, each rat was individually placed beneath an inverted plastic box (207 mm × 132 mm × 136 mm) with an elevated wire mesh bottom. A series of VFFs were applied perpendicularly to the medial surface of the hindpaw. Data were analyzed using the up-and-down method of Dixon and Mood [2]. To investigate mechanical hyperalgesia in the calf muscle, each rat was restrained with a sock from the head to the pelvis. Pressure stimulation of the calf muscle was performed using a push–pull gauge algometer (Aikoh Engineering, Japan). A cone-shaped pusher with a rounded tip (diameter, 2.4 mm) was applied to the calf muscle belly with linearly increasing pressure (10 g/s), and the minimum pressure required to elicit foot withdrawal was measured. Pressure stimulation of the calf muscle was performed 4 times at intervals of at least 30 s, and the median value of the last 3 trials was defined as the pain threshold.

2.6. Statistical analysis

Results are presented as mean ± SEM. Statistical significance was determined by mixed-design two-way repeated measures analysis of variance (two-way ANOVA) for multi-group comparisons, or by one-way repeated measures ANOVA (one-way ANOVA), as appropriate. Dunnett's test was used for post hoc comparisons when the F value was significant ($P < 0.05$). Tukey–Kramer's post hoc test was used to compare the values of experimental groups. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. MRI changes after cast removal

Fig. 1 illustrates the characteristic MRI patterns of both the immobilized and contralateral hindlimbs after cast removal. T2WI (A) and T1WI with Gd enhancement (C) showed dense hyperintensity in the calf skin (arrowheads) and interstitial region surrounding blood vessels (arrows) in the immobilized hindlimb. These high signals in the calf skin were observed 2 h after and 1 day after cast removal. In the interstitial region surrounding blood vessels, high signals appeared just after cast removal (2 h) and diffused into the muscle and intermuscular region 1 day after cast removal. By the third day after cast removal, all these changes had disappeared. There was, however, no signal change on T1WI (B). These results suggest that the high signals in the calf skin and interstitial region surrounding blood vessels were the result of muscular edema (i.e., inflammatory change) in the immobilized hindlimb. Based on these results, exercise trials (TR and SS) were initiated 3 days after cast removal, when inflammatory changes in the immobilized hindlimb were nearly resolved.

3.2. Effects of exercise

3.2.1. Long-lasting mechanical hyperalgesia

Fig. 2A shows the time course of mechanical threshold in the calf skin after cast removal for each group. In rats with CPCP, the withdrawal threshold decreased quickly (indicating mechanical hyperalgesia), reaching a minimum 1–3 days after cast removal and persisting for 7 weeks. The withdrawal thresholds in both the CPCP + TR and CPCP + SS groups were also significantly decreased after cast removal. Under subsequent TR or SS trial, however, the decreased mechanical threshold was clearly inhibited. Two-way ANOVA identified a significant treatment effect (treatment: $F_{(2,22)} = 31.5$; time: $F_{(10,220)} = 206.3$; treatment × time: $F_{(20,220)} = 5.4$; $P < 0.001$ for all). The withdrawal thresholds of both CPCP + TR and CPCP + SS rats were significantly greater than those of CPCP rats after exercise trials. Decrease in withdrawal threshold after cast removal was also observed in the hindpaw (Fig. 2B), and this mechanical hyperalgesia was clearly inhibited by subsequent TR and SS trials. Two-way ANOVA identified a significant treatment effect (treatment: $F_{(2,22)} = 30.5$; time: $F_{(10,220)} = 197.2$; treatment × time: $F_{(20,220)} = 6.8$; $P < 0.001$ for all). Fig. 2C shows the time course of mechanical threshold in the calf muscle after cast removal for each group. In the CPCP rats, the withdrawal threshold to push–pull mechanical stimulation significantly decreased after cast removal, indicating mechanical hyperalgesia. This threshold change also appeared quickly, and was then gradually attenuated; however, the decreased level was still apparent at 7 weeks after cast removal. The withdrawal thresholds in both the CPCP + TR and CPCP + SS rats were also significantly decreased after cast removal. Subsequent TR or SS trial enhanced the gradual attenuation of the decreased mechanical threshold (treatment: $F_{(2,22)} = 14.5$; time: $F_{(10,220)} = 255.5$; treatment × time: $F_{(20,220)} = 4.5$, by two-way ANOVA; $P < 0.001$ for all). These results indicate that both active (TR) and passive (SS) exercise inhibited the mechanical hyperalgesia evident in the calf skin, hindpaw, and calf muscle after cast removal.

3.2.2. Joint ROM limitations

Fig. 3A shows the time course of knee joint ROM after cast removal for each group. In the CPCP rats, ROM decreased as early as 1 day after cast removal and was sustained for 7 weeks after cast immobilization. One-way ANOVA indicated a significant decrease in ROM ($F_{(6,42)} = 51.4$, $P < 0.001$). The knee joint ROMs in both the CPCP + TR and CPCP + SS groups were also significantly decreased after cast removal. Two-way ANOVA identified a significant treatment effect (treatment: $F_{(2,22)} = 40.9$, time: $F_{(6,132)} = 209$, treatment × time: $F_{(12,132)} = 5.7$, $P < 0.001$ for all). Under subsequent TR or SS trial the ROMs were significantly restored as early as 4 days after beginning TR and SS (1 week after cast removal). This inhibitory effect of the exercises on ROM limitation was also evident in the ankle joint. The ankle joint ROMs also decreased as early as 1 day after cast removal ($F_{(6,42)} = 53.3$, $P < 0.001$ by one-way ANOVA). The ROMs in CPCP + TR and CPCP + SS rats were significantly greater than those in CPCP rats as early as 4 days after beginning TR and SS (1 week after cast removal). Two-way ANOVA identified a significant treatment effect (treatment: $F_{(2,22)} = 18.9$, time: $F_{(6,132)} = 125.2$, treatment × time: $F_{(12,132)} = 7.1$, $P < 0.001$ for all). These results indicate that both TR and SS improved ROM limitations in the knee and ankle joints after cast removal.

3.3. Muscle atrophy

Fig. 3B shows the time course of calf width after cast removal for each group. In the CPCP rats, calf width significantly decreased after cast removal, indicating muscle atrophy. This decrease appeared as early as 1 day after cast removal and was then gradually attenuated. One-way ANOVA with Dunnett's post hoc tests revealed that

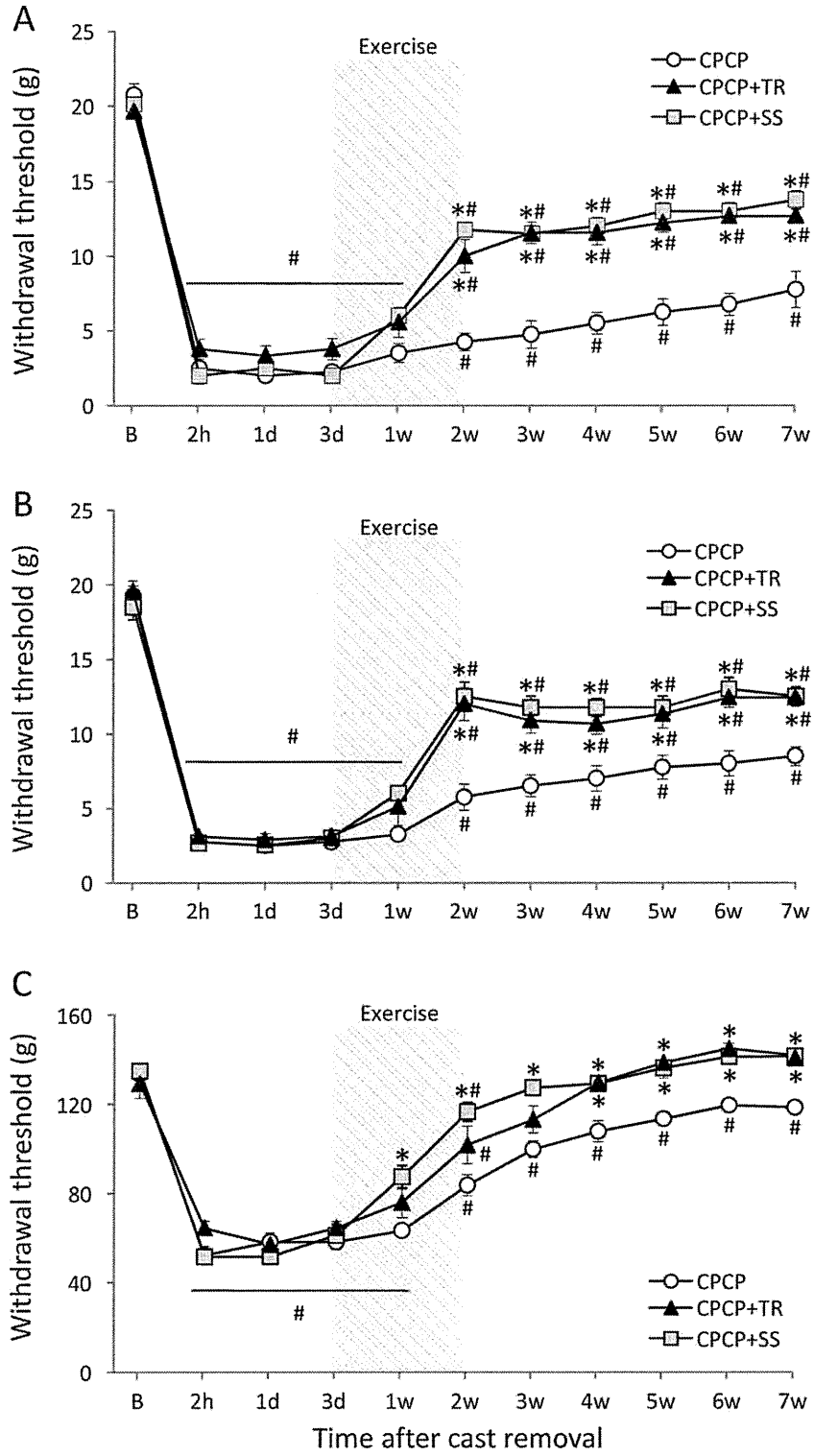

Fig. 2. Time course of changes in withdrawal thresholds in the calf skin (A), hindpaw (B), and calf muscle (C) of the CPCP rats without exercise (CPCP, $n=8$), with treadmill exercise (CPCP+TR, $n=9$), and with static stretching (CPCP+SS, $n=8$). In the CPCP rats, the withdrawal thresholds in these body parts significantly decreased 2 h after cast removal compared to pre-cast thresholds; calf muscle [$F_{(10,70)}=88.3$, $P<0.001$], calf skin [$F_{(10,70)}=61.4$, $P<0.001$] and hindpaw [$F_{(10,70)}=46.2$, $P<0.001$] by one-way ANOVA with Dunnett's tests. *$P<0.05$, compared with CPCP values (Tukey–Kramer's post hoc test). #$P<0.05$, compared with associated baseline values (Dunnett's post hoc test). The horizontal axis indicates the measurement time points. Data are presented as mean ± SEM.

significant decrease was apparent up to 3 weeks after cast removal ($F_{(6,42)}=40.3$, $P<0.001$). The calf widths in both the CPCP+TR and CPCP+SS rats were also significantly decreased 1 day after cast removal. In these cases, however, the calf widths had returned to pre-cast levels within a maximum of 3 weeks, faster than those of the CPCP rats. At 7 weeks after cast removal, both groups' calf widths were even greater than they had been before casting.

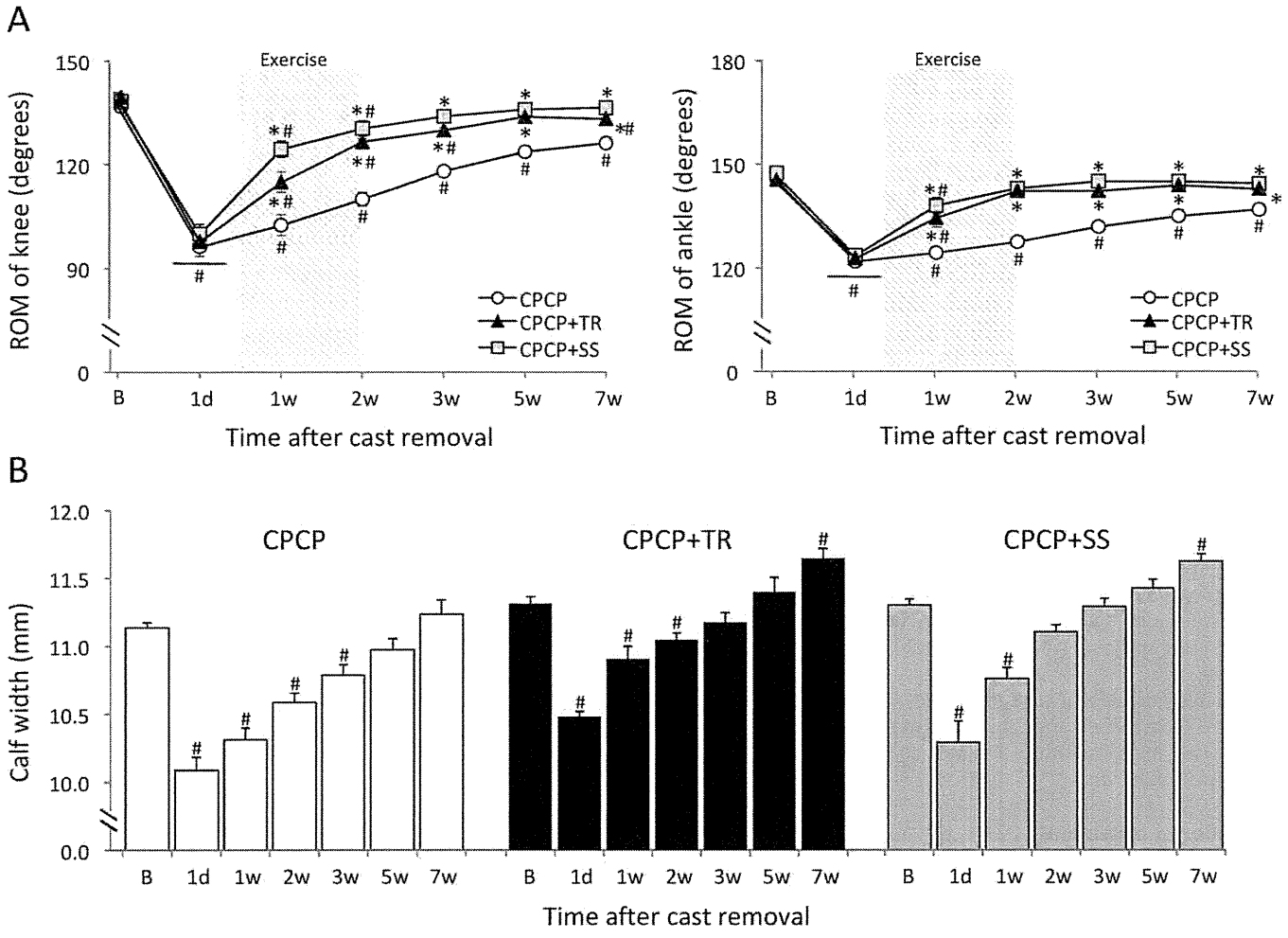

Fig. 3. Time course of changes in ROM (A) and calf width (B) of the CPCP rats without exercise (CPCP, $n=8$), those with treadmill exercise (CPCP+TR, $n=9$), and those with static stretching (CPCP+SS, $n=8$). $^{*}P<0.05$, compared with CPCP rats (Tukey–Kramer's post hoc test). $^{\#}P<0.05$, compared with associated baseline values (Dunnett's post hoc test). The horizontal axis indicates the measurement time points. Data are presented as mean ± SEM.

4. Discussion

The present results are consistent with our recent observation that rats exposed to 2-week cast immobilization of the hindlimb exhibited local inflammatory changes, muscle atrophy, and long-lasting mechanical hyperalgesia in the immobilized hindlimb [14]. The present study further demonstrates that both TR (active exercise) and SS (passive exercise), which were conducted after muscle edema had subsided, reduced mechanical hyperalgesia in the calf, ROM limitation in the knee and ankle joints, and muscle atrophy induced by cast immobilization.

We have demonstrated that oxygen free-radical scavengers administered 24 h after cast removal reversed mechanical hyperalgesia in both hindlimbs of CPCP rats [13,14], suggesting the contribution of ischemia/reperfusion injury occurring just after cast removal to the development of widespread hyperalgesia. In addition, when a sciatic nerve block was performed upon initial appearance of local inflammation (24 h after cast removal), the mechanical hyperalgesia in both hindlimbs of CPCP rats was significantly inhibited. More recently, we have found that astrocytes in both spinal dorsal horns were dramatically activated 5 weeks after cast removal, and an oxygen free-radical scavenger administered 24 h after cast removal reversed this phenomenon [13]. Taken together, our recent results suggest that cast immobilization induces ischemia/reperfusion injury-induced inflammatory events in the immobilized hindlimb; this leads to astrocyte activation in the dorsal horn and, as a result, long-lasting hyperalgesia in CPCP rats.

Two hypotheses are suggested as mechanisms for the inhibitory effects of TR and SS on long-lasting hyperalgesia in the immobilized hindlimb. The first possibility is that TR and SS decrease oxygen free radicals in the immobilized hindlimb. It is reported that prolonged disuse of hindlimb induces muscle atrophy with production of oxygen free radicals in the immobilized hindlimb [16]. It has been also shown that aerobic and endurance exercises increased the activity of one or more endogenous scavengers of reactive oxygen metabolites in skeletal muscle as well as increased oxidative capacity [5]. These results suggest that exercise performed soon after cast removal attenuates ischemia/reperfusion injury in the cast-immobilized hindlimb and prevents development of hyperalgesia in the CPCP model. We do not know if TR and SS had similar effects in our CPCP rats; this might be an avenue for further investigation.

The second possibility is that TR and SS induce analgesia through a central nervous system mechanism. Several partly overlapping mechanisms are suggested to play a role in active exercise-induced analgesia in humans, including release of endogenous opioids [11], growth factors [11], and catecholamine [15] and activation of supraspinal nociceptive inhibitory mechanisms orchestrated by the brain [12,17]. Recent animal studies have reported that treadmill exercise attenuates chronic mechanical hyperalgesia induced by muscle injury [1] and neuropathy [19]; the underlying mechanism involves endogenous opioids. These results suggest that TR

improves long-lasting hyperalgesia by increasing endogenous opioids in the central nervous system.

Our present study shows that SS, a passive exercise, also has an analgesic effect on chronic pain in CPCP rats. It has been demonstrated that spine manipulation, another passive exercise, produces immediate analgesia in human subjects [22]. In an animal study, Skyba et al. demonstrated that knee joint manipulation produces a non-opioid form of analgesia mediated by spinal serotonergic and noradrenergic receptors utilizing descending pain inhibitory pathways [18]. Although these findings suggest that SS attenuates long-lasting hyperalgesia in CPCP rats through the analgesic mechanism in the central nervous system, there is no direct evidence to support this hypothesis. Endogenous opioid measurements would allow direct testing of this hypothesis in future studies.

It is not clear which type of exercise is advantageous as therapy for post-cast pain. In fact, both TR and SS showed comparable inhibitory effects on pain, joint ROM limitations, and muscle atrophy induced by cast immobilization. SS appears to be a better therapy because it does not require supplementary apparatus. On the other hand, the advantage of the TR is that this active exercise does not require anesthesia. However, in human therapy, SS can be performed without anesthetization. In addition, patients often have difficulty in moving because of pain. Therefore, SS appears to be a better therapy, especially when patients experience pain during motion.

In conclusion, our present study indicates that both TR (active exercise) and SS (passive exercise) ameliorated mechanical hyperalgesia in the calf, joint limitation, and muscle atrophy induced by cast immobilization in rats.

Conflict of interest statement

None of the authors have any conflicts of interest to declare.

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特集
膝蓋骨脱臼の評価と治療

新鮮膝蓋骨脱臼に対する治療

Treatment for acute traumatic patellar dislocation

大森　豪

G. Omori：新潟大学研究推進機構超域学術院

Key words
- 新鮮膝蓋骨脱臼(acute traumatic patellar dislocation)
- 保存療法(conservative treatment)
- 手術療法(surgical treatment)

はじめに

膝蓋骨脱臼には複数の因子が関与し病態も複雑であるため，治療法についても一定の見解が得られているとはいえない。新鮮膝蓋骨脱臼(いわゆる初回脱臼)例では，一般的に保存療法が選択されることが多いが，再脱臼の割合が20～40%，膝蓋骨不安定性の残存は60%に達するという報告[1),2)]もあることから，近年，新鮮例に対する手術療法の報告も散見される。

本稿では，新鮮膝蓋骨脱臼(初回脱臼)例に対する保存療法と手術療法の内容および成績について概説する。

保存療法

まず，臨床症状，理学所見に加えて単純X線，MRI，CT検査から膝蓋骨関節面骨軟骨損傷や内側膝蓋大腿靱帯(medial patello-femoral ligament；MPFL)の膝蓋骨および大腿骨付着部の剥離骨折など，急性期の手術療法を必要とする合併症がないことを確認する。膝関節内の血腫を穿刺吸引後，局所の安静を目的にknee braceによる膝伸展位固定と松葉杖による非荷重歩行を行う。knee braceによる固定は2週間とし，部分荷重は疼痛および関節腫脹が軽減したことを確認後(通常，受傷後1週間程度)許可し，疼痛が自制内であれば全荷重とする。患肢挙上および大腿四頭筋セッティングは早期から行う。knee brace除去後可動域訓練を開始し，筋力訓練は内側広筋(VM，VMO)の強化を積極的に行う。可動域回復後膝蓋骨脱臼用の装具を装着し，スポーツ復帰をめざす[3)]**(図1，2)**。

著者らの治療方針

著者らは，新鮮膝蓋骨脱臼例に対しては膝蓋骨関節面の骨軟骨損傷，もしくはMPFL付着部剥離損傷で遊離骨片が大きいものを除いては，既述した保存療法を行っている。そして，再脱

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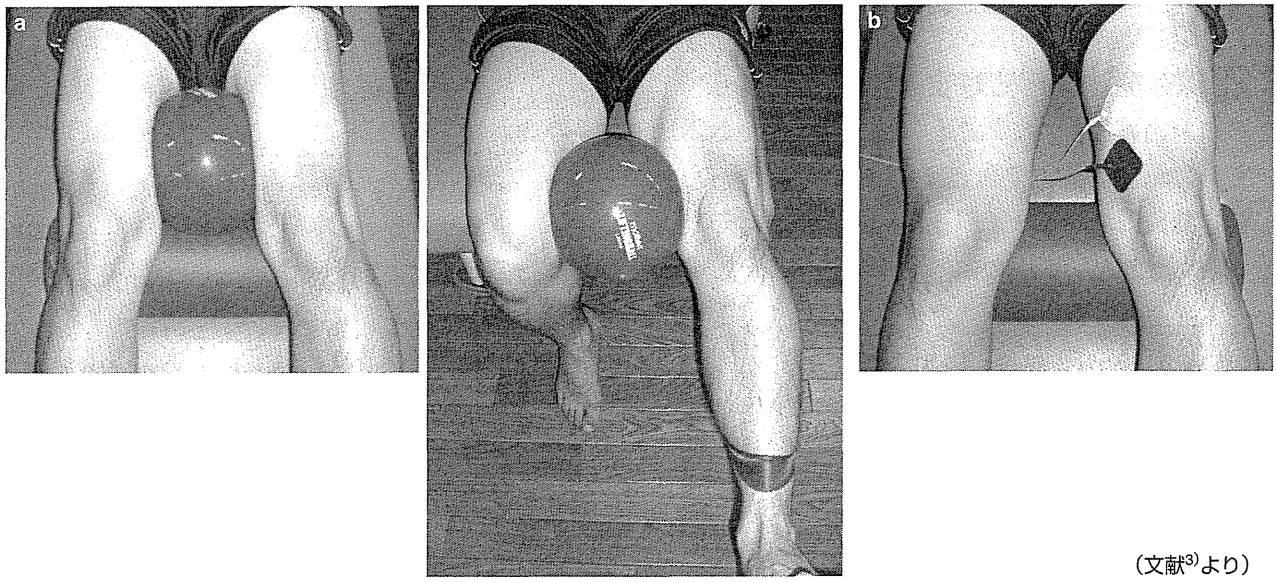
図1 内側広筋訓練

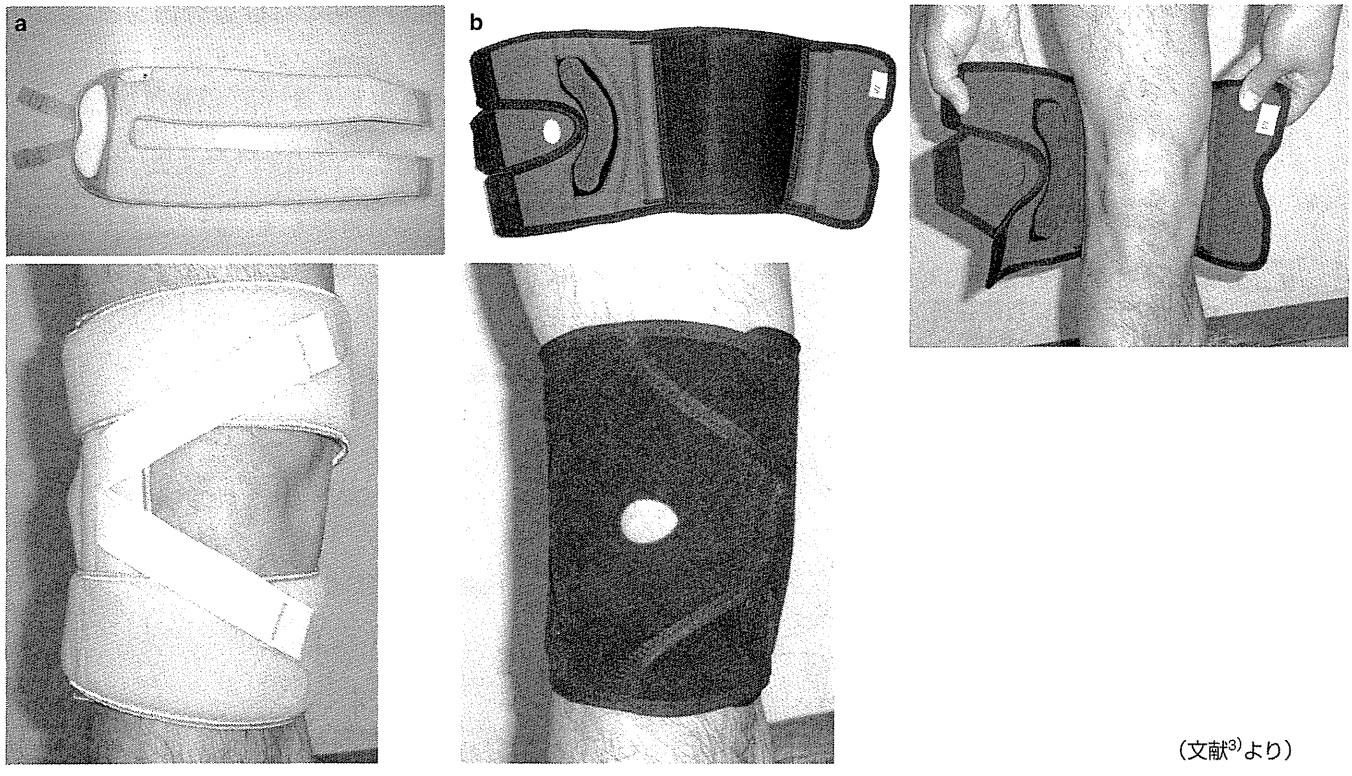
a：ボールを用いた内側広筋と内転筋の同時収縮訓練。
b：低周波を用いた内側広筋の選択的収縮。



（文献[3]より）

図2 膝蓋骨脱臼防止用装具

a：FAST WRAP
b：GELTEX PATELLA



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