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Visualization of Painful Experiences Believed to Trigger the Activation of Affective and Emotional Brain Regions in Subjects with Low Back Pain

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Abstract

In the management of clinical low back pain (LBP), actual damage to lower back areas such as muscles, intervertebral discs etc. are normally targeted for therapy. However, LBP may involve not only sensory pain, but also underlying affective pain which may also play an important role overall in painful events. Therefore we hypothesized that visualization of a painful event may trigger painful memories, thus provoking the affective dimension of pain. The present study investigated neural correlates of affect processing in subjects with LBP (n = 11) and subjects without LBP (n = 11) through the use of virtual LBP stimuli. Whole brain functional magnetic resonance imaging (MRI) was performed for all subjects while they were shown a picture of a man carrying luggage in a half-crouching position. All subjects with LBP reported experiencing discomfort and 7 LBP subjects reported experiencing pain. In contrast to subjects without LBP, subjects with LBP displayed activation of the cortical area related to pain and emotions: the insula, supplementary motor area, premotor area, thalamus, pulvinar, posterior cingulate cortex, hippocampus, fusiform, gyrus, and cerebellum. These results suggest that the virtual LBP stimuli caused memory retrieval of unpleasant experiences and therefore may be associated with prolonged chronic LBP conditions.

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Introduction

Psychological factors are known to affect the subjective experience of pain. Pain catastrophizing is one such maladaptive response to pain that is characterized by heightened pain intensity [1], increased disability [2]and difficulty disengaging from pain [3]. Recently, functional neuroimaging techniques have been developed that allow the neural correlates of psychological states to be explored. The blood oxygenation level-dependent contrast (BOLD-fMRI) is currently the most popular tool for mapping human brain activity [4]. Pain-related brain activations which could be considered as psychological factors have been reported in various studies. In healthy volunteers, several brain regions, including the primary and secondary somatosensory cortices, insula, anterior cingulate cortex (ACC), thalamus, and motor cortex, respond to real noxious stimuli and are regarded as part of the "pain matrix" [5,6]. However, it is also known that the expectation of pain can evoke brain activation patterns resembling that of a real pain experience [7].

In a previous study [8,9], Ogino reported that the imagination of pain even without physical injury engages the cortical representations of the pain-related neural network. Also, we reported that prior pain experiences can strongly affect pain anticipation and associated brain activations. We have also found that the anticipation of painful stimuli can cause the activation of cortical areas underlying pain-related affect in chronic neuropathic pain patients [10]. Activation in the brain during the visualization of a painful experience was found in the ACC and the medial prefrontalcortex (MPFC), which are regions known to be areas associated with pain and affect processing. Similar activations were found to be correlated with pain catastrophizing in individuals with fibromyalgia [11]. In that study, pain catastrophizing was associated with greater activity in the dorsolateral prefrontal cortex, rostral ACC, and MPFC, regions implicated in pain vigilance, attention and awareness [12,13,14,15]. These results suggest that pain-related neuronal activities might reflect the development and maintenance of chronic pain syndromes.

Low back pain (LBP) is one of the most common chronic pain syndromes. A recent fMRI study in humans reported actual LBP-related cerebral substrates [16]. Abnormal activations were identified in the prefrontal cortex, insula, thalamus, posterior cingulate cortex (PCC), supplementary motor area (SMA), and premotor areas (PMA) – predominantly in the right hemisphere.

Table 1. Evaluations of task-related discomfort and pain.

	LBP group (n=11)	non-LBP group(n=11)
Experiences evoked by tasks		
Discomfort (range)	3.5 (1–6)	0
Pain (range)	2.1 (0–6)	0
RDQ (mean ± SD)	3.1±3.1	0
ODI (mean ± SD)	19.8±7.8%	0

RDQ, Roland-Morris Disability Questionnaire; ODI, Oswestry Disability Index 2.0.
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We hypothesized that visualization of a painful experience would provoke unpleasant emotions, and these emotions might have a role in the maintenance of chronic pain syndromes. The present study investigated neural correlates of affect processing in subjects with nonspecific LBP and subjects without LBP by using virtual visual stimuli.

Results

Self-reported discomfort and pain (Table 1)

All subjects in the LBP group reported discomfort associated with viewing the simulated back pain (mean NRS score, 3.5; range, 1–6). 7 of the 11 subjects in the LBP group described pain associated with the task. However, no subjects in the non-LBP group reported any discomfort or pain resulting from viewing the picture of back pain.

fMRI results

Compared with the non-LBP group, the LBP group demonstrated significantly more activation in the left fusiform, as well as left inferior temporal gyrus, bilateral precentral gyrus, left middle frontal gyrus, left superior frontal gyrus, bilateral thalamus, bilateral caudate, right insula, left postcentral gyrus, bilateral lingual gyrus, bilateral parahippocampal gyrus, right superior temporal gyrus, left angular gyrus, left superior occipital gyrus, left precuneus, left middle temporal gyrus, left posterior cingulate cortex (PCC), and left cerebellum (Table 2,

Table 2. Talairach coordinates and Broadmann's areas for regions of statistically significant activation ($p<0.0005$ at voxel level uncorrected threshold) in response to virtual LBP stimulation (task – control condition).

Anatomical region	Side	Coordinate	Broadmann area	Z score
LBP group as compared to non-LBP group				
Fusiform gyrus	Lt	−46, −34, −13	Area 20	4.53
Inferior temporal gyrus	Lt	−57, −43, −15	Area 37	3.60
Precentral gyrus	Lt	−32, 8, 38	Area 9	4.38
	Rt	28, −24, 56	Area 4	4.03
Middle frontal gyrus	Lt	−46, 20, 43	Area 8	3.68
		−32, 11, 60	Area 6	3.50
Superior frontal gyrus	Lt	−40, 16, 53	Area 8	3.56
Thalamus	Lt	−24, −25, 7	-	4.34
	Rt	24, −27, 0	-	3.40
Caudate	Lt	−28, −32, 13	-	3.57
	Rt	38, −35, −3	-	3.91
Insula	Rt	28, −27, 12	Area 13	4.30
	Rt	34, −20, 18	Area 13	3.50
Postcentral gyrus	Lt	−8, −55, 64	Area 7	4.07
Lingual gyrus	Rt	18, −62, 0	Area 19	3.99
	Lt	−6, −72, −5	Area 18	3.81
Parahippocampal gyrus	Lt	−36, −43, 0	Area 19	3.96
	Rt	32, −53, −4	Area 19	3.91
	Rt	28, −41, −10	Area 36	3.62
Superior temporal gyrus	Rt	40, −35, 4	Area 41	3.78
Angular gyrus	Lt	−32, −74, 30	Area 39	3.88
Superior occipital gyrus	Lt	−38, −80, 33	Area 19	3.78
Precuneus	Lt	−42, −72, 35	Area 19	3.42
Middle temporal gyrus	Lt	−60, −35, −5	Area 21	3.62
Posterior cingulate gyrus	Lt	−10, −41, 30	Area 31	3.61
	Lt	−4, −43, 37	Area 31	3.55
Cerebellum	Lt	−24, −30, −20	-	3.88
non-LBP group as compared to LBP group				
Caudate	Rt	22, −34, 20	-	3.61

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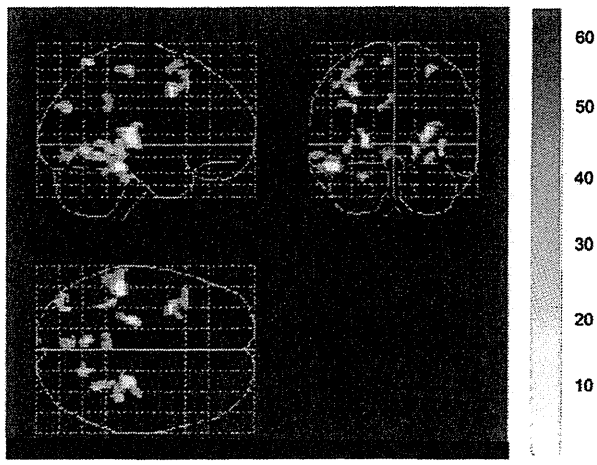

Figure 1. Areas of cortical activation in the LBP group compared with the non-LBP group in response to virtual LBP stimuli (task – control condition) detected by fMRI($p<0.0005$, Z score>3.4, uncorrected threshold).
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Fig. 1). The reverse contrast showed that the LBP group had lower activations than the non-LBP group in a single cluster in right caudate (Table 2).

In the LBP group, activations related to discomfort were found in the bilateral thalamus, bilateral medial frontal gyrus, right claustrum, left cerebellum (Table 3, Fig. 2). Activations associated with self-reported pain were found in the right thalamus and right lingual gyrus. RDQ scores were associated with activation in the left ACC, and ODI scores were associated with activations in the right insula (Table 3, Fig. 3).

Table 3. Cortial areas showing a linear signal increase with the discomfort rating, pain rating, RDQ scores and ODI scores.

Anatomical region	Side	Coordinate	Broadmann area	Z score
Discomfort				
Thalamus	Rt	20, −23, 5	-	4.19
	Lt	−4, −17, 3	-	3.78
Medial frontal gyrus	Rt	10, −22, 58	Area 6	3.85
	Lt	−12, −28, 53	Area 6	3.70
	Lt	−50, 1, 28	Area 6	3.38
Claustrum	Rt	30, 3, 13	-	3.75
Cerebellum	Lt	0, −53, −6	-	3.57
Pain				
Thalamus	Rt	20, −31, 7	-	4.27
Lingual gyrus	Rt	8, −86, −11	Area 18	3.62
RDQ				
Anterior cingulate gyrus	Lt	−6, 9, 27	Area 24	3.99
ODI				
Insula	Rt	40, −8, −5	Area 13	3.67

RDQ, Roland-Morris Disability Questionaire; ODI, Oswestry Disability Index 2.0.
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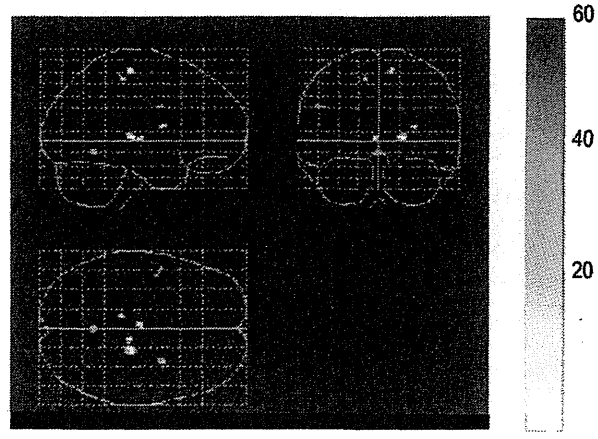

Figure 2. Areas of cortical activation showing an association with perceived discomfort.
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Discussion

Our results demonstrate that viewing images of simulated back pain evoke unpleasant feelings, and specific brain activations in individuals with LBP. According to the International Association for the Study of Pain, pain is defined as, "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". As this definition suggests, both real pain stimuli and virtual pain experiences such as the visual stimuli in our study may play an important role in pain recognition and interpretation in the brain.

Functional MRI results showed that many of the areas described as being part of the "pain matrix" are also active during virtual pain. These results suggest that previous experiences of low back pain can sensitize an individual to pain anticipation. Activation in the insular cortex is associated with pain discrimination [17,18,19]. Additionally, the posterior insular cortex also plays a role in directing appropriate motor behaviors [20]. Furthermore, the insular cortex has projections to the SMA [21,22]. The SMA and PMA are commonly activated by pain [19,23], and usually associated with motor preparation. Activation in those areas might be associated with preparation for protective behavior against pain. In addition, we found virtual LBP stimuli led to increased activation in cerebellum. Activity in the cerebellum is frequently found in pain neuroimaging studies. Cerebellar activation is considered to be primarily associated with motor responses [13]. The need for temporally precise information may also be relevant for brain areas involved in initiating, propagating, and executing defensive motor responses to noxious stimuli [11,13,24,25].

The thalamus and the pulvinar are heavily interconnected with the visual and parietal cortices. Neuroimaging studies suggest responses in the pulvinar have a spatiotopic organization that are modulated by visual attention [26,27,28]. These results suggest that low back pain experiences may make individuals pay more attention to pain-related visual stimuli.

Many reports identify a role of the PCC in negative emotion [29,30,31,32,33,34], visuospatial orientation, and assessment of self-relevant sensation [35]. Exaggerated cerebral activation by pain stimuli may also be associated with pathologic pain states such as allodynia [36,37]. Together with its possible role in inflammatory pain [38], PCC activation could possibly reflect the negative emotion and the pathologic state of pain.

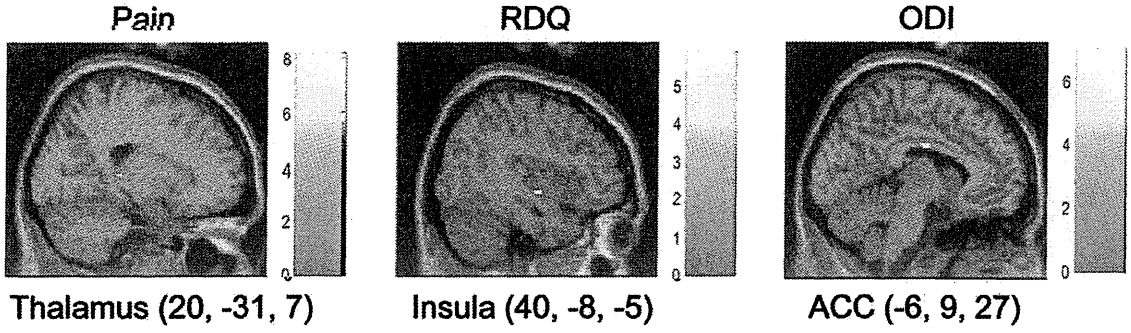

Figure 3. Sagittal sections showing cortical clusters where activity was linearly correlated with perceived pain, RDQ scores and ODI scores.
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We found other regions with heightened activity in LBP participants, in areas outside of the classic pain matrix. Those regions included the hippocampus, fusiform gyrus and angular gyrus. While not typically considered a nociceptive processing region, activation in the hippocampus has been previously reported to be activated in response to painful heat [14,39] and laser stimulation [40]. The hippocampus has been traditionally associated with recent memory consolidation [41], spatial memory [42], and fear-initiated avoidance behavior [43]. The hippocampus might also play a role in memorizing the pain stimulation and preparing fear-initiated avoidance. The fusiform gyrus is often associated with facial recognition [44]. It is conceivable, therefore, that our visual stimuli (which included a human face) may have been responsible for observed activations in the fusiform gyrus. However, our visual stimuli included a human facewithout any faical expression. This might suggest that the fusiform gyrus plays another important role in the cognitive neuroscience field. The angular gyrus is associated with empathy and 'theory of mind' [45]. Visual stimuli may cause subjects in the LBP group to imagine self pain or feel empathy towards the individual in pain in the picture.

Via parametric analyses in the LBP group, we identified several regional activations that were associated with discomfort rating, pain rating, RDQ scores and ODI scores. The SMA and PMA were related to the discomfort rating. As indicated previously, the SMA and PMA are involved in motor preparation. Activation in those areas might therefore be associated with preparation of protective behaviors against discomfort and pain. Thalamic activation was associated with both discomfort and pain ratings. Greater insula activation was associated with higher ODI scores. The thalamus and insula are considered part of the sensory component of pain processing [46]. But, a recent study suggests that imagining oneself in painful situations is sufficient to trigger some pain sensory regions [47]. The ACC was associated with RDQ scores. The ACC is an important part of affective pain processing [48,49] and can be activated in tasks of pain empathy [47,50,51,52,53,54,55]. It is unknown, therefore, whether the ACC activations, which were observed in the LBP group, were due to imagined self pain, or empathetic pain for the individual in the picture.

In this study, we showed that pain-related visual stimuli can activate several regions of the pain matrix in LBP patients, but not normal volunteers. Moreover, the pain questionnaire scores in the LBP patients were associated with greater activation of pain-processing brain regions. Functional MRI and the virtual visual tasks are non-invasive methods for probing pain-related fear and catastrophizing. These results might be applied to the evaluation of chronic pain syndromes, such as low back pain, in the future.

Materials and Methods

We recruited subjects with nonspecific LBP (LBP group) (n = 11, 6 male, 5 female, mean age 20.4 years) and subjects without LBP (non-LBP group) (n = 11, 5 male, 6 female, mean age 21.5 years). All participants were right-handed, had no history of cerebrovascular disease, and were free from any medication within 24 hours of the study. Scores for the Roland-Morris Disability Questionnaire (RDQ) and Oswestry Disability Index 2.0 (ODI) were obtained for all participants. Participants in the LBP group reported low back pain, and a RDQ or ODI score greater than zero. Participants in the non-LBP group had never experienced low back pain lasting longer than 1 week, and their RDQ and ODI scores were zero. No participants in either group displayed any evidence of structural abnormality in the lumbar spine on MRI, or any neurologic symptoms. None reported having a history of psychiatric disorders, or currently using any psychoactive medications.

We used virtual LBP stimuli depicting a man who is carrying luggage in a half-crouching position (Fig. 4). This picture represents an action that would likely cause pain in an individual with low back pain, and may therefore cause pain anticipation in the LBP group. Participants were also shown a picture depicting a man standing in front of luggage, providing the baseline stimulation (control condition) (Fig. 4). Participants in the LBP group had painful experiences in the half-crouching posture but did not have any pain in the standing posture. In addition, the participants in the LBP group currently feel little pain in daily life. During the fMRI session, trials were presented in a fixed block design. The distance between the participants' eyes and the screen was 12.5 cm, with a visual angle of 7.4×11.3°. The trials were applied eight times in each series, with each trial presentation lasting 3 seconds. The entire functional experiment lasted 150 seconds (see details of the experimental paradigm in Fig. 4). Self-reported discomfort and pain measures were collected using a numerical rating scale after the experimental session.

Images of the entire brain were acquired using GE SIGNA 3.0 Tesla scanner. Blood oxygenation level-dependent (BOLD) signals were collected with a T2-weighted, multi-slice, gradient echo-planar imaging (EPI) sequence (TE = 35 ms, TR = 3000 ms, flip angle = 90°, slice width = 4 mm, gap = 0 mm, 36 axial slices). Participants were scanned in the supine position, with the head

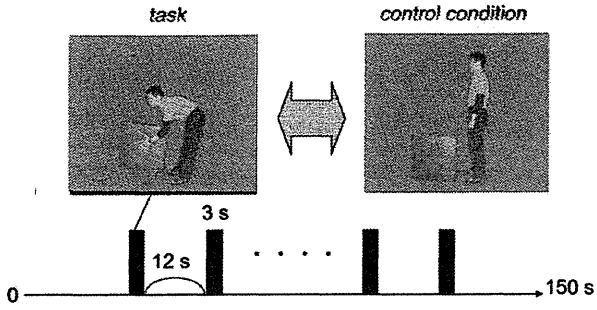

Figure 4. Experimental design. Subjects enrolled in the experiment were shown a picture demonstrating a man holding luggage in a half-crouching position (task picture) and a picture demonstrating a man standing in front of luggage, providing the baseline stimulation (control condition picture).
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fixed to minimize movement artifact. During the experiment, participants were simply instructed to observe the picture on screen.

The study was approved by the Ethical Committee of Kochi Medical School. All participants were informed of the study purpose beforehand and provided written consent to participate.

Results were analyzed on a Unix workstation using SPM2 (Statistical Parametric Mapping) software; Wellcome Department of Cognitive Neurology, Institute of Neurology, London: http://www.fil.ion.ucl.ac.uk/spm). The acquired images were realigned, spatially normalized to a standard EPI template and finally smoothed with an isotropic Gaussian kernel of 6 mm FWHM (full width at half maximum). Significance was assessed using the box car approach, convolved with the canonical hemodynamic response function. Activation maps represent t-test contrasts between the different experimental conditions. To identify the neural substrates for the virtual pain task, we contrasted the task condition and control condition in the LBP and non-LBP groups. Thresholds for activation were set at $p<0.0005$ for the voxel level of activation, and were further corrected for multiple comparisons at the cluster extent threshold of $p<0.05$. The Talairach atlas was used to anatomically localize foci of significant activation [56]. Brain activation between the LBP group and the non-LBP group was statistically compared to identify the neural processing specific to the LBP group ($p<0.05$, corrected, one-way ANOVA).

For the LBP group only, parametric analyses were also performed to determine associations between brain activity and perceived discomfort, perceived pain, RDQ score and ODI score. Normalized ratings were introduced at the subject level, taking into account only trials from the LBP group.

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Author Contributions

Conceived and designed the experiments: KS T. Ushida. Performed the experiments: KS T. Ushida. Analyzed the data: KS T. Ueno JY MN SI T. Ushida. Contributed reagents/materials/analysis tools: TI ST. Wrote the paper: KS T. Ueno JY T. Ushida.

References

1. Sullivan MJ, Rodgers WM, Kirsch I (2001) Catastrophizing, depression and expectancies for pain and emotional distress. Pain 91: 147–154.
2. Sullivan MJ, Lynch ME, Clark AJ (2005) Dimensions of catastrophic thinking associated with pain experience and disability in patients with neuropathic pain conditions. Pain 113: 310–315.
3. Van Damme S, Crombez G, Eccleston C (2004) Disengagement from pain: the role of catastrophic thinking about pain. Pain 107: 70–76.
4. Apkarian AV, Darbar A, Krauss BR, Gelnar PA, Szeverenyi NM (1999) Differentiating cortical areas related to pain perception from stimulus identification: temporal analysis of fMRI activity. J Neurophysiol 81: 2956–2963.
5. Qiu Y, Noguchi Y, Honda M, Nakata H, Tamura Y, et al. (2006) Brain processing of the signals ascending through unmyelinated C fibers in humans: an event-related functional magnetic resonance imaging study. Cereb Cortex 16: 1289–1295.
6. Wager TD, Rilling JK, Smith EE, Sokolik A, Casey KL, et al. (2004) Placebo-induced changes in FMRI in the anticipation and experience of pain. Science 303: 1162–1167.
7. Koyama T, McHaffie JG, Laurienti PJ, Coghill RC (2005) The subjective experience of pain: where expectations become reality. Proc Natl Acad Sci U S A 102: 12950–12955.
8. Ogino Y, Nemoto H, Inui K, Saito S, Kakigi R, et al. (2007) Inner experience of pain: imagination of pain while viewing images showing painful events forms subjective pain representation in human brain. Cereb Cortex 17: 1139–1146.
9. Ushida T, Ikemoto T, Tanaka S, Shinozaki J, Taniguchi S, et al. (2008) Virtual needle pain stimuli activates cortical representation of emotions in normal volunteers. Neurosci Lett 439: 7–12.
10. Ushida T, Ikemoto T, Taniguchi S, Ishida K, Murata Y, et al. (2005) Virtual pain stimulation of allodynia patients activates cortical representation of pain and emotions: a functional MRI study. Brain Topogr 18: 27–35.
11. Gracely RH, Geisser ME, Giesecke T, Grant MA, Petzke F, et al. (2004) Pain catastrophizing and neural responses to pain among persons with fibromyalgia. Brain 127: 835–843.
12. Bornhovd K, Quante M, Glauche V, Bromm B, Weiller C, et al. (2002) Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. Brain 125: 1326–1336.
13. Buchel C, Bornhovd K, Quante M, Glauche V, Bromm B, et al. (2002) Dissociable neural responses related to pain intensity, stimulus intensity, and stimulus awareness within the anterior cingulate cortex: a parametric single-trial laser functional magnetic resonance imaging study. J Neurosci 22: 970–976.
14. Derbyshire SW, Jones AK, Gyulai F, Clark S, Townsend D, et al. (1997) Pain processing during three levels of noxious stimulation produces differential patterns of central activity. Pain 73: 431–445.
15. Valet M, Sprenger T, Boecker H, Willoch F, Rummeny E, et al. (2004) Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain–an fMRI analysis. Pain 109: 399–408.
16. Kobayashi Y, Kurata J, Sekiguchi M, Kokubun M, Akaishizawa T, et al. (2009) Augmented cerebral activation by lumbar mechanical stimulus in chronic low back pain patients: an FMRI study. Spine (Phila Pa 1976) 34: 2431–2436.
17. Henderson LA, Gandevia SC, Macefield VG (2007) Somatotopic organization of the processing of muscle and cutaneous pain in the left and right insula cortex: a single-trial fMRI study. Pain 128: 20–30.
18. Korotkov A, Ljubisavljevic M, Thunberg J, Kataeva G, Roudas M, et al. (2002) Changes in human regional cerebral blood flow following hypertonic saline induced experimental muscle pain: a positron emission tomography study. Neurosci Lett 335: 119–123.
19. Svensson P, Minoshima S, Beydoun A, Morrow TJ, Casey KL (1997) Cerebral processing of acute skin and muscle pain in humans. J Neurophysiol 78: 450–460.
20. Berthier M, Starkstein S, Leiguarda R (1988) Asymbolia for pain: a sensory-limbic disconnection syndrome. Ann Neurol 24: 41–49.
21. Augustine JR (1996) Circuitry and functional aspects of the insular lobe in primates including humans. Brain Res Brain Res Rev 22: 229–244.
22. Luppino G, Matelli M, Camarda R, Rizzolatti G (1993) Corticocortical connections of area F3 (SMA-proper) and area F6 (pre-SMA) in the macaque monkey. J Comp Neurol 338: 114–140.
23. Hsieh JC, Stahle-Backdahl M, Hagermark O, Stone-Elander S, Rosenquist G, et al. (1996) Traumatic nociceptive pain activates the hypothalamus and the periaqueductal gray: a positron emission tomography study. Pain 64: 303–314.
24. Peyron R, Kupers R, Jehl JL, Garcia-Larrea L, Convers P, et al. (2007) Central representation of the RIII flexion reflex associated with overt motor reaction: an fMRI study. Neurophysiol Clin 37: 249–259.
25. Sullivan MJ, Thorn B, Haythornthwaite JA, Keefe F, Martin M, et al. (2001) Theoretical perspectives on the relation between catastrophizing and pain. Clin J Pain 17: 52–64.
26. Cotton PL, Smith AT (2007) Contralateral visual hemifield representations in the human pulvinar nucleus. J Neurophysiol 98: 1600–1609.

27. Fischer J, Whitney D (2009) Precise discrimination of object position in the human pulvinar. Hum Brain Mapp 30: 101–111.
28. Smith AT, Cotton PL, Bruno A, Moutsiana C (2009) Dissociating vision and visual attention in the human pulvinar. J Neurophysiol 101: 917–925.
29. Benuzzi F, Lui F, Duzzi D, Nichelli PF, Porro CA (2008) Does it look painful or disgusting? Ask your parietal and cingulate cortex. J Neurosci 28: 923–931.
30. Britton JC, Phan KL, Taylor SF, Welsh RC, Berridge KC, et al. (2006) Neural correlates of social and nonsocial emotions: An fMRI study. Neuroimage 31: 397–409.
31. Maddock RJ, Garrett AS, Buonocore MH (2003) Posterior cingulate cortex activation by emotional words: fMRI evidence from a valence decision task. Hum Brain Mapp 18: 30–41.
32. Mantani T, Okamoto Y, Shirao N, Okada G, Yamawaki S (2005) Reduced activation of posterior cingulate cortex during imagery in subjects with high degrees of alexithymia: a functional magnetic resonance imaging study. Biol Psychiatry 57: 982–990.
33. Sander K, Frome Y, Scheich H (2007) FMRI activations of amygdala, cingulate cortex, and auditory cortex by infant laughing and crying. Hum Brain Mapp 28: 1007–1022.
34. Sinha R, Lacadie C, Skudlarski P, Wexler BE (2004) Neural circuits underlying emotional distress in humans. Ann N Y Acad Sci 1032: 254–257.
35. Vogt BA (2005) Pain and emotion interactions in subregions of the cingulate gyrus. Nat Rev Neurosci 6: 533–544.
36. Freund W, Wunderlich AP, Stuber G, Mayer F, Steffen P, et al. (2010) Different activation of opercular and posterior cingulate cortex (PCC) in patients with complex regional pain syndrome (CRPS I) compared with healthy controls during perception of electrically induced pain: a functional MRI study. Clin J Pain 26: 339–347.
37. Lorenz J, Cross DJ, Minoshima S, Morrow TJ, Paulson PE, et al. (2002) A unique representation of heat allodynia in the human brain. Neuron 35: 383–393.
38. Ruehle BS, Handwerker HO, Lennerz JK, Ringler R, Forster C (2006) Brain activation during input from mechanoinsensitive versus polymodal C-nociceptors. J Neurosci 26: 5492–5499.
39. Ploghaus A, Narain C, Beckmann CF, Clare S, Bantick S, et al. (2001) Exacerbation of pain by anxiety is associated with activity in a hippocampal network. J Neurosci 21: 9896–9903.
40. Bingel U, Quante M, Knab R, Bromm B, Weiller C, et al. (2002) Subcortical structures involved in pain processing: evidence from single-trial fMRI. Pain 99: 313–321.
41. Alvarez P, Squire LR (1994) Memory consolidation and the medial temporal lobe: a simple network model. Proc Natl Acad Sci U S A 91: 7041–7045.
42. Clark RE, Broadbent NJ, Squire LR (2007) The hippocampus and spatial memory: findings with a novel modification of the water maze. J Neurosci 27: 6647–6654.
43. Hollup SA, Kjelstrup KG, Hoff J, Moser MB, Moser EI (2001) Impaired recognition of the goal location during spatial navigation in rats with hippocampal lesions. J Neurosci 21: 4505–4513.
44. Radua J, Phillips ML, Russell T, Lawrence N, Marshall N, et al. (2010) Neural response to specific components of fearful faces in healthy and schizophrenic adults. Neuroimage 49: 939–946.
45. Vollm BA, Taylor AN, Richardson P, Corcoran R, Stirling J, et al. (2006) Neuronal correlates of theory of mind and empathy: a functional magnetic resonance imaging study in a nonverbal task. Neuroimage 29: 90–98.
46. Bushnell MC, Duncan GH, Hofbauer RK, Ha B, Chen JI, et al. (1999) Pain perception: is there a role for primary somatosensory cortex? Proc Natl Acad Sci U S A 96: 7705–7709.
47. Jackson PL, Brunet E, Meltzoff AN, Decety J (2006) Empathy examined through the neural mechanisms involved in imagining how I feel versus how you feel pain. Neuropsychologia 44: 752–761.
48. Apkarian AV, Bushnell MC, Treede RD, Zubieta JK (2005) Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain 9: 463–484.
49. Davis KD, Taylor KS, Hutchison WD, Dostrovsky JO, McAndrews MP, et al. (2005) Human anterior cingulate cortex neurons encode cognitive and emotional demands. J Neurosci 25: 8402–8406.
50. Botvinick M, Jha AP, Bylsma LM, Fabian SA, Solomon PE, et al. (2005) Viewing facial expressions of pain engages cortical areas involved in the direct experience of pain. Neuroimage 25: 312–319.
51. Gu X, Han S (2007) Attention and reality constraints on the neural processes of empathy for pain. Neuroimage 36: 256–267.
52. Jackson PL, Meltzoff AN, Decety J (2005) How do we perceive the pain of others? A window into the neural processes involved in empathy. Neuroimage 24: 771–779.
53. Lamm C, Batson CD, Decety J (2007) The neural substrate of human empathy: effects of perspective-taking and cognitive appraisal. J Cogn Neurosci 19: 42–58.
54. Morrison I, Lloyd D, di Pellegrino G, Roberts N (2004) Vicarious responses to pain in anterior cingulate cortex: is empathy a multisensory issue? Cogn Affect Behav Neurosci 4: 270–278.
55. Singer T, Seymour B, O'Doherty J, Kaube H, Dolan RJ, et al. (2004) Empathy for pain involves the affective but not sensory components of pain. Science 303: 1157–1162.
56. Talairach J, Tournoux P (1988) Co-planar Stereotaxic Atlas of the Human Brain. New York: Thime Medical Publishers. 122 p.

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Health Survey of Numbness/Pain and Its Associated Factors in Kotohira, Japan

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Abstract

We conducted a survey of adults in Kotohira, a town of about 10,000 people located in the Nakatado District of Kagawa Prefecture, Japan. The survey was distributed to 8184 individuals, and effective responses were received from 3863 persons (response rate, 47.2%) during the survey period. Results regarding numbness and pain showed numbness alone in 7.7%, pain alone in 7.2%, both numbness and pain in 6.0%, and neither numbness nor pain in 79.6%. Spine and spinal cord damage was reported present by 5.4%, and absent by 94.6%. Analysis using the Short-Form Health Survey questionnaire, with comparison between subjects reporting both numbness and pain in the extremities and subjects with either numbness or pain alone, showed lower scores for in Short-Form Health Survey subscales (physical functioning, role [physical, emotional], bodily pain, vitality, and mental health). Subjects with numbness alone generally reported no disability in daily life. In a secondary survey, analysis of neurological findings by specialists identified 6 cases of "pain following spinal cord damage" in which spinal cord-related pain developed in the hands or feet. This represented 0.15% of the survey population starting from the primary survey.

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Introduction

Limb (arm and leg) numbness and pain can occur not only due to spine/spinal cord disorder, entrapment syndromes, diabetes, and neuropathy causing nerve dysfunction, but also due to muscle and vascular diseases. Because individuals with numbness or pain may experience great discomfort, elucidating the underlying mechanisms and developing effective treatments are very important.

Pain is an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [1]. However, patients with neurological dysfunction due to spine/spinal cord disorder often first complain of "shibirekan" [2], or "numbness" in English. Numbness is listed in ICD10 section R20 "disturbances of skin sensation"; and anesthesia, paresthesia, and dysesthesia (which can clearly be defined), as well as hypesthesia and some symptoms that cannot be specified, are often referred to as "numbness." Moreover, even when pain is also present, this is sometimes expressed as "numbness." In particular, in refractory and difficult-to-treat diseases such as cervical myelopathy, ossification of the posterior longitudinal ligament (OPLL), and syringomyelia, as well as after spinal cord injury; limb numbness and pain (allodynia or pressure sensation in the body) is severe, pain may be resistant to treatment, and quality of life (QOL) and activities of daily living (ADLs) are markedly diminished [2,3,4,5]. Therefore, in pain following spinal cord damage, with symptoms of pain and numbness, elucidating the neuropathological and pharmacological mechanisms involved and developing effective treatments are of paramount importance.

According to recent nationwide surveys [6,7], the prevalence of chronic pain with neuropathic characteristics is reported to be 7 - 8%. However, numbness information and impacts of pain and numbness on health status are largely unknown. In addition, pain directly attributable to spinal cord damage may include allodynia, in which pain is triggered by tactile stimulation that ordinarily does not cause pain, and spontaneous pressure-like pain below the level of the damaged spinal cord. Some drugs, such as anticonvulsants, are effective in some patients, but the same treatment is often ineffective in other patients with similar symptoms. Many cases are treatment-resistant, and much remains unknown about this disease population [8].

In Kotohira where this survey was conducted, a high level of cooperation exists among the council of social welfare, welfare commissioners, women's groups, and local liaison councils; and the area is very small (8.46 km^2) with only a small degree of population shift. This provides conditions under which the current status of town residents can very easily be ascertained (http://www.town.kotohira.kagawa.jp/english/data/index.html). The aim of this study was to clarify the prevalence of numbness and pain and their impacts on health status in a rural community in

Japan, particularly spine-related symptoms were evaluated. The present study was undertaken as part of a survey on spinal-related pain (number of patients, percentage of population, symptom characteristics) (MHLW Research) in Kotohira, a town with a population of about 10,000 located in Kagawa Prefecture, Japan.

Results

Primary Survey Results (Fig. 1, Table 1)

Among the 119 neighborhood associations, surveys were collected from 108 neighborhood associations (2728 households, 8184 persons), and effective responses were received from 3863 persons (47.2%). This included 2141 women (55.4%) and 1722 men (44.6%). Age was <65 years in 2124 (54.5%), 65 to <75 years (young-old elderly) in 21.8%, and ≥75 years (old-old elderly) in 23.7%. Regarding limb numbness and pain, numbness alone was present in 297 (7.7%), pain alone in 280 (7.2%), both numbness and pain in 234 (6.1%), and neither numbness nor pain in 3052 (79.0%).

With regard to symptoms, 215 respondents (5.6%) had been diagnosed with spine/spinal cord disorder at a hospital, while 3648 persons (94.4%) had not. In addition, 372 individuals had a history of diabetes. Taken together, the number of persons with both spinal disorder and diabetes, spinal disorder only, diabetes only, and neither spinal disorder nor diabetes was 32, 183, 346, and 3308, respectively.

2691 individuals (32.8%) responded to SF-36 questionnaire. Analysis of SF-36 subscale scores showed that the group with both limb numbness and pain, as compared to the group with either pain alone or numbness alone, showed lower scores for all SF-36 subscale items except general health. Moreover, the group with either numbness or pain showed lower scores for each SF-36 item compared to the group with neither numbness nor pain. Scores for general health, physical functioning, and mental health were lower in the pain-alone group than in the numbness-alone group (Fig. 2). Among those individuals diagnosed with both diabetes and spine disease, the group with numbness or pain showed decreased health status as compared to the group without numbness or pain. This trend was stronger among individuals with a history of spine/spinal cord disorder (Fig. 3).

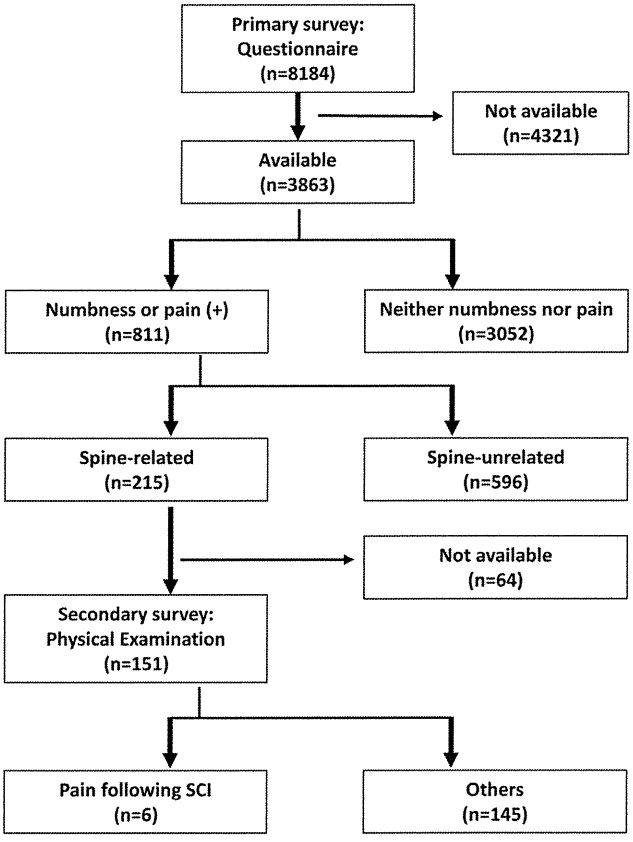

Figure 1. A flow diagram showing an outline of the study.
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Table 1. Limb numbness and pain according to sex and age.

		Male	
Age	−64	65–75	75–
n	1008	358	356
Numbness+	73 (7.2)	36 (10.1)	35 (9.8)
Pain+	26 (2.6)	24 (6.7)	23 (6.5)
Both+	37 (3.7)	23 (6.4)	39 (11.0)
		Female	
Age	−64	65–75	75–
n	1097	484	560
Numbness+	69 (6.3)	36 (7.4)	48 (8.6)
Pain+	70 (6.4)	54 (11.2)	83 (14.8)
Both+	39 (3.6)	29 (6.0)	67 (12.0)

(Values in parentheses represent percentages).
doi:10.1371/journal.pone.0060079.t001

Secondary Survey Results (Fig. 1)

Among those individuals with limb numbness or pain at the primary survey who had been diagnosed with spine/spinal cord disorder at a hospital, the number from whom cooperation for the secondary survey was obtained. Among the 215 residents targeted for the secondary survey was 151 persons. Based on a medical examination and a detailed interview survey in these cases, 6 individuals who have intractable spinal cord-related numbness and pain in extremities were judged to have "pain following spinal cord damage" that was resistant to ordinal treatment such as non-steroidal anti-inflammatory drugs. This represented 0.15% of the survey population starting from the primary survey. However, there were 29 persons with lumbosacral-related numbness and pain such as spinal canal stenosis or a herniated lumbar disk. In addition, cases of another cause of numbness and tingling, even though spine/spinal cord disorder had been diagnosed at a hospital, included 5 persons with limb trauma and 16 persons with arthropathy (including rheumatoid arthritis and lateral epicondylitis of the humerus).

Discussion

Numbness is a sensory abnormality and the word is often used to describe abnormal sensations such as paresthesia, dysesthesia, and hypesthesia. Numbness is seen not only in spine/spinal cord disorder, but also often in carpal tunnel syndrome. Tay et al. reported paresthesias in 70.1% of patients diagnosed with this syndrome [9]. However, because the etiology is multifaceted with regard to the population in whom symptoms of limb numbness and pain are frequently observed, the effects of these symptoms on

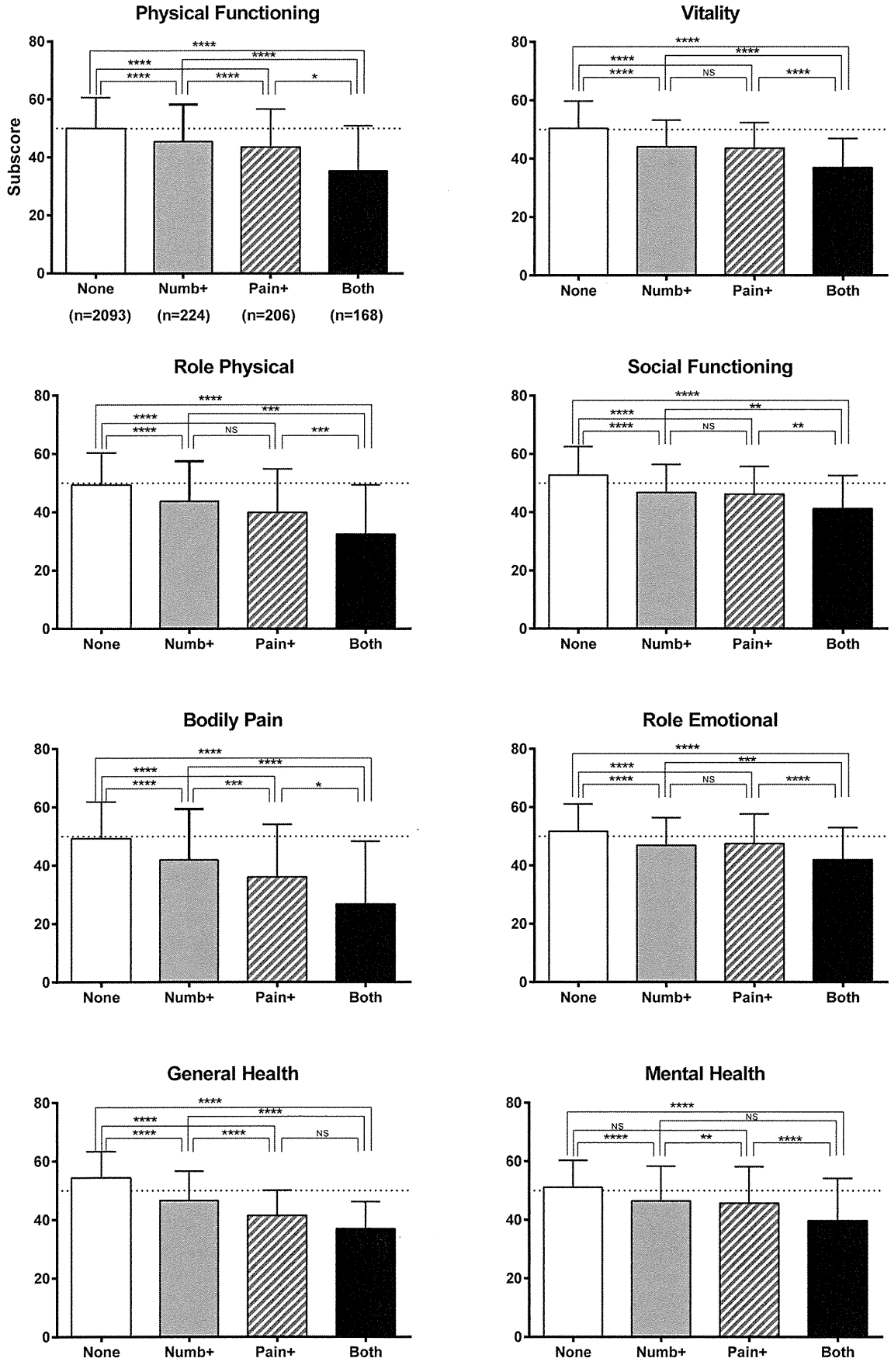

Physical Functioning
Subscore
None (n=2093)
Numb+ (n=224)
Pain+ (n=206)
Both (n=168)
Vitality
Role Physical
Social Functioning
Bodily Pain
Role Emotional
General Health
Mental Health
NS

Figure 2. SF-36 subscale scores with presence or absence of numbness or pain. In the group with both numbness and pain, scores were significantly decreased as compared to the group with neither. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
doi:10.1371/journal.pone.0060079.g002

health status remain unclear, and thorough surveys have not been conducted to date.

In the present survey used in Kotohira, 7.7% of the population had limb numbness alone, 7.3% had pain alone, and 6.0% had both. Many individuals show symptoms, and prevalence increases with aging. One reason for an increase in numbness (a sensory abnormality caused by multiple etiologies, as described above) in older persons is that the population with spine/spinal cord disorders such as lumbar spinal canal stenosis [10,11] and cervical spondylotic myelopathy [12], which can cause these symptoms, increases with older age. Regarding decreased sensory function, a decrease in the number of peripheral mechanoreceptors has been reported with aging, even in the absence of disease, and hypofunction [13] and myelin degeneration might be due to the involvement of such mechanisms [14]. This is thought to be linked to the mechanism by which numbness is increased in older persons. The percentage of the population of Kotohira aged 65 or older is relatively high (32%) compared with that of Japan as a whole (23%). Therefore, it is possible that the prevalence of symptoms in this study is higher than the national average.

Our survey showed that in persons with both limb numbness and pain, SF-36 subscores (physical functioning, role physical, bodily pain, vitality, social functioning, role emotional, and mental health) were lower than in participants with numbness alone or pain alone. Individuals with numbness alone generally reported no disability in daily life. However, the characteristics of numbness were not available because the questionnaire simply asked for the presence or absence of numbness in this study. It is possible that "numbness" in this study included abnormal sensation such as paresthesia and dysesthesia. A more detailed survey that can be linked to development of treatment for numbness may thus be necessary.

In a previously conducted cohort survey overseas, in all SF-36 domains except mental health, health status was impaired in the diabetes group compared to healthy persons [15]. In our survey, SF-36 subscale scores were markedly decreased in participants who had been diagnosed with spine/spinal cord disorder. In diabetes as well, when there was limb pain, each of the subscale scores tended to be decreased. This demonstrates the importance of maintaining locomotor function to control medical diseases such as diabetes and prevent chronic pain. Future development of intervention strategies to promote health status is needed [16].

Spinal cord-related pain, as pain caused by direct damage to the spinal cord, and the effects on ADL associated with this pain represent conditions caused by many diseases. Because of difficult-to-treat symptoms, treatment strategy is challenging even at facilities specializing in spine/spinal cord disorder. Causative disorders include not only spinal cord injury, but also a wide range of a smaller number of cases such as compressive myelopathy due to OPLL, syringomyelia, and spinal cord tumors. Ascertaining the whole clinical picture may thus be difficult. In our survey conducted in about half of the population of Kotohira, the data showed 6 such cases (0.15%) among about 4000 adults. The population shift in Kotohira is small, and cooperation between the town, council of social welfare and neighborhood associations is high. In this area, neighborhood associations function with support centering on the council of social welfare. Because patients with spinal cord injury usually need social support, it is unlikely that we missed a certain number of patients with severe pain related to spinal cord injury.

Mechanisms of numbness and pain in spinal cord-related pain syndromes include: 1) damage at the dorsal root level [17]; 2) damage to the dorsal horn (synapse region) (including effects of inhibition and facilitation of propagation, sprouting, and glial activation) [18]; 3) damage to spinothalamic tract [19]; 4) damage to descending inhibition pathways [20]; 5) muscle pain due to nerve damage [21]; and 6) psychosocial factors together with brain memory mechanisms [22]. To further analyze these neuropathological mechanisms and develop new treatments, a network must first be established to collect these types of patients.

Methods

The study was conducted in cooperation among the Kotohira Council of Social Welfare, the Federation of Neighborhood Associations comprised of neighborhood association presidents, Kotohira Women's Association, welfare commissioners, and Kotohira Town Office. All participants gave their informed written consent to the study. The requirements of data protection and medical professional secrecy were respected by all study investigators. All consent and protocols for both primary and secondary surveys had been specifically approved by the ethical committee of the Aichi Medical University.

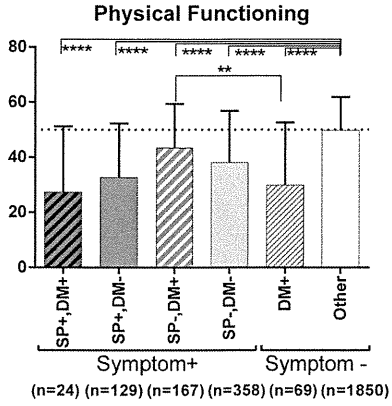
Primary Survey

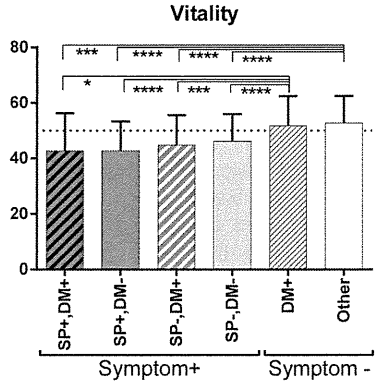
The survey questionnaire, through the Kotohira Council of Social Welfare and Federation of Neighborhood Associations, was distributed and collected by neighborhood association presidents to 119 neighborhood associations in Kotohira Town using the placement survey method. For areas where distribution was difficult, the council of social welfare officers, welfare commissioners, and the women's club provided assistance. Because the study would be hindered if persons in charge of distributing and collecting the surveys were unable to explain the survey, opinions of the Federation of Neighborhood Associations were sought during the stage of questionnaire creation to enable the survey to also be conducted among elderly persons. The questionnaire included items about limb numbness and pain, history of spine/spinal cord disorder, a history of diabetes, and the Short-Form Health Survey (SF-36). Because it simply asked for the presence or absence of symptoms and disease history, details of symptom and disease severity were not obtained from the primary survey. The surveys were distributed beginning on January 21, 2010 and collected by March 3, 2010.

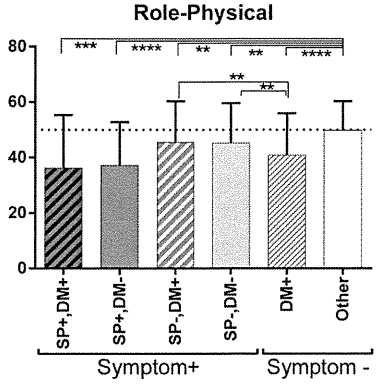
For survey results, national standard norm-based scoring (NBS) was used for the data obtained from the SF-36. The results, including physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotional, and mental health, were analyzed using the Kruskal-Wallis test. Items with significant differences were examined with Dunn's multiple comparison test.

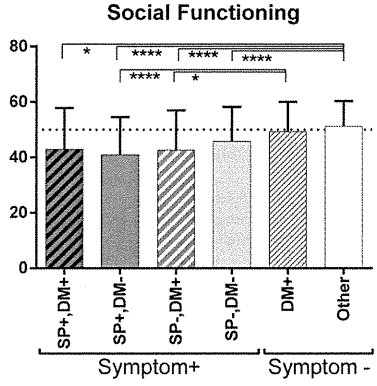
Secondary Survey

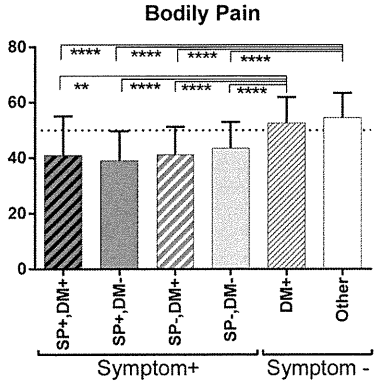
Among respondents to the primary survey with limb numbness or pain and who reported previous diagnosis of with spine/spinal cord disorder in a hospital, in those from whom cooperation was obtained, a secondary survey was conducted by specialists in spine/spinal cord disorder or neurological diagnosis. This survey was conducted as a secondary screening, or for non-participants in screening, by a telephone interview, to obtain detailed neurolog-

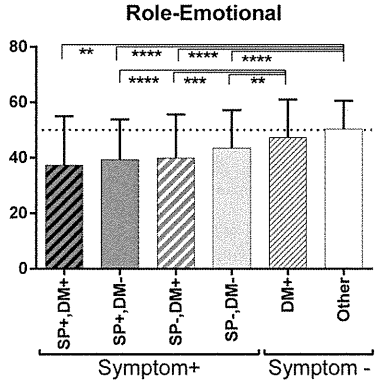

Physical Functioning
80
60
40
20
0
SP+,DM+
SP+,DM-
SP-,DM+
SP-,DM-
DM+
Other
Symptom+
Symptom -
(n=24) (n=129) (n=167) (n=358) (n=69) (n=1850)

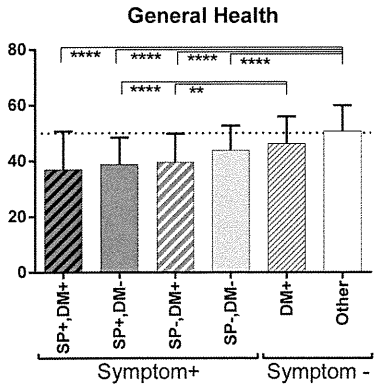

Vitality
80
60
40
20
0
SP+,DM+
SP+,DM-
SP-,DM+
SP-,DM-
DM+
Other
Symptom+
Symptom -


Role-Physical
80
60
40
20
0
SP+,DM+
SP+,DM-
SP-,DM+
SP-,DM-
DM+
Other
Symptom+
Symptom -


Social Functioning
80
60
40
20
0
SP+,DM+
SP+,DM-
SP-,DM+
SP-,DM-
DM+
Other
Symptom+
Symptom -


Bodily Pain
80
60
40
20
0
SP+,DM+
SP+,DM-
SP-,DM+
SP-,DM-
DM+
Other
Symptom+
Symptom -


Role-Emotional
80
60
40
20
0
SP+,DM+
SP+,DM-
SP-,DM+
SP-,DM-
DM+
Other
Symptom+
Symptom -


General Health
80
60
40
20
0
SP+,DM+
SP+,DM-
SP-,DM+
SP-,DM-
DM+
Other
Symptom+
Symptom -

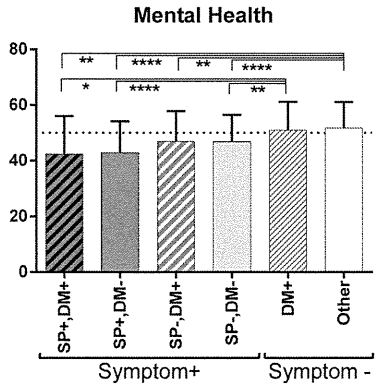

Mental Health
80
60
40
20
0
SP+,DM+
SP+,DM-
SP-,DM+
SP-,DM-
DM+
Other
Symptom+
Symptom -

Figure 3. SF-36 subscale scores for presence or absence of spine/spinal cord-related disorder and diabetes. Diagnosed with spine/spinal cord-disorder (SP+), with diabetes (DM+), positive for numbness or pain (symptom +). Among individuals diagnosed with diabetes and spinal disease, health status was lower in the group with numbness or pain as compared to the group with neither. This trend was strong in those diagnosed with spine/spinal cord disorder. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
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ical findings. These patients were narrowed down to cases of refractory spinal-related pain following spinal cord damage based, when necessary, on the results of specialist examinations and imaging studies. The secondary survey was conducted from August 2010 to December 2010.

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References

1. Taxonomy ITFo (1994) Part III: Pain Terms: A Current List with Definitions and Notes on Usage. In: Merskey H, Bogduk N, editors. Classification of Chronic Pain. 2 ed. Seattle: IASP Press. pp. 209–214.
2. Todor DR, Mu HT, Milhorat TH (2000) Pain and syringomyelia: a review. Neurosurg Focus 8: E11.
3. Attal N, Bouhassira D (2006) Chapter 47 Pain in syringomyelia/bulbia. Handb Clin Neurol 81: 705–713.
4. Robert R, Perrouin-Verbe B, Albert T, Bussel B, Hamel O (2009) Chronic neuropathic pain in spinal cord injured patients: what is the effectiveness of surgical treatments excluding central neurostimulations? Ann Phys Rehabil Med 52: 194–202.
5. Chang MH, Liao KK, Cheung SC, Kong KW, Chang SP (1992) "Numb, clumsy hands" and tactile agnosia secondary to high cervical spondylotic myelopathy: a clinical and electrophysiological correlation. Acta Neurol Scand 86: 622–625.
6. Bouhassira D, Lantéri-Minet M, Attal N, Laurent B, Touboul C (2008) Prevalence of chronic pain with neuropathic characteristics in the general population. Pain 136: 380–387.
7. Torrance N, Smith BH, Bennett MI, Lee AJ (2006) The epidemiology of chronic pain of predominantly neuropathic origin. Results from a general population survey. The journal of pain: official journal of the American Pain Society 7: 281.
8. Lucchetta M, Pazzaglia C, Padua L, Briani C (2011) Exploring neuropathic symptoms in a large cohort of Italian patients with different peripheral nervous system diseases. Neurol Sci 32: 423–426.
9. Tay LB, Urkude R, Verma KK (2006) Clinical profile, electrodiagnosis and outcome in patients with carpal tunnel syndrome: a Singapore perspective. Singapore Med J 47: 1049–1052.
10. Vogt MT, Cawthon PM, Kang JD, Donaldson WF, Cauley JA, et al. (2006) Prevalence of symptoms of cervical and lumbar stenosis among participants in the Osteoporotic Fractures in Men Study. Spine 31: 1445.
11. Hicks GE, Gaines JM, Shardell M, Simonsick EM (2008) Associations of back and leg pain with health status and functional capacity of older adults: Findings from the retirement community back pain study. Arthritis Care & Research 59: 1306–1313.
12. Young WF (2000) Cervical spondylotic myelopathy: a common cause of spinal cord dysfunction in older persons. American family physician 62: 1064.
13. Sherrington C (1906) The Integrative Action of the Nervous System. Cambridge: Cambridge University Press.
14. Sato A, Sato Y, Suzuki H (1985) Aging effects on conduction velocities of myelinated and unmyelinated fibers of peripheral nerves. Neuroscience letters 53: 15–20.
15. Chittleborough CR, Baldock KL, Taylor AW, Phillips PJ (2006) Health status assessed by the SF-36 along the diabetes continuum in an Australian population. Qual Life Res 15: 687–694.
16. Hayden JA, van Tulder MW, Tomlinson G (2005) Systematic review: strategies for using exercise therapy to improve outcomes in chronic low back pain. Ann Intern Med 142: 776–785.
17. Bigbee A, Hoang T, Havton L (2007) At-level neuropathic pain is induced by lumbosacral ventral root avulsion injury and ameliorated by root reimplantation into the spinal cord. Experimental neurology 204: 273–282.
18. Wieseler J, Ellis AL, McFadden A, Brown K, Starnes C, et al. (2010) Below level central pain induced by discrete dorsal spinal cord injury. Journal of neurotrauma 27: 1697–1707.
19. Defrin R, Ohry A, Blumen N, Urca G (2001) Characterization of chronic pain and somatosensory function in spinal cord injury subjects. Pain 89: 253–263.
20. Weng HR, Lee J, Lenz F, Schwartz A, Vierck C, et al. (2000) Functional plasticity in primate somatosensory thalamus following chronic lesion of the ventral lateral spinal cord. Neuroscience 101: 393–401.
21. Finnerup NB, Baastrup C (2012) Spinal Cord Injury Pain: Mechanisms and Management. Current pain and headache reports: 1–10.
22. Wollaars MM, Post MWM, van Asbeck FWA, Brand N (2007) Spinal cord injury pain: the influence of psychologic factors and impact on quality of life. The Clinical journal of pain 23: 383.

Author Contributions

Conceived and designed the experiments: TU SI MN CK. Performed the experiments: KO TI MK MI TT. Analyzed the data: TU MN CK. Contributed reagents/materials/analysis tools: TU SI MN CK. Wrote the paper: SI MI TU.

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エルボーバンドによる上腕骨外側上顆炎の治療成績 —アンケート調査—

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上腕骨外側上顆炎の治療は多岐に渡っているが決定的な治療は無い．今回著者らはアンケート調査により，上腕骨外側上顆炎の治療実態及びエルボーバンドの有効性を検討した．対象は2008～2010年の間に本疾患にてバンドを処方された158人中，アンケートの返信があった53人で，主なアンケート項目は，年齢，職業，性別，発症原因，施行された治療，バンドの装着様式と全装着期間，予後などである．集計後，難治化の原因を特定する為，患者を完治群と非完治群に分け，それらを従属変数，その他の項目を独立変数として，カイ二乗検定およびロジスティック回帰分析を施行した．結果，15か月後の完治率は44％と低く，難治症例は20％以上存在した．バンドは，装着コンプライアンスが不良である一方，効果を実感している患者も30％ほど存在した．統計学的検討の結果，ステロイド注射歴，発症原因，年齢，性別等は予後に影響していなかったが，一日の中でバンドを常時装着していた患者は完治率が高い傾向にあった．

【緒　言】

上腕骨外側上顆炎は近年，短橈側手根伸筋（ECRB）の上腕骨付着部における腱付着部症（enthesopathy）が病態の主体とされているが[1]，未だ不明な点も多く，治療法に関しても，NSAIDs内服投与，湿布，ステロイド局所注射，針灸，レーザー，理学療法（マッサージ，ストレッチ，筋力増強訓練），超音波，対外衝撃波，ギプス固定，エルボーバンド，手術的治療など多岐に渡るが，決定的な治療は無い[2)3)4)]．今回著者らはエルボーバンドの処方患者リストをもとに上腕骨外側上顆炎患者にアンケートを送付し，上腕骨外側上顆炎治療の実態調査を行うと共に，上腕骨外側上顆炎におけるエルボーバンドの有効性を統計学的に検討した．

【対象と方法】

対象は2008～2010年の間に我々の病院を受診し「上腕骨外側上顆炎」にてエルボーバンド（アルケア社，テニスエルボーサポーター）を処方された158人中，アンケートの返信があった53人で，年齢は32～78平均58.3歳，平均経過観察期間は15.3か月であった．この間エルボーバンドは，「治療法の説明を聞いた後に，患者が処方を希望された場合」にのみ処方された．主なアンケート項目は，年齢，職業，性別に加え，発症原因，エルボーバンドに併用された治療，一日の中でエルボーバンドを装着していた時間帯と全装着期間，そして最終治療成績などである．アンケート集計後，難治化の原因を特定するため，患者を完治群と非完治群に分け，それらを従属変数，また，性別，重労働，発症原因の負荷強度，一日の中でエルボーバンドを装着していた時間帯，全装着期間，対側肢の症状の有無，腱鞘炎の有無，肩こりの有無，ステロイド注射歴，ストレッチ歴などを独立変数としてカイ二乗検定などの単変量解析を行い，傾向があったものを，ロジスティック回帰分析で解析した．P値が0.05未満を有意な差とした．

【結　果】

発症原因としては，ゴルフや草むしりが多かったが，パソコンなどの弱負荷作業が40％存在した．また発症原因の負荷強度と完治率の間に関連はなく，家事などの弱負荷でも完治率は良くない結果となった（図1）．エルボーバンドに併用された各治療法の人数は，湿布治療が最多で，以下はストレッチ，ステロイド局所注射，NSAIDS内服，理学療法

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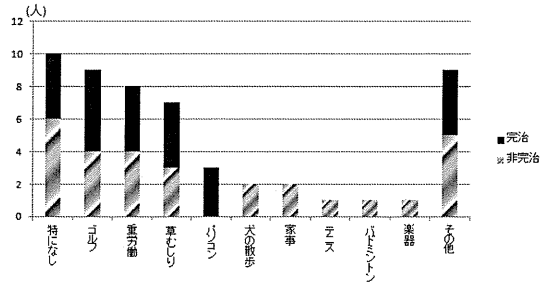

図1　上腕骨外側上顆炎の発症原因

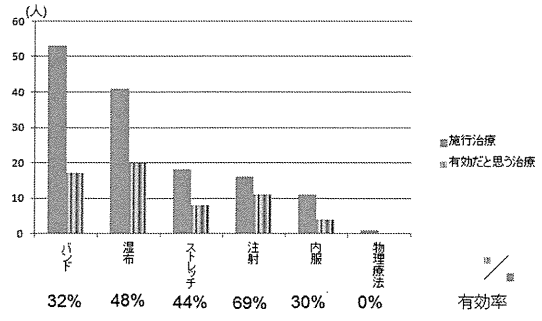

図2　実施された治療および有効だと患者が思う治療（複数回答可）

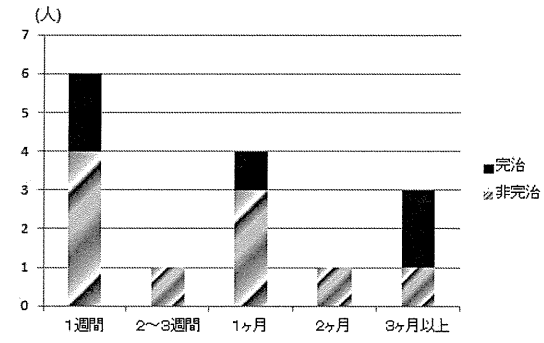

図3　一回のステロイド注射の効果持続期間

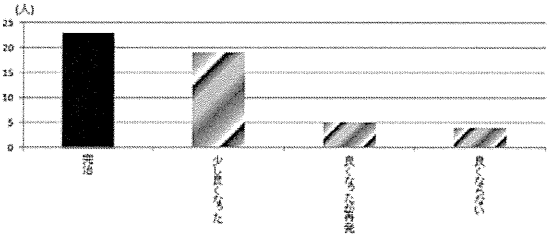

図4　最終治療成績

の順であったが，その中で，有効であると患者が感じた割合は，ステロイド局所注射（ベタメタゾン2mgと1%リドカイン2ml）が69%と最高で，その他の治療法はエルボーバンドを含め，どれも30〜40%台であった（図2）．注射療法は「効いている」という実感は強いものの，実際には全体の40%の患者が1週間で，さらには全体の70%が1か月で効果が切れており，過去の報告[5]と同様の結果となった（図3）．次に，対側肢の同症状の有無であるが，これは12人（22%）に認められた．同じ上肢で痛かった部位は，頚部や肩が7人（14%），手指や手関節が11人（22%）と多く，その中で腱鞘炎患者が6人存在した．平均15か月後の最終経過観察時には，完治した患者は44%であった．「再発した」や「良くならない」など，難治性といえる患者が合計22%存在した（図4）．エルボーバンドの装着状況であるが，80%の患者は「仕事やスポーツの時のみ時間限定で装着している」という状況であり，常時装着していた患者は20%に留まった（図5）．バンドの全装着期間つまりコンプライアンスであるが，26%が直ちに，60%が2か月までに辞めており，直ちに辞めている患者の86%は，完治していないにも関わらず「痛い，面倒，効かない」などの理由で辞めてしまっていた（図6）．統計解析の結果であるが，カイ二乗検定では性別，対側肢の症状の有無，肩こりの有無，注射歴などは完治率に影響していなかったものの，重労働者は完治率が低い傾向にあり（P値0.09），バンド常時装着者は完治率が高い傾向にあった（P値0.06）（表1）．また，続いて施行したロジステッィク回帰分析では，バンド常時装着者の完治率が高い傾向があるのみであった（P値0.07）．

【考　察】

今回，対側肢の症状を全体の22%の患者に，また，首から肩にかけての痛みを全体の14%の患者に，手指や手関節の痛みを全体の22%の患者に認めた．この事に関して，Fernandezら[6]は「上腕骨外側上顆炎患者のうち，幾らかにはcentral sensitizationが起こり筋肉の痛みが発生しやすい状態にある」と

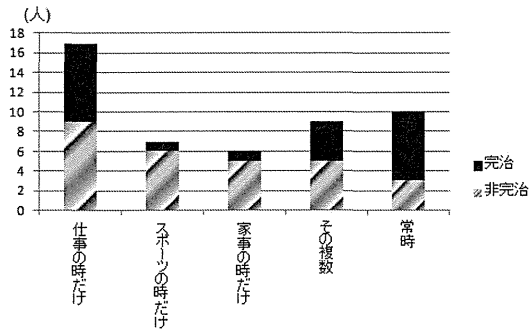

図5 エルボーバンドの装着様式

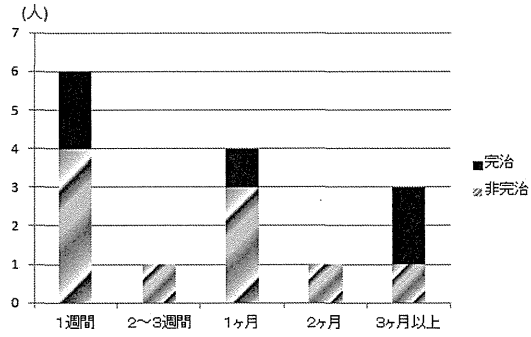

図6 エルボーバンドの装着期間

表1 カイ二乗検定及びロジスティック回帰分析の結果

	カイ二乗検定		
	オッズ比	95%信頼区間	P値
性別(女、男)	1.329	0.43-4.03	0.61
仕事(重労働、非重労働)	0.182	0.20-1.63	0.09
発症負荷(強負荷、弱負荷)	1.19	0.39-3.59	0.75
バンド装着(常時、非常時)	3.846	0.97-15.20	0.06
バンド装着期間(二か月以下、以上)	0.6	0.19-1.86	0.37
対側肢の症状(有、無)	0.35	0.08-1.48	0.14
手指腱鞘炎症状(有、無)	0.313	0.05-1.67	0.16
肩こり(有、無)	0.6	0.13-2.71	0.5
ステロイド注射歴(有、無)	0.706	0.21-2.34	0.56
ストレッチ治療歴(有、無)	1.19	0.39-3.59	0.75
	ロジスティック回帰分析		
			P値
仕事(重労働)			0.283
バンド装着(常時)			0.075

述べている．肘が痛い為，肩や手指の動きで代償し，その結果，それらの部位にも疼痛が発生しているということも考えられる．今後さらに検討していきたいと考えている．

治療の有効性に関する過去の論文では，短期的にはステロイド注射が有効であるものの，6か月以上の長期では，経過観察のみ群と大きく変わる治療法はなく，ストレッチや筋力トレーニングがわずかに優れているだけという報告がある[7]．今回の著者らの調査でも注射は大半が2か月以内の効果に留まっており，長期にわたり有効な治療というのはその他の中でも存在しなかった．

今回のアンケートでは，バンドは「痛い，面倒，効かない」などの理由から装着コンプライアンスが良くない事が分かったが，一方で，32%の患者には「効いている」という実感があり，「一日中，常時装着すると完治率が高い」という傾向が統計学的にも認められた．2007年の月村ら[8]の報告でも，手関節装具ではあるが「一日の中で6時間以上装着していた群が6時間以下の群に比べ最終時の痛みのVAS値が有意に低かったとしている．バンドがどのような患者に対し有効であるかについては，Walther[9]らは「エルボーバンドは外上顆への加速振幅と加速度の積分値を減少させるので，テニス，ゴルフなどには有効であるが，弱負荷作業には適していない」と述べており，月村ら[10]は「弱負荷発症の外上顆炎にはエルボーバンドよりも中指伸展制限付き手関節装具の方が効果ある」と報告している．今回の研究では，発症原因の負荷強度による完治率の差は認められなかったが，今後はエルボーバンド使用群と対照群を比較するrondomized controlled trialを行い，バンドの有効性をより詳細に検討すると共に，どのような患者に対しバンドが有効であるのかも検討していきたい．

【まとめ】

・エルボーバンド処方患者にて，上腕骨外側上顆炎の治療の実態を調査した．

・短期的には注射治療が効果的であったが，長期的には完治率が有意に高い治療は認められなかった．上腕骨外側上顆炎の15か月後の完治率は44%と低く，難治症例は20%以上存在した．

・エルボーバンドは，装着コンプライアンスが不良である一方，効果を実感している患者も30%ほど存在し，一日の中で常時装着している患者は完治率が高い傾向にあった．

【文　献】

1) 薄井正道ほか．テニス肘の病態と手術的（Nirshl-Pettorone法）治療．Orthopaedics 11(4): 81-89, 1998.
2) Struijs PA, et al. Orthotic devices for tennis elbow: a systematic review. British Journal of General Practice 51(472): 924-929, 2001.
3) Solveborn SA, et al. Radial epicondylalgia (tennis elbow): treatment with stretching or forearm band. A prospective study with long-term follow-up including range-of-motion measurements. Scand J Med Sci Sports 7(4): 229-237, 1997.
4) Okcu G, et al. The comparison of single dose versus multi-dose local corticosteroidinjections for tennis elbow. Clin Res 13: 158-163, 2002.
5) Smidt N, et al. Corticosteroid injections for lateral epicondylitis : a systematic review. Pain 96(1-2): 23-40, 2002.
6) Fernandez-Carnero J, et al. Bilateral myofascial trigger points in the forearm muscles in patients with chronic unilateral epicondylalgia. A blinded controlled study. Clin J Pain 24(9): 802-807, 2008.
7) Smidt N, et al. Corticosteroid injections, physiotherapy, or a wait-and-see policy: A randomised controlled trial. Lancet 359: 657-662, 2002.
8) 月村規子ほか．上腕骨外側上顆炎患者に対する装具療法の至適使用時間について．臨床スポーツ医学 24(3): 349-353, 2007.
9) Walther M, et al. Biomechanical evaluation of braces used for the treatment of epicondylitis. J Shoulder Elbow Surg 11: 265-270, 2002.
10) 月村規子ほか．テニス肘患者の主観的受傷機転と装具療法の効果の関連性について．臨床スポーツ医学 23(10): 1263-1268, 2006.

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Chronological Changes in Astrocytes Induced by Chronic Electrical Sensorimotor Cortex Stimulation in Rats

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Abstract

Motor cortex stimulation (MCS) is a treatment option for various disorders such as medically refractory pain, poststroke hemiplegia, and movement disorders. However, the exact mechanisms underlying its effects remain unknown. In this study, the effects of long-term chronic MCS were investigated by observing changes in astrocytes. A quadripolar stimulation electrode was implanted on the dura over the sensorimotor cortex of adult rats, and the cortex was continuously stimulated for 3 hours, 1 week, 4 weeks, and 8 weeks. Immunohistochemical staining of microglia (ionized calcium-binding adaptor molecule 1 [Iba1] staining) and astrocytes (glial fibrillary acidic protein [GFAP] staining), and neuronal degeneration histochemistry (Fluoro-Jade B staining) were carried out to investigate the morphological changes following long-term chronic MCS. Iba1 staining and Fluoro-Jade B staining showed no evidence of Iba1-positive microglial changes or neurodegeneration. Following continuous MCS, GFAP-positive astrocytes were enlarged and their number increased in the cortex and the thalamus of the stimulated hemisphere. These findings indicate that chronic electrical stimulation can continuously activate astrocytes and result in morphological and quantitative changes. These changes may be involved in the mechanisms underlying the neuroplasticity effect induced by MCS.

Key words: motor cortex stimulation, neural plasticity, trophic function, pain, cingulate gyrus

Introduction

Continuous electrical stimulation of the human brain and the spinal cord is known to have therapeutic effects on various disorders. In particular, motor cortex stimulation (MCS) has been increasingly receiving attention since the first application to intractable thalamic pain.[28–30] Nowadays, MCS is applied for both thalamic and peripheral neuropathic pain, movement disorders, and neurorehabilitation.[1,3,15,20,21] Continuous electrical stimulation may induce neuroplasticity and reorganization of the neural networks.[3,14,27]

We previously investigated the neural activities in the rat brain following unilateral chronic MCS utilizing c-Fos immunopositivity as a functional marker.[27] Both astrocytes and neurons were activated as shown by the observation of c-Fos-immunopositive cells in the sensorimotor cortex (SMC) and deep brain structures.[27] On the other hand, recent studies have shown that astrocytes are important in neural networks, and that abnormalities of astrocytes are associated with various disorders.[8,13] Several studies demonstrated that electrical stimulation activates astrocytes in vitro,[12,13] and we also found unusually large astrocytes in a patient with Parkinson's disease who had long-term continuous subthalamic nucleus stimulation (manuscript in preparation). On the basis of these findings, we extended our previous investigation to evaluate the chronological changes in the astrocytes following continuous MCS.

In this study, the SMC in the left hemisphere in rats was chronically stimulated for 3 hours, 1 week, 4 weeks, and 8 weeks. The motor cortex has direct

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and indirect connections with deep brain structures such as the thalamus (TH), basal ganglia, anterior cingulate cortex, and periaqueductal gray matter.[6,22] Therefore, chronological changes in the number of c-Fos-immunopositive astrocytes in the SMC and two deep brain structures (i.e. TH and cingulate gyrus [CG]) were observed utilizing immunohistochemical techniques. In addition, the number and area of astrocytes in the CG were investigated, as the CG has crucial roles in mood and pain control.[6,7,22,23] We discuss the possible mechanisms underlying pain suppression and neuroplasticity effects induced by MCS.

Materials and Methods

This study was carried out in accordance with the Guide for Animal Experimentation of the Faculty of Medicine, Nihon University and Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1985), and approved by the Animal Care and Use Committee of Nihon University.

The surgical procedure and the parameters of electrical stimulation were described in detail in our previous study.[27] The experimental animals were 12 adult male Wistar rats (body weight, approximately 500 g). The animal was anesthetized by intramuscular injection of ketamine hydroxylase (100 mg/kg body weight), and a mixture of 0.5% epinephrine hydrochloride and lidocaine hydrochloride (1 ml each) was injected under the skin and external ear canals to numb the areas. Then intraperitoneal injection of sodium pentobarbital (Nembutal; Abbot Laboratories, Chicago, Illinois, USA) (20 mg/kg body weight) was carried out. The rat was positioned in a stereotactic frame (Narishige, Tokyo) and a cranial burr hole (2 mm × 5 mm) was drilled on the left coronal suture, 3.5 mm lateral to the midline. A quadripolar stimulation electrode 2 mm wide and 5 mm long (Unique Medical, Tokyo) was positioned on the dura over the SMC in the left hemisphere. The electrode with four contact points numbered 0 to 3 sequentially from the most distal contact (0) to the most proximal contact (3) was placed, so that contact 0 was located in the rostral portion of the SMC. Each contact of the electrode was 0.7 mm long and the contacts were 0.7 mm apart. The optimal location was confirmed by test stimulation that causes forelimb muscle contraction. An extension wire was then passed from the head to the back subcutaneously and connected to an implantable pulse generator (Soletora Model 7426 IPG; Medtronic Inc., Minneapolis, Minnesota, USA).

Bipolar stimulation was applied at 25 Hz with the anode on the rostral contact and the cathode on the caudal contact beginning on postoperative day 1. A stimulation voltage of approximately 2-3 V was applied with a pulse width of 0.2 msec. The voltage was determined as less than 80% of that required for forelimb muscle contraction. These parameters were lower than the threshold level that induces seizure activity.[27,32] The stimulation was applied for 3 hours, 1 week, 4 weeks, and 8 weeks, and the stimulation parameters were not changed during these periods. Following each stimulation period, it was confirmed that the SMC was continuously stimulated by test stimulation before sacrificing the rat. The same operative procedures were carried out except for electrical stimulation was not performed as controls.

Following the chronic stimulation period, the rats were sacrificed by intraperitoneal injection of pentobarbital (60 mg/kg body weight) and perfused with 4% paraformaldehyde in 0.1 M phosphate-buffered saline, pH 7.4, following perfusion of 0.15 M NaCl. The brains were removed, postfixed in the same fixative for 12 hours with constant shaking, and then immersed in 20% sucrose for approximately 48 hours at 4°C until the brains sank. Brain slices were made at 5 mm anterior to the vertical zero point, which was immediately below the stimulated part of the SMC, for determining the number, area, and density of cells in each slice. The brains were embedded in the OCT compound, frozen, and sectioned (40 μm thick) on a freezing sliding microtome (Yamato Kohki Industrial Co., Ltd., Tokyo).

Immunohistochemical analyses used fluorescent double-staining methods to identify c-Fos-immunopositive astrocytes, and an immunoenzymatic staining method to detect glial fibrillary acidic protein (GFAP)-immunopositive cells to measure the areas of astrocytes. Selected sections were pretreated with 0.3% hydrogen peroxide solution in methanol and incubated in normal goat serum and 10% fish gelatin in 0.1 M phosphate buffer, pH 7.4 (FG-PB). Then, the sections were incubated with a mixture of an anti-c-Fos goat antibody (1:100; Santa Cruz Biotechnology, Santa Cruz, California, USA) and anti-GFAP rabbit antibody (1:100; Sigma, St. Louis, Missouri, USA) diluted in FG-PB for 48 hours at 4°C. After several washes with PB, the sections were incubated with secondary antibodies. The sections were first reacted with a fluorescein isothiocyanate-labeled anti-goat immunoglobulin G (IgG) donkey antibody (1:200; Chemicon, Billerica, Massachusetts, USA), and following several washes, an Alexa Fluor-labeled anti-rabbit IgG goat antibody (1:200; Invitrogen, Carlsbad, California, USA) diluted in FG-PB was used. The sections were set on slide

Fig. 1 Photomicrographs of double immunostaining of c-Fos (A) and glial fibrillary acidic protein (B) of cells in the lateral thalamus following 1-week stimulation. Arrows indicate double-immunopositive cells in the merged photograph (C). Original magnification ×400.

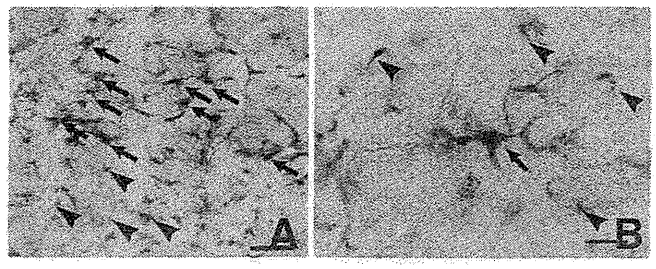

Fig. 3 Photomicrographs of giant astrocytes in rats stimulated for 4 and 8 weeks. Glial fibrillary acidic protein immunohistochemistry showed unusually large astrocytes (*arrows*) compared with other astrocytes (*arrowheads*) in the cingulate gyrus following 4-week stimulation (A) and the thalamus following 8-week stimulation (B). Original magnification A: ×80, B: ×160.

glasses and mounted with nonfluorescent glycerine (Merck, Darmstadt, Germany). The sections were photographed using a Coolscope CCD camera (Nikon, Tokyo) attached to an Eclipse microscope (Nikon). Then, the numbers of c-Fos/GFAP-double-immunopositive cells in the SMC, TH, and CG were counted (Fig. 1). In this process, GFAP-positive cells in the capillary walls were excluded.

Selected sections were pretreated with 0.3% hydrogen peroxide solution in methanol and normal goat serum, then incubated with only anti-GFAP rabbit antibody diluted in FG-PB for 48 hours at 4°C. Following several washes with PB, the sections were incubated with a biotinylated goat anti-rabbit IgG antibody (Vector Laboratories, Burlingame, California, USA) and reacted with avidin-biotin complex solution (Vector Laboratories). Then, the sections were reacted with 0.02% diaminobenzidine and 0.03% H_2O_2 for 10 minutes. Select sections were stained with Nissl for counterstaining. The sections were mounted on gelatin-coated glass slides, and air-dried, then dehydrated with ethanol, cleared in xylene, and coverslipped. The sections were examined and photographed utilizing a Coolscope CCD camera attached to an Eclipse microscope. First, the CG, TH, and SMC sections were examined to search for giant astrocytes. Then, the number of GFAP-immunopositive cells in the CG was counted and the area was also measured utilizing a Neurolucida system (MicroBrightField, Inc., Williston, Vermont, USA) installed on a personal computer attached to an AX-10 microscope (Olympus, Tokyo) and a 2400c CCD camera (Hamamatsu Photonics, Hamamatsu, Shizuoka).

Cell density (number of c-Fos/GFAP-double-immunopositive cells/0.1 mm²) was first calculated,

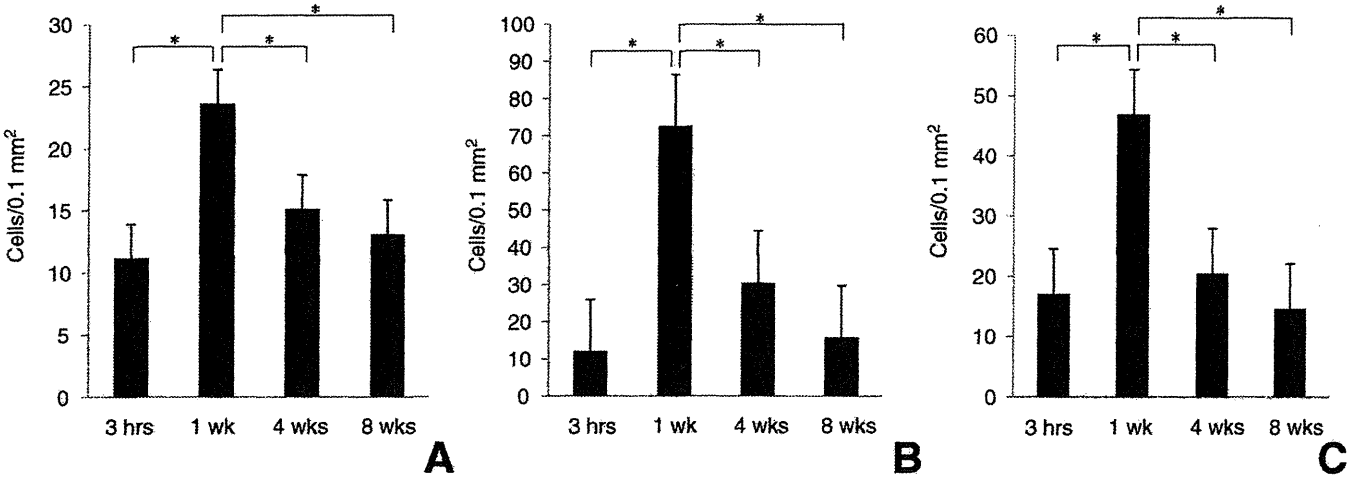

Fig. 2 Mean number of c-Fos-immunopositive astrocytes over time. The number of activated (c-Fos-immunopositive) astrocytes peaked following 1 week of continuous stimulation in the cingulate gyrus (A), thalamus (B), and sensorimotor cortex (C). Values are mean and standard deviation. *$p < 0.01$.

and then compared between time points utilizing the Wilcoxon signed-rank test. To investigate the chronological changes in astrocytes, differences in area were compared between time points as well as between the left and right hemispheres. Two-way analysis of variance (ANOVA) was used to test the null hypothesis of no chronological differences in the number and area. SAS Enterprise Guide 4.1 (SAS Institute, Cary, North Carolina, USA) was used for the statistical analyses.

To validate the experimental procedures, we confirmed that electrical stimulation did not cause neuronal damage by performing double staining using c-Fos and ionized calcium-binding adaptor molecule 1 (Iba1). Fluoro-Jade B (FJB) staining was also carried out to detect degenerating neurons in accordance with the method of Schmued et al.[26] Iba1 was used as a marker of microglia/macrophages, and the staining method was as for the c-Fos/GFAP double staining method except that anti-Iba1 rabbit antibody (1:250; Wako Pure Chemical Industries, Ltd., Osaka) was used instead of the anti-GFAP rabbit antibody as the primary antibody. For FJB histochemistry, the sections were incubated with 1% sodium hydroxide in 80% ethanol for 5 minutes and in 0.06% potassium permanganate for 10 minutes on a shaker. The sections were then incubated in freshly mixed 0.0004% FJB (Cosmo Bio Co., Ltd., Tokyo) in 0.1% acetic acid for 20 minutes, washed in distilled water, and air-dried at 50°C for 10 minutes. The sections were cleaned using xylene and coverslipped.

Results

The number of c-Fos-immunopositive astrocytes was counted in one sham-operated rat (control) and two stimulated rats at each time point. Twelve high-power fields (HPFs) were examined for the CG, 4 HPFs for the TH, and 4 HPFs for the SMC in each animal. The area of each HPF was 0.1 mm^2. The chronological changes in the mean number of c-Fos-immunopositive astrocytes showed that the number peaked following 1 week stimulation in all areas (Fig. 2). Only a few c-Fos-immunopositive astrocytes were observed in control rats, whereas a significantly larger number of c-Fos-immunopositive astrocytes were found in stimulated rats.

Several unusually large astrocytes (larger than 50 μm^2) were observed; in particular, we observed supergiant astrocytes with areas larger than 100 μm^2 (*arrows* in Fig. 3) in the CG and TH of rats stimulated for more than 4 weeks. The mean areas and numbers of measured astrocytes are summarized in Fig. 4. Two-way ANOVA revealed that the mean area of astrocytes peaked following 4 weeks of continuous stimulation (Fig. 5), and the mean area was larger in the left hemisphere (stimulated side) than in the right hemisphere (nonstimulated side) throughout the 8 weeks (Fig. 4).

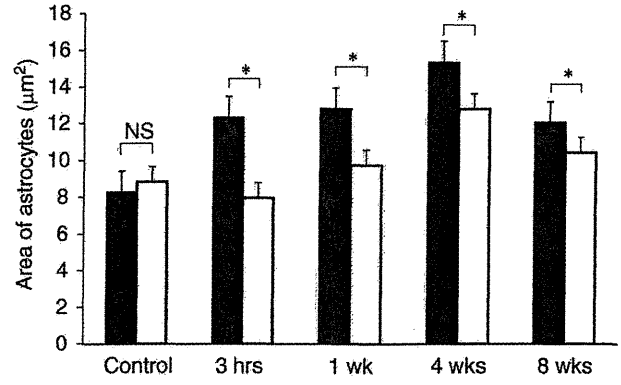

Fig. 4 Comparison of the area of astrocytes between stimulated (left, *closed columns*) and nonstimulated (right, *open columns*) cingulate gyri. The mean area of astrocytes in the stimulated hemisphere was significantly larger than that in the nonstimulated hemisphere. NS: not significant. Values are mean and standard deviation. *p < 0.01.

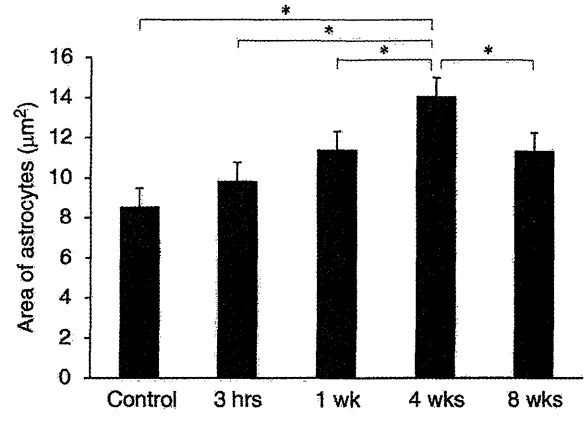

Fig. 5 Area of astrocytes over time in the cingulate cortex. The mean area of astrocytes peaked following 4 weeks of continuous stimulation. Values are mean and standard deviation. *p < 0.01.

No abnormal seizure movements were observed during the surgical procedure or observation periods up to 8 weeks. Perfused brains did not show obvious macroscopic hemorrhage or contusion under the electrodes. A few cells were positive for both c-Fos and Iba1 in both the control rats and stimulated rats up to 8 weeks, as observed in a previous study.[24] Moreover, there were no FJB-stained cells in the cortex, hippocampus, and TH. These findings indicate no evidence of neurodegeneration or injury