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

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ABSTRACT

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

1. Introduction

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

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

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

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

2. Materials and methods

2.1. Subjects

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

2.2. Stimulus

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

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

Table 1
Stimulus intensity for the muscle and cutaneous stimuli.

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

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

2.3. Imaging procedure

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

2.4. Image processing and analyses

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

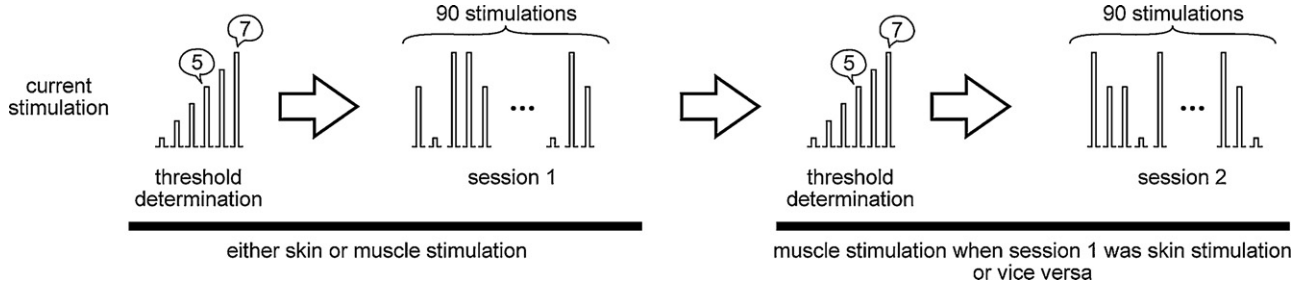

Fig. 1. Schematic diagram of stimulus application. Electric current stimulation was applied to the left leg for each subject. Subjects received two fMRI scanning sessions (skin and muscle stimulations. The order was randomized). Before each session, determination of pain threshold was carried out. Balloons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

Table 2
Predefined contrasts for fMRI analysis.

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

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

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

3. Results

3.1. Pain perception

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

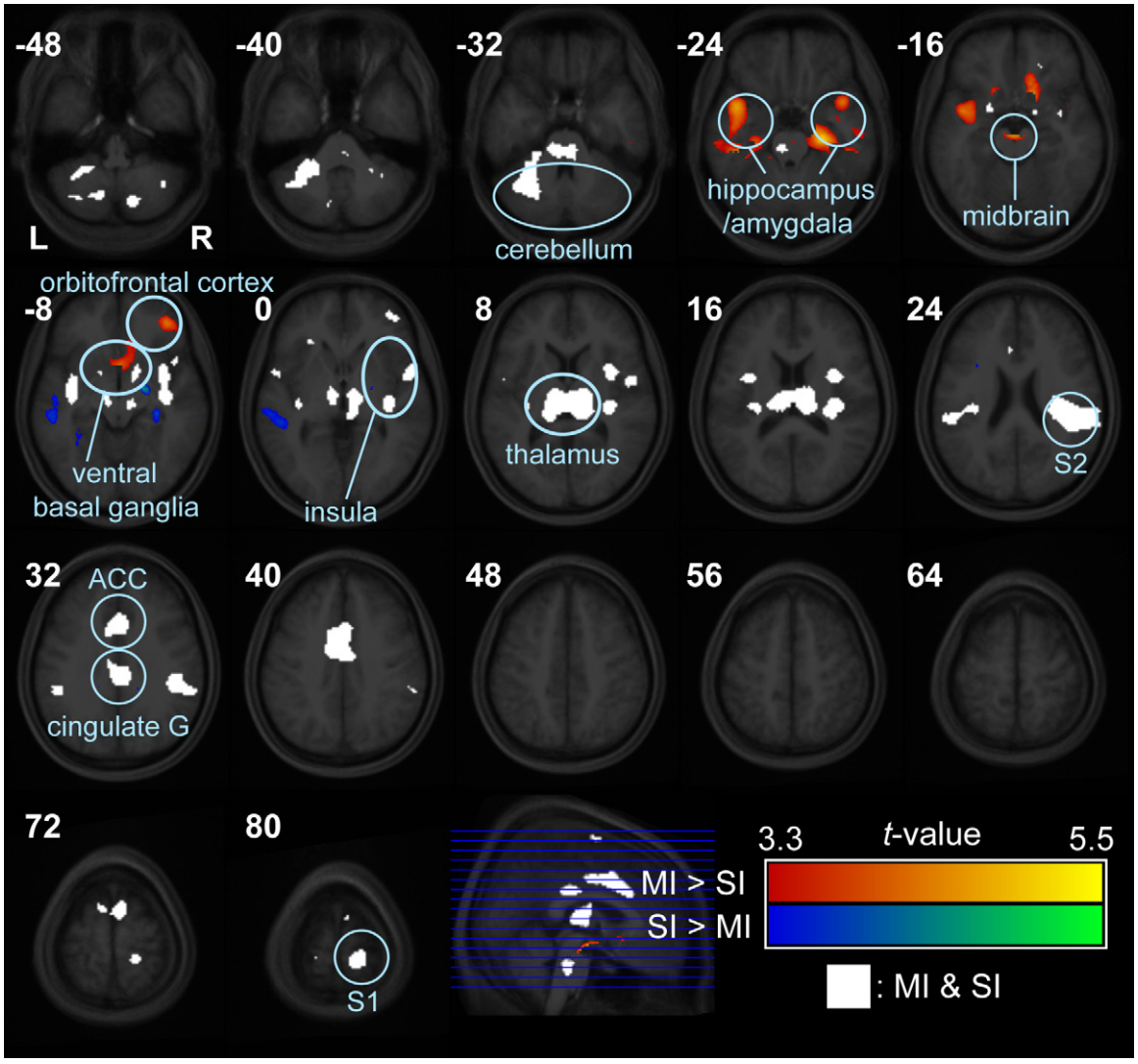

Fig. 2. Sequential brain maps of t-scores representing brain activities in response to electrical painful stimuli. Color scales indicate signal intensity increases during painful stimuli. *Red-yellow*: specific response to painful muscle stimulation; *Blue-green*: specific response to painful skin stimulation.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

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

3.2. Response to painful muscle stimulation

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

Table 3
Brain regions responding to painful electric stimuli.

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

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

* False discovery rate corrected $p < 0.05$.

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

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

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

3.3. Response to painful skin stimulation

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

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

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

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

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

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

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

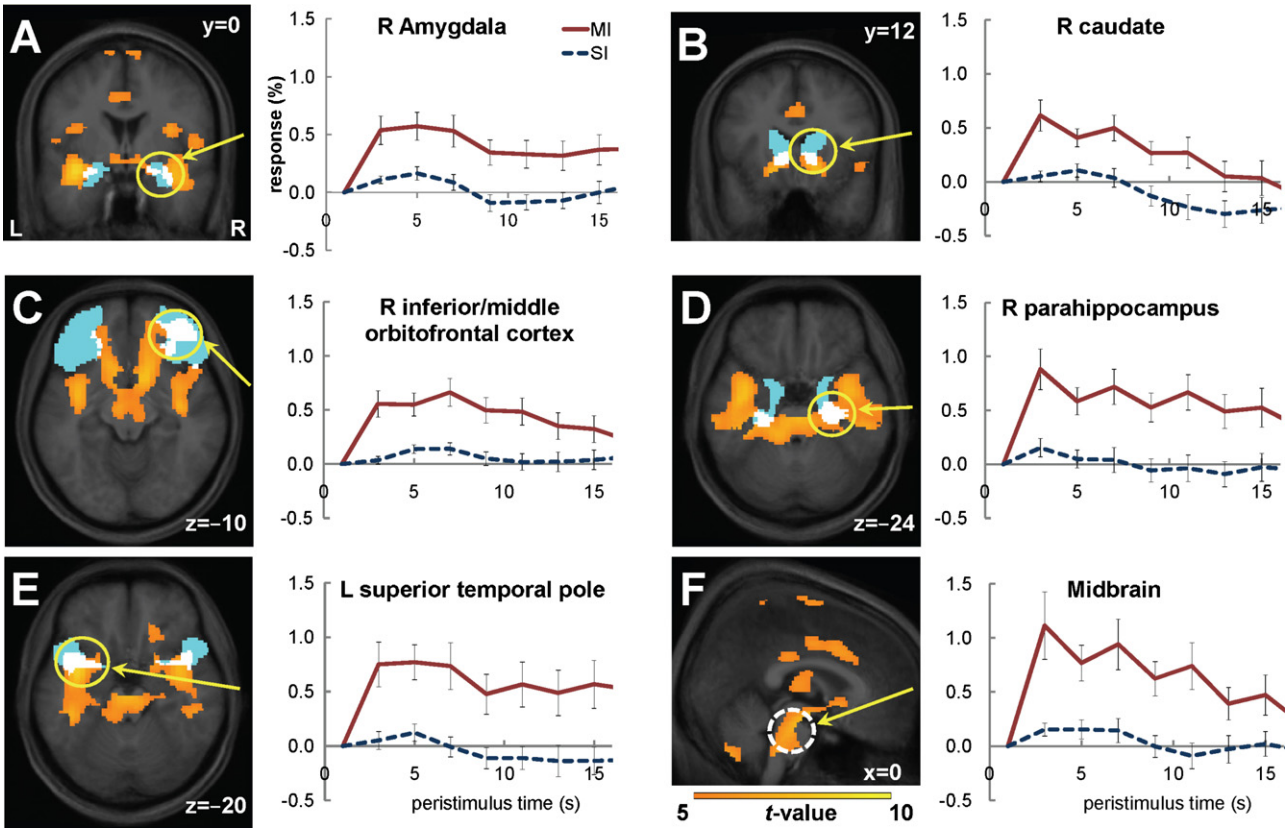

Fig. 3. Activation maps and peristimulus time–response curves at the brain regions that showed greater activities for painful muscle stimulation than for painful skin stimulation. (A) amygdala, (B) caudate, (C) inferior and middle orbitofrontal cortex, (D) parahippocampus, (E) superior temporal pole,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

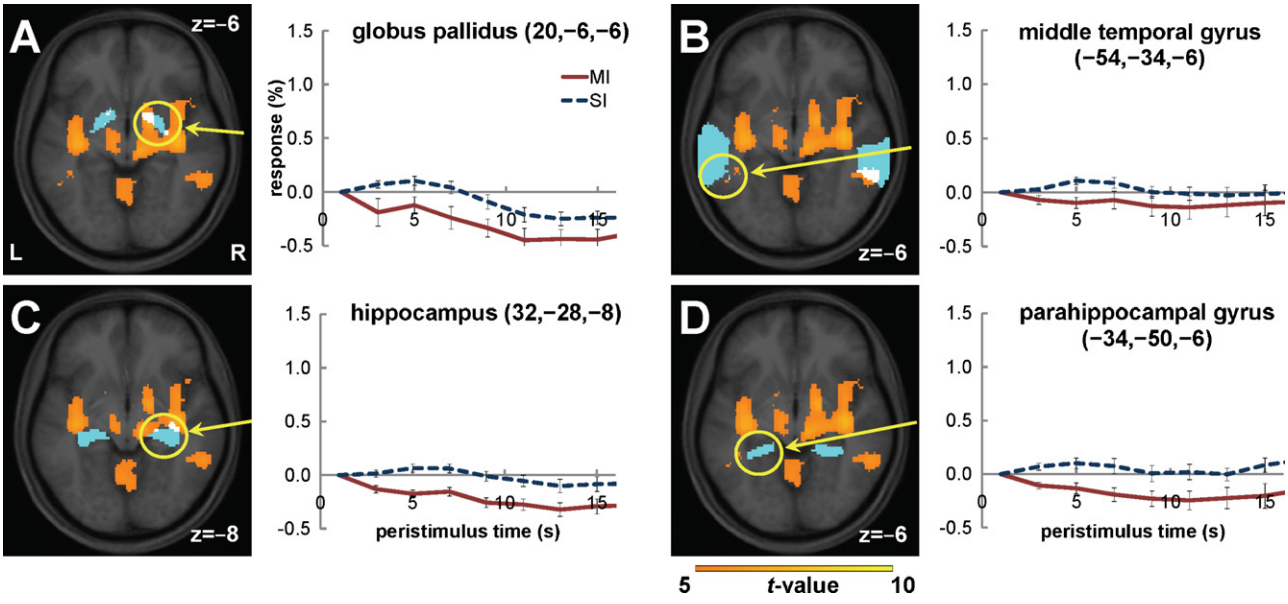

Fig. 4. Peristimulus time–response curves at brain regions that showed greater response to painful skin stimulation than to muscle stimulation. (A) globus pallidus, (B) middle temporal gyrus, (C) hippocampus, and (D) parahippocampus. These anatomical regions are indicated by blue regions in the activation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

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

4. Discussion

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

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

4.1. Midbrain

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

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

4.2. Amygdala/hippocampal regions

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

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

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

4.3. Orbitofrontal cortex

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

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

4.4. Ventral Basal ganglia

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

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

4.5. Superior temporal pole

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

4.6. Brain regions preferentially respond to skin pain

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

4.7. Limitation of the present study

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

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

References

Baumgartner, U., Buchholz, H.G., Bellosevich, A., Magerl, W., Siessmeier, T., Rolke, R., Hohnemann, S., Piel, M., Rosch, F., Wester, H.J., Henriksen, G., Stoeter, P., Bartenstein, P., Treede, R.D., Schreckenberger, M., 2006. High opiate receptor binding potential in the human lateral pain system. Neuroimage 30, 692–699.

Brandao, M.L., Troncoso, A.C., de Souza Silva, M.A., Huston, J.P., 2003. The relevance of neuronal substrates of defense in the midbrain tectum to anxiety and stress: empirical and conceptual considerations. Eur. J. Pharmacol. 463, 225–233.

Brett, M., Anton, J.L., Valabregue, R., Poline, J.B., 2002. Region of interest analysis using an SPM toolbox. In: 8th International Conference on Functional Mapping of the Human Brain, HBM'2002 , Sendai, Japan.

Davidson, R.J., 2002. Anxiety and affective style: role of prefrontal cortex and amygdala. Biol. Psychiatry 51, 68–80.

Derbyshire, S.W., 2003. A systematic review of neuroimaging data during visceral stimulation. Am. J. Gastroenterol. 98, 12–20.

Fanselow, M.S., Gale, G.D., 2003. The amygdala, fear, and memory. Ann. NY Acad. Sci. 985, 125–134.

Friston, K.J., Fletcher, P., Josephs, O., Holmes, A., Rugg, M.D., Turner, R., 1998. Event-related fMRI: characterizing differential responses. Neuroimage 7, 30–40.

Friston, K.J., Penny, W., Phillips, C., Kiebel, S., Hinton, G., Ashburner, J., 2002. Classical and Bayesian inference in neuroimaging: theory. Neuroimage 16, 465–483.

Henderson, L.A., Bandler, R., Gandevia, S.C., Macefield, V.G., 2006. Distinct forebrain activity patterns during deep versus superficial pain. Pain 120, 286–296.

Ikemoto, T., Ushida, T., Taniguchi, S., Tani, T., Morio, K., Sasaki, T., Tanaka, S., 2006. The difference of brain cortical activation between superficial pain and deep pain. Pain Res. 21, 117–125.

Kniffki, K.D., Mizumura, K., 1983. Responses of neurons in VPL and VPL-VL region of the cat to algesic stimulation of muscle and tendon. J. Neurophysiol. 49, 649–661.

Kupers, R.C., Svensson, P., Jensen, T.S., 2004. Central representation of muscle pain and mechanical hyperesthesia in the orofacial region: a positron emission tomography study. Pain 108, 284–293.

Liu, X., Powell, D.K., Wang, H., Gold, B.T., Corbly, C.R., Joseph, J.E., 2007. Functional dissociation in frontal and striatal areas for processing of positive and negative reward information. J. Neurosci. 27, 4587–4597.
Lovick, T.A., 2008. Pro-nociceptive action of cholecystokinin in the periaqueductal grey: a role in neuropathic and anxiety-induced hyperalgesic states. Neurosci. Biobehav. Rev. 32, 852–862.
Lu, C.L., Wu, Y.T., Yeh, T.C., Chen, L.F., Chang, F.Y., Lee, S.D., Ho, L.T., Hsieh, J.C., 2004. Neuronal correlates of gastric pain induced by fundus distension: a 3T-fMRI study. Neurogastroenterol. Motil. 16, 575–587.
Matharu, M.S., Cohen, A.S., McGonigle, D.J., Ward, N., Frackowiak, R.S., Goadsby, P.J., 2004. Posterior hypothalamic and brainstem activation in hemicrania continua. Headache 44, 747–761.
McBeth, J., Jones, K., 2007. Epidemiology of chronic musculoskeletal pain. Best Pract. Res. Clin. Rheumatol. 21, 403–425.
Morgane, P.J., Galler, J.R., Mokler, D.J., 2005. A review of systems and networks of the limbic forebrain/limbic midbrain. Prog. Neurobiol. 75, 143–160.
Nichols, T., Brett, M., Andersson, J., Wager, T., Poline, J.B., 2005. Valid conjunction inference with the minimum statistic. Neuroimage 25, 653–660.
Niddam, D.M., Yeh, T.C., Wu, Y.T., Lee, P.L., Ho, L.T., Arendt-Nielsen, L., Chen, A.C., Hsieh, J.C., 2002. Event-related functional MRI study on central representation of acute muscle pain induced by electrical stimulation. Neuroimage 17, 1437–1450.
Oshiro, Y., Quevedo, A.S., McHaffie, J.G., Kraft, R.A., Coghill, R.C., 2007. Brain mechanisms supporting spatial discrimination of pain. J. Neurosci. 27, 3388–3394.
Peyron, R., Kupers, R., Jehl, J.L., Garcia-Larrea, L., Convers, P., Barral, F.G., Laurent, B., 2007. Central representation of the RIII flexion reflex associated with overt motor reaction: an fMRI study. Neurophysiol. Clin. 37, 249–259.
Peyron, R., Laurent, B., Garcia-Larrea, L., 2000. Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiol. Clin. 30, 263–288.
Rempel-Clower, N.L., 2007. Role of orbitofrontal cortex connections in emotion. Ann. NY Acad. Sci. 1121, 72–86.
Rolls, E.T., 2000. The orbitofrontal cortex and reward. Cereb. Cortex 10, 284–294.
Rolls, E.T., O'Doherty, J., Kringelbach, M.L., Francis, S., Bowtell, R., McGlone, F., 2003. Representations of pleasant and painful touch in the human orbitofrontal and cingulate cortices. Cereb. Cortex 13, 308–317.
Sato, A., Sato, Y., Schmidt, R.F., 1997. The impact of somatosensory input on autonomic functions. Rev. Physiol. Biochem. Pharmacol. 130, 1–328.
Schneider, F., Habel, U., Holthusen, H., Kessler, C., Posse, S., Muller-Gartner, H.W., Arndt, J.O., 2001. Subjective ratings of pain correlate with subcortical-limbic blood flow: an fMRI study. Neuropsychobiology 43, 175–185.
Schreckenberger, M., Siessmeier, T., Viertmann, A., Landvogt, C., Buchholz, H.G., Rolke, R., Treede, R.D., Bartenstein, P., Birklein, F., 2005. The unpleasantness of tonic pain is encoded by the insular cortex. Neurology 64, 1175–1183.
Strigo, I.A., Duncan, G.H., Boivin, M., Bushnell, M.C., 2003. Differentiation of visceral and cutaneous pain in the human brain. J. Neurophysiol. 89, 3294–3303.
Suka, M., Yoshida, K., 2005. Burden of musculoskeletal pain in Japan. Mod. Rheumatol. 15, 48–51.
Suka, M., Yoshida, K., 2009. The national burden of musculoskeletal pain in Japan: projections to the year 2055. Clin. J. Pain 25, 313–319.
Svensson, P., Beydoun, A., Morrow, T.J., Casey, K.L., 1997a. Human intramuscular and cutaneous pain: psychophysical comparisons. Exp. Brain Res. 114, 390–392.
Svensson, P., Minoshima, S., Beydoun, A., Morrow, T.J., Casey, K.L., 1997b. Cerebral processing of acute skin and muscle pain in humans. J. Neurophysiol. 78, 450–460.
Taguchi, T., Hoheisel, U., Mense, S., 2008. Dorsal horn neurons having input from low back structures in rats. Pain 138, 119–129.
Vandenberghe, J., Dupont, P., Van Oudenhove, L., Bormans, G., Demyttenaere, K., Fischler, B., Geeraerts, B., Janssens, J., Tack, J., 2007. Regional cerebral blood flow during gastric balloon distention in functional dyspepsia. Gastroenterology 132, 1684–1693.
Viveros, M.P., Marco, E.M., Llorente, R., Lopez-Gallardo, M., 2007. Endocannabinoid system and synaptic plasticity: implications for emotional responses. Neural Plast. 2007, 52908.
Wood, J.N., Romero, S.G., Knutson, K.M., Grafman, J., 2005. Representation of attitudinal knowledge: role of prefrontal cortex, amygdala and parahippocampal gyrus. Neuropsychologia 43, 249–259.