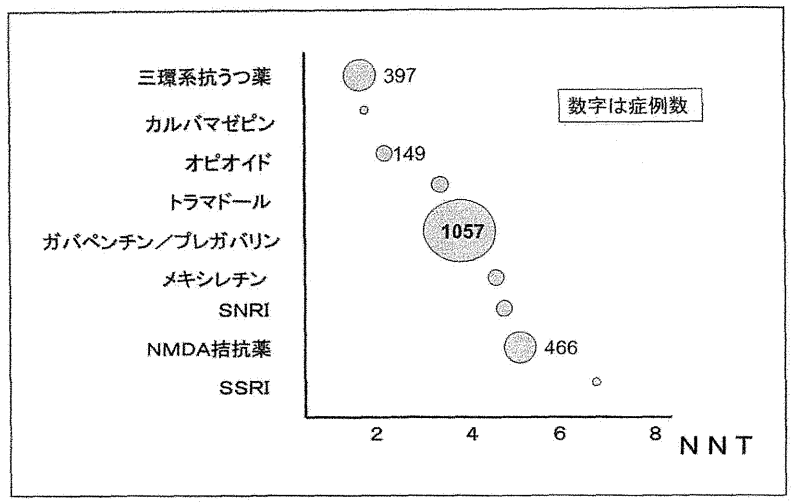

図5　神経障害性疼痛に用いる薬物のNNT

〔文献7)より引用．著者訳〕

hypothesisが出された．彼らは，さまざまな基礎実験から，神経線維の障害が起こると，末梢からの非侵害性刺激によっても隣接する神経線維の自発的発火が起こり，その発火は一次的増強を見た後，頻度依存性抑制機序により自然消滅することを明らかにした．末梢からの刺激を遮断する(神経ブロック)ことにより，痛みの発作が出なくなる理由も説明できる．

おわりに

ペインクリニックにおける慢性難治性疼痛のうち，特に治療に難渋することが多い末梢性神経障害性疼痛に重点をおいてその機序，薬物治療を中心に述べた．本症は末梢性と中枢性に分類されるが，この両者においてもその発生機序には多くの一致した発生機序と同じく，多くの異なった発生機序が存在しているものと考えられる．さまざまな疼痛性疾患の発生機序はいまだ不明な点が多いが，今後それらの機序が明らかになるにつれ，その治療法にも新しい方法が開発されてくることを期待したい．

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Diagnosis and Treatment of Neuropathic Pain

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It is recognized that neuropathic pain is very difficult to treat in pain-clinic practice. The reason that it is difficult to treat is considered a complexity of the mechanisms. Therefore, treatment of this condition must be done with adequate treatment strategies and/or pharmacological application.

In this article, the author describes nerve fibers, ectopic ion-channel, the contribution of nerve growth factor and lysophosphatidic acid to the development of ephapse and allodynia, the contribution of Glia to development of neuropathic pain, etc., concerning the mechanisms of neuropathic pain. Pharmacological management is also described.

Key Words : Neuropathic pain, Pain mechanisms, Nerve fiber, Pharmacotherapy

The Journal of Japan Society for Clinical Anesthesia Vol.31 No.7, 2011

Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2011, Article ID 939023, 11 pages
doi:10.1155/2011/939023

Review Article

The Animal Model of Spinal Cord Injury as an Experimental Pain Model

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Received 13 September 2010; Accepted 19 December 2010

Academic Editor: Andrea Vecchione

Pain, which remains largely unsolved, is one of the most crucial problems for spinal cord injury patients. Due to sensory problems, as well as motor dysfunctions, spinal cord injury research has proven to be complex and difficult. Furthermore, many types of pain are associated with spinal cord injury, such as neuropathic, visceral, and musculoskeletal pain. Many animal models of spinal cord injury exist to emulate clinical situations, which could help to determine common mechanisms of pathology. However, results can be easily misunderstood and falsely interpreted. Therefore, it is important to fully understand the symptoms of human spinal cord injury, as well as the various spinal cord injury models and the possible pathologies. The present paper summarizes results from animal models of spinal cord injury, as well as the most effective use of these models.

1. Introduction

Spinal cord injury (SCI) often results in severe motor dysfunction, such as complete paralysis. These patients typically cannot only walk, but also lose bowel, bladder, and sexual functions. Pain impact following SCI has been reported as 37% of higher-level SCI patients with pain and 23% of lower-level SCI patients with pain; given the choice, these patients would trade pain relief for loss of bladder, bowel, or sexual functions [1]. Pain management is, therefore, an important health problem and topic of study.

Pain experiments with human subjects have proven to be practically challenging, fundamentally subjective, and ethically self-limiting. For these reasons, there remains a need for the use of laboratory animal models of pain. Pain is subjective in humans, and interpretation of animal model results requires careful attention. In fact, some have called for the abandonment of animal pain studies in favor of more extensive human testing.

A number of animal models of SCI exist and are primarily used to determine mechanisms of motor dysfunctions [2–4]. Recently, these various SCI animal models have been utilized for pain studies [5]. However, when SCI animal models are used for pain research, special attention should be paid to the concomitant conditions. The present paper discussed the various SCI animal models as models for pain, with an emphasis on the complexities and limitations, as well as strategies for improvement and future use.

2. Pain in SCI Patients

2.1. SCI and the Social Impact. SCI occurs in most countries at an annual rate of 20–40 individuals per million. SCI is a devastating event that results in motor dysfunction below the level of lesion, as well as development of chronic pain syndromes. Studies have reported the prevalence of pain in SCI patients. A summary of results from 10 studies indicates that an average of 69% of the patients experienced pain, and nearly one-third of patients in pain rated their pain as severe [6]. The stakes are enormous, given the impact of pain on the economy (pain-related treatment costs 1 trillion US dollars

per year in developed countries) [7]. If SCI pain could be eliminated, the quality of life could be greatly improved in patients; they would no longer suffer from pain and could take part in social aspects of life or earn money.

2.2. Spinal Cord Injury and Chronic Pain. Following mechanical injury to the spinal cord, a wave of secondary pathological changes occurs and amplifies the extent of initial damage. Apoptosis is critical for triggering collateral damage following primary injury to the spinal cord. Spontaneous and evoked pain is frequent in traumatic or ischemic spinal cord injury.

In complete and partial spinal lesions, chronic pain develops within months following injury [8]. Up to 80% of patients experience clinically significant pain, which is described as burning, stabbing, and/or electric-like [9, 10]. Post-SCI pain results in drastically impaired daily routines and quality of life to a greater extent than motor impairment [11]; it is refractory to clinical treatments, despite a variety of neurosurgical, pharmacological, and behavioral therapeutic strategies [12, 13]. The pain so greatly affects quality of life that depression and suicide frequently result [14, 15].

3. Chronic Pain Classification in SCI (Tables 1 and 2)

Siddall and colleagues [16] classified SCI pain from spinal cord injury into two broad types, with three regions of pain.

3.1. Nociceptive Pain. It is crucial for a pain clinician to distinguish between nociceptive or neuropathic pain, because the clinical approach for each is different. The first choice for nociceptive pain treatment following SCI is often a nonsteroidal, anti-inflammatory drug, or opiate, which often results in sufficient pain control.

3.1.1. Musculoskeletal Pain. Musculoskeletal pain is very common in SCI patients. In chronic states, secondary overuse or abnormal use of structures, such as the arm and shoulder, occurs [17]. Muscle spasm pain is a commonly observed type of musculoskeletal pain and is refractory for treatment of common musculoskeletal pain; analgesics are sometimes helpful, but antispasticity treatment may be needed in many cases [18].

3.1.2. Visceral Pain. Pathology in visceral structures, such as urinary tract infection, bowel impaction, and renal calculi, generally results in nociceptive pain. Visceral pain usually exhibits a delayed onset following SCI, which could be due to normal afferent input *via* sympathetic or vagal nerves in paraplegics or *via* the vagus nerve in tetraplegics [19, 20]. Patients with upper thoracic injury or cervical SCI may present with autonomic dysreflexia headache, because of bowel impaction or bladder distension.

3.2. Neuropathic Pain. SCI often results in neuropathic pain, which is difficult to treat and exhibits various patterns due to its pathology.

Table 1: Classification of the Spinal Cord Injury Pain Task Force of the International Association of the Study of Pain.

Broad type	Broad system	Affected structures/Pathologies
Nociceptive	Musculoskeletal	Bone, joint, muscle trauma, or inflammation
		Mechanical instability
		Muscle spasm
		Secondary overuse
	Visceral	Renal calculus (kidney stones)
		Bowel and sphincter dysfunctions
		Headache by autonomic dysreflexia
Neuropathic	Above-level	Compression mononeuropathy
		Complex Regional Pain Syndrome
	At-level	Nerve root compression (cauda equine)
		Syringomyelia
		Spinal cord trauma/ischemia
		Dual-level cord and root trauma (double-lesion syndrome)
	Below-level	Spinal cord trauma/ischemia

Table 2: SCI pain classification by Bryce and Ragnarsson.

Location		Type	Etiologic subtypes
Above-level	nociceptive	1	Mechanical and musculoskeletal
		2	Autonomic dysreflexia headache
		3	Others
	neuropathic	4	Compressive neuropathy
		5	Others
At-level	nociceptive	6	Mechanical and musculoskeletal
		7	Visceral
	neuropathic	8	Central
		9	Radiculopathy
		10	Compressive neuropathy
		11	Complex Regional Pain Syndrome
Below-level	nociceptive	12	Mechanical and musculoskeletal
		13	Visceral
	neuropathic	14	Central
		15	Other

3.2.1. At-Level Pain. At-level pain occurs in dermatomes near the spinal injury and develops shortly after the injury. The pain is often characterized as stabbing or stimulus-independent and is accompanied by allodynia [21, 22].

3.2.2. Below-Level Pain. Below-level pain is localized to dermatomes distal to the injury site and develops more gradually than at level pain; it is often classified as a stimulus-independent, continuous, burning pain [21, 22].

3.2.3. Above-level Pain. Above-level pain occurs at dermatomes cranial to the injury site [21, 22].

3.3. Other Classification of SCI Pain (Table 2). Bryce et al. classified SCI pain by location of the pain [23]. In terms of animal behavior, this classification helps to provide a better understanding of pain pathology. In basic pain research, pain is defined as neuropathic or nociceptive. Similarly, SCI pain is complex and the pathology should be taken into consideration at the same time. It is important to understand the pathologies in each model.

4. The Role of Animal Model

Human self-ratings of pain, using questionnaires and scales, are reliable, accurate, and versatile for measuring experimental and clinical pain [24]. Nonetheless, the subjectivity of these measures has led to a decade-long search for surrogate biomarkers. To date, an objective surrogate with acceptable high sensitivity and specificity has not been identified. However, individual function-imaging scans could provide a reliable and objective measurement of subjective pain perception [25]. In addition, genetic biomarkers could prove to be useful. However, it is likely that too many genes are involved [26]. Moreover, genomic DNA variants could predict trait sensitivity to pain rather than ongoing levels of pain. Only a small percentage of injuries, infections, or others causes that results in chronic pain syndrome actually develop chronic pain. Therefore, in human studies, it will be difficult to determine the correlation between genetic background and pain severity. Furthermore, common clinical pain conditions, such as back pain, are too polygenic to be effectively modeled and genetically understood.

Animal models cannot self-report. In response to noxious stimuli, behaviors can be reliably and objectively scored, although these simple reflexes or innate responses (such as licking an inflamed paw) seem to lack clinical validity. Indeed, experiments with behavioral measurements of pain in animal models have become more common. According to studies published in flagship journals, pain studies comprise approximately 25% of total studies, more than any other field of study [27].

The animal model of pain plays a central role in analgesic drug development and the fundamental mechanisms that drive it. Despite the development of human imaging studies, such as functional MRI, the use of animal models of pain is a continuing necessity [5].

5. Spinal Cord Injury Dynamics and Procedures

Several models of neuropathic pain due to spinal cord injury have been simulated in rats. These studies have primarily focused on spinal cord injury caused by contusion or weight dropping, spinal cord compression, excitatory neurotoxins, photochemical-induced ischemia, spinal cord transaction, or crushing of the spinal cord. These models have also been adapted for mice [28–31]. The development of reliable neurotrauma mouse models provides great promise for evaluating overexpression or inactivation of certain genes on lesion pathophysiology and functional outcome. However, more attention should be focused on motor recovery while evaluating pain behavior, because of the delayed motor recovery in mice compared with rats [32, 33]. The utility for each model summarizes in Table 3.

5.1. Contusive or Hemicontusive Models. Spinal contusion is the oldest and most widely used animal model. In addition to motor dysfunction, this injury elicits sensory dysfunction, including neuropathic pain, tactile allodynia, and thermal hyperalgesia [34, 35]. Cervical contusion is rarely reported, because life-threatening adverse effects could occur. Therefore, cervical hemicontusion, following hemilaminectomy, is used to analyze the unilateral spinal cord contusion model. Because motor dysfunction appears in the forelimbs, pain-related behavior is difficult to estimate, and for this reason, cervical contusion is often utilized for motor functional analysis [2, 3]. The thoracic spinal cord contusion model is the most popular pain research model and is induced with impactors, such as the weight-drop impactor [36]. In brief, the exposed spinal cord is injured by dropping a 10.0-g rod from specified heights [37, 38]. After 2 or 3 weeks, motor dysfunction is recovered and pain behavior can be analyzed. The impact of the injury tends to vary. Therefore, especially in short distances from the rod to spinal cord, pain behavior does not always appear. It is difficult to bilaterally drop the rod onto the spinal cord. Following injury, motor function analysis is needed to exclude unilateral paralysis and the possibility of unilateral contusion. Abnormal sensations due to mechanical, thermal, or cold stimuli are observed for several weeks or longer [32, 33, 39–52], and all regions (at-, above-, below-level) of allodynia are analyzed [53–56].

5.2. Transection or Hemisection Models. The complete spinal transection injury model reflects symptoms of complete SCI patients. Following laminectomy, spinal cord transection is performed with spring scissors. Occasionally, to attach the two ends for regeneration, a sterile, gel foam is placed between the two resected spinal cord ends. At-level and below-level neuropathic pains are then analyzed [57, 58]. Many studies have reported muscle spasms in the spinal complete transection model [18, 59, 60], and musculoskeletal pain pathology during spasticity could help to clarify the use of this model.

The partial spinal transection injury model (hemisection) has become popular in neuropathic pain studies [61–78]. Motor dysfunction appears only in the ipsilateral injured side and persists from 5 days to 4 weeks [64, 75]. Mechanical allodynia and thermal hyperalgesia are bilaterally observed in above-level and below-level cases [61, 76–81].

5.3. Photochemical Model. Over the past two decades, the photochemical model of spinal cord injury, developed by Watson et al. [82], has proven to be one of the most reliable and reproducible graded experimental rat models of spinal cord injury [83–94] and has been widely used to study neurotrauma in mice [88]. The biggest advantage of

Table 3: Animal spinal cord injury models and symptoms.

Impact to spinal cord	Laterality and devices	Injury area			Sensory abnormality			Duration	
		Cervical	Thoracic	Lumbar	At-level	Below-level	Above-level	Allodynia	Maximal motor dysfunction
Transection	Bilateral		○	○	○	○	×	Several weeks or more	Less than 4 weeks
	Unilateral	◎	◎	◎		◎	◎	1–5 weeks or more	Ipsilateral to injury: 4 weeks
Compression	Contusion		○		○	○	○	Weeks to months	1-2 weeks
	Hemi-contusion	○							3 weeks
	Clip		○		○	○: severe injury impossible		4 weeks	4 weeks severe injury
	Displacement					◎		4–6 weeks	2 weeks
	Canal stenosis			○		○ or ×		8 days or hypoalgesia	
Photochemically			◎		◎			10–20 days	Various
Excitotoxic			○		○	○	○?	5 weeks or more	
Spinothalamic tract lesions			◎		◎		◎	Several weeks	Less than 1week

Many spinal cord injury models exist for pain research. Pain behavior should not be measured in injured animals during maximal motor dysfunction.

this method is that the resulting injury does not induce mechanical trauma to the cord, because there is no need for laminectomy. Instead, an intravascular photochemical reaction occurs through the use of a dye that is activated by an argon ion laser to produce single oxygen molecules at the endothelial surface of spinal cord vessels. This results in an intense platelet response, as well as subsequent vessel occlusion and parenchymal tissue infarction [83]; the pathology is of a purely ischemic origin. Motor deficits are related to irradiation duration, as well as mechanical allodynia (cold, not thermal), which lasts for several days [91]. Following application of the von Frey filament to the trunk, behavioral analysis is performed according to vocalization threshold. Antiallodynic effects of analgesics have been determined using this model [84, 85, 90]. However, extent of injury is difficult to control. Therefore, motor deficit scores, such as BBB [95] and CBS [96], have been widely utilized [86, 90].

5.4. Excitotoxic Models. Intraspinal or intrathecally injection of some excitotoxins, such as quisqualic acid or other excitatory amino acids (glutamate, N-methylasparate, and kainic acid), produces long-lasting spontaneous pain, mechanical allodynia, and thermal hyperalgesia in rats and mice [97, 98]. Following excitotoxin injections, neuronal loss, cavity formation, astrocytic scaring, and prominent inflammation occur. The advantage of this model is the ability to correlate specific areas of tissue damage with behavioral changes. Moreover, the percentage of animals that exhibit pain-related behaviors following injury is greater than with other models; induced mechanical allodynia was 67% in the contusion injury model [99], in contrast to 44% chronic allodynia following ischemic injury [86]. In excitotoxic animal models, nearly 100% animals develop varying degrees of hypersensitivity to mechanical and thermal stimuli [98].

5.5. Other Mechanical Spinal Cord Injuries

5.5.1. Clip Compression Injury. Clip compression injury resembles spinal contusion injury at the point of the injury caused by pressure to the spinal cord. Following laminectomy, compression injury is induced with clips calibrated to exert a force of 50 or 35 g. The 50-g clip induces severe injury and the 35-g clip induces moderate injury. Either clip is dorsoventrally closed over the entire cord for 1 min and then subsequently removed [58, 100–102]. A vascular clip is used for this procedure in mice [103]; the spinal cord becomes ischemic and mimics common clinical injuries and outcomes.

5.5.2. Spinal Cord Displacement. The spinal cord displacement model attempts to regulate trauma impact by controlling displacement length of the spinal cord. Through the use of this model, a cutoff for normal sensory function has been determined [104]. In human SCI, trauma severity is not proportional to pain severity, because the method of injury varies. The unique features of controlled displacement and monitoring of biomechanical parameters at the time of impact help to reduce outcome variability [105].

5.5.3. Canal Stenosis. Lumbar canal stenosis is due to entrapment of the cauda equine and/or lumber nerve roots by hypertrophy of osseous and soft tissue structures

surrounding the lumbar spinal canal. A typical pathology is reduced blood flow to the peripheral nerve, resulting in demyelination or axonal degeneration, depending on the magnitude of ischemic injury. Canal stenosis can also be termed a spinal cord injury model, in which square-shaped pieces of silicon are placed into the epidural space in the rat [106, 107]. However, these procedures also induce mechanical hypoalgesia [107]. Nevertheless, this model could help to clarify pathophysiology of chronic, light pressure to the spinal cord.

5.5.4. Spinothalamic Tract Lesions. The spinothalamic tract is the core pain pathway in the spinal cord. This model is designed to lesion only the spinothalamic tract area using a tungsten microelectrode. Although this model injures the unilateral spinothalamic tract, bilateral above- and below-level hyperalgesia, as well as allodynia, is induced and can persist for many weeks. These features resemble allodynia and hyperalgesia experienced by humans suffering from central pain syndromes following spinal cord injury. Therefore, this model could provide useful and novel insights into the underlying biological mechanisms of spinal cord injury [108].

6. Pain-Related Behavior As an Evaluation of Symptoms

Pain-related behavior is recorded using various devices applied to the forelimbs, hindlimbs, trunk, and face. If pain behavior appears in the face, it is considered to reflect the reaction to supraspinal mechanisms, because sensory function in the face is regulated by the trigeminal nerve (a cranial nerve). In thoracic spinal cord injury, trunk allodynia reflects at-level neuropathic pain, and allodynia in the hindlimb reflects below-level neuropathic pain. Forelimb allodynia reflects at-level neuropathic pain in cervical injury and above-level neuropathic pain in other injuries.

Abnormal pain behavior is a result of three different stimulations: mechanical, thermal, and cold.

6.1. Mechanical Allodynia. Mechanical allodynia can be measured in various ways using the von Frey hair. In one of the methods, the "up-down method" [109], each von Frey hair is applied to the test area for 2-3 s, with a 1-2-minute interval between stimuli. The trial begins with application of the 15-mN von Frey probe to the hindpaws. A positive response is defined as a rapid withdrawal and/or licking of the paw immediately upon application of the stimulus. The von Frey hair can also be used to determine vocalization threshold to graded mechanical allodynia as a means to evaluate at-level neuropathic pain in the trunk [92]. When a positive response to stimulus occurs, the next smaller von Frey hair is applied. If a negative response occurs, the next higher force is applied. Testing continues for five or more stimuli after the first change in response, and the pattern of responses is converted to a 50% von Frey threshold using a previously described technique [109]. If the animal shows no response to the highest von Frey hair (160 mN), a von Frey threshold of 260 mN, corresponding to the next log increment in potential von Frey probes, is assigned to the threshold.

Touch-evoked agitation is another evaluation of mechanical allodynia [110] and can be used to test the animal response to tactile stimulation. The animal skin is briskly stroked with a pencil point in a rostral to caudal direction. The animal response is graded with a score of 0: no response, 1: moderate efforts to avoid the probe, transient vocalization, and 2: vigorous efforts to escape the stimulus, frequent and sustained vocalization in response to the probe.

Pathological reactions between the von Frey probe and pencil point vary due to reactions to the von Frey hair (caused by A-delta-fiber and C-fiber) or the pencil (A-beta fiber).

6.2. Thermal Hyperalgesia. Thermal hyperalgesia can be measured by latency of paw withdrawal in response to a radiant heat source [111]. Briefly, animals are placed in Plexiglas boxes on an elevated glass plate heated by a radiant heat source directed by a beam of light to the planter surface of each paw through the glass plate (47°C). The light beam is automatically turned off by a photocell upon limb-lift, allowing for measurement of time between stimulus start and paw withdrawal (paw withdrawal latency). Three to five minutes are allowed between each trial, and three trials are averaged for each limb.

6.3. Cold Allodynia. Cold sensitivity to acetone can be quantified by foot withdrawal frequency [112]. A total of 100 μL acetone is applied to the paw planter surface using a plastic tubule connected to a 1 ml syringe. Acetone is applied 5 times to each paw at an interval of at least 5 minutes. The number of brisk foot withdrawals is recorded.

7. Evaluation of Motor Functions in the Spinal Cord Injury Model

Locomotor function is observed and recorded using the Basso, Beattie, and Bresnahan (BBB) Locomotor Rating Scale [95]. Briefly, the BBB is a 22-point ordinal scale ranging from 0 (no discernable hindlimb movement) to 21 (consistent and coordinated gait with parallel paw placement of the hindlimb and consistent trunk stability). Scores from 0 to 7 rank early phase of recovery, with return of isolated movements from three joints (hip, knee, and ankle); scores from 8 to 13 describe the intermediate recovery phase with, return of paw placement, stepping, and forelimb-hindlimb coordination; and scores from 14 to 21 represent late phase of recovery, with return of toe clearance during the step phase, predominant paw position, trunk stability, and tail position. Scores are tabulated and considered to be an indicator of motor recovery.

The Basso Mouse Scale (BMS), a 9-point rating scale, has been specially developed for mouse models [113]. An additional scoring systems, described by Gale et al. [96] and termed the Combined Behavioral Score (CBS) (Table 4), has been used to measure locomotor function.

Following cervical spinal cord injury, recovery of forelimb function can be measured [114] by indicators such as the grooming test and forelimb asymmetry test [115]. Forelimb grooming function has been assessed using a scoring system originally developed to examine recovery in a rat brachial plexus reconstruction model [116]. The forelimb asymmetry, or paw preference test, is sensitive to asymmetries produced by a variety of CNS insults [117]. In addition, forelimb motor function recovery and pain behavior should be coanalyzed, because behavior is a result of motor functions [118].

8. Future Direction and Conclusions

8.1. Spinal Cord Injury As a Musculoskeletal Pain Model. Spinal cord injury leads to immediate impaired motor and sensory functions, which are also manifested over time. Following an initial period of spinal shock, reflexes become reduced and a disturbing hyperreflexia develops, which is often referred to as spasticity [119].

Spasticity is a disabling complication that affects individuals with spinal cord injury [18, 120]. Approximately 75% of individuals with SCI exhibit spasticity 1 year after injury and half undergo antispasticity treatment [121]. Significant scientific interest has been devoted to spasticity over the past 10–15 years as an example of plastic changes occurring distal to a central lesion.

The primary mechanisms hypothesized to be responsible for spasticity are increased motoneuron excitability [122, 123] and increased synaptic input, as a result of muscle stretch and reduced inhibitory mechanisms (presynaptic [124] and reciprocal inhibitions [125]). The mechanisms underlying decreased inhibition below the lesion remain poorly understood [59].

The most commonly proposed mechanisms to account for decreased inhibition following spinal cord injury include disruptions of facilitatory supraspinal input to inhibitory interneurons [59, 126]. Motoneuron and sensory neurons are often regulated by common mechanisms [127], and common molecular mechanisms could be responsible for below-level neuropathic pain and spasticity [18, 37].

The spinal cord injury model, in particular the spinal transaction model, is considered useful for spasticity research. Because spasticity results in musculoskeletal pain, the spinal cord injury model could be considered a musculoskeletal pain model.

8.2. Spinal Cord Injury As a Visceral Pain Model. Visceral pain in spinal cord injury commonly triggers autonomic dysreflexia, a potentially life-threatening hypertensive syndrome due to high thoracic spinal cord injury. Pathology correlates with increased sprouting of primary afferent c-fibers into the spinal cord. During motor dysfunction, visceral pain-related behavior is difficult to analyze. However, based on the above-described mechanisms, a morphological approach to spinal complete transection injury has been utilized [128].

Table 4: Combined Behavioral Score (CBS), as reported by Gale et al. [96].

General description		Points
Motor score		
0	Normal walking	0
1	Walks with mild deficit	5
2	Hindlimb can support weight	15
3	Frequent movement of hindlimb, no weight support	25
4	Minor movement in hindlimb, no weight bearing	40
5	No movement in hindlimb, no weight bearing	45
Toe spread		
0	Normal, full, toe spread	0
1	Partial spreading of toes	2.5
2	No spreading of toes	5
Righting		
0	Normal righting, counter to direction of roll	0
1	Weakened attempt to right	5
2	Delayed attempt to right	10
3	Delayed attempt to right itself	15
Extension withdrawal		
0	Normal	0
1	Weak and slow reflex to withdraw hindlimb	2.5
2	No withdrawal reflex	5
Placing		
0	Normal placing	0
1	Weak attempt to place foot	2.5
2	No attempt to place foot	5
Inclined plate		
0	65~70/deg	0
1	55~60	5
2	40~50	10
3	<40	15

8.3. Limitations of Animal Models of Chronic Pain. Limited success in the pain field during the past few decades has resulted in a plethora of basic scientific data. The use of animal models has increased our knowledge of novel, effective, and safe clinical analgesics. Experimental failures with novel drugs are associated with adverse side effects and the lack of efficacy in humans. In addition, psychosocial aspects of chronic pain due to spinal cord injury have been completely omitted, despite a large body of knowledge emphasizing the importance of these factors in chronic pain. Future studies should extend the scope of inquiry to include the psychosocial aspects of chronic pain and spinal cord injury.

8.4. Conclusion. By widening the number of animal models of spinal cord injury, new challenges have emerged. Although experimental methods of spinal cord injury pain lead to various behavioral outcomes, it is clear that some models respond similarly to pharmacological agents. This suggests that common mechanisms could underlie specific symptoms derived from various injury conditions. Etiologies of spinal cord injury pain could vary. However, by focusing on various symptoms of spinal cord injury pain, treatment possibilities for pathologies of spinal cord injury pain could emerge.

Continuous basic and clinical studies focused on different aspects of spinal cord injury pain are needed to better understand the mechanisms involved.

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J Anesth (2011) 25:523–530
DOI 10.1007/s00540-011-1173-9

ORIGINAL ARTICLE

Human brain activity associated with painful mechanical stimulation to muscle and bone

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Received: 8 February 2010 / Accepted: 12 May 2011 / Published online: 2 June 2011

Abstract
Purpose The purpose of this study was to elucidate the central processing of painful mechanical stimulation to muscle and bone by measuring blood oxygen level-dependent signal changes using functional magnetic resonance imaging (fMRI).
Methods Twelve healthy volunteers were enrolled. Mechanical pressure on muscle and bone were applied at the right lower leg by an algometer. Intensities were adjusted to cause weak and strong pain sensation at either target site in preliminary testing. Brain activation in response to mechanical nociceptive stimulation targeting muscle and bone were measured by fMRI and analyzed.
Results Painful mechanical stimulation targeting muscle and bone activated the common areas including bilateral insula, anterior cingulate cortex, posterior cingulate cortex, secondary somatosensory cortex (S2), inferior parietal lobe, and basal ganglia. The contralateral S2 was more activated by strong stimulation than by weak stimulation. Some areas in the basal ganglia (bilateral putamen and caudate nucleus) were more activated by muscle stimulation than by bone stimulation.
Conclusions The putamen and caudate nucleus may have a more significant role in brain processing of muscle pain compared with bone pain.

Keywords Pain · Muscle · Bone · Neuroimaging · fMRI

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Introduction

Physical pain originating from deep tissues—including sprains, fibromyalgia, rheumatic polymyalgia, and other muscle-derived pain, and bone-derived pain such as fractures, spondylosis, and bone tumors—is very commonly encountered. Consequently, understanding how these conditions come to be painful through brain processing is clinically important. Recent imaging research using the blood oxygen level-dependent-based (BOLD) functional magnetic resonance imaging (fMRI) method successfully revealed cognitive mechanisms in response to painful stimulation.

In the field of anesthesiology, fMRI has been on trial as a tool for investigating cerebral pain processing [1–5]. Previous reports, however, have mainly been studies of

heat stimulation to skin [6, 7]. More recently, studies targeting brain activation when muscle pain is caused using electric stimulation or hypertonic saline have successfully demonstrated that some areas are differently activated, including the contralateral primary somatosensory cortex (S1), the ipsilateral anterior insula, the contralateral motor cortex, the cingulate motor area, and the perigenual cingulate [8–10]. However, there are few studies of the brain processing activated by mechanical stimulation to deep tissues [11]. To elucidate the central processing of painful mechanical stimulation to deep tissues (muscle and bone), we compared the brain activation induced by two different intensities of stimulation (strong and weak) at two different targets (muscle and bone), using fMRI.

Materials and methods

Subjects

All the procedures were approved by the Osaka University Hospital Institutional Review Board. Twelve healthy volunteers (7 men, 5 women; aged 24–56 years) agreed to receive painful stimulation while their brain activation was evaluated. They had no neurological disorders or detectable MRI abnormalities in the brain and were free from any medication within 24 h before the study. In written informed consent, each acknowledged that they were willing to receive experimental painful stimulation. Before the protocols were carried out, each volunteer was familiarized with the experimental protocol, the types of stimulation, and the tasks performed.

Painful stimulation

To determine suitable stimulation intensities for each subject, a preliminary testing was performed immediately before the fMRI study. Perpendicularly applying a round 10-mm solid tip of an algometer probe (Pressure Algometer NPA-1, Shinko, Japan) on the surface (skin) at the medial point of the right tibia (Fig. 1), the experimenter gradually increased the pressure until the subject verbally indicated that the stimulation was painful. At that point, the pain intensity was taken to be '3' on a subjective 10-point numerical rating scale (NRS). The three median values of five trials were averaged to determine the weak stimulation to be applied to the tibia of particular volunteers. Similarly, the subject was asked to verbally indicate when the pain was such that it would probably be intolerable for more than 20 s without withdrawal movement. At this point, pain intensity was scored as '8' on the volunteer's subjective 10-point NRS. Similar grading of muscle pain was also carried out [12]. Here the tip of the probe was applied to the skin on the gastrocnemius muscle at a medial point 3–5 cm from the stimulation point of the tibia (Fig. 1). For each volunteer, as in the bone protocol, subjective NRS pain scores of '3' and '8' were obtained. These procedures were conducted by one experimenter (M.S.) who, in each instance and so far as possible, endeavored to consistently apply the required level of pressure perpendicularly.

Protocol

Each subject participated in a trial comprising 12 fMRI task sessions. At each right-leg site that was evaluated in the preliminary testing, three trials of 20 s of strong or weak stimulation were applied in each session. The site and strength of stimulation were pseudo random for each series. After each series of three stimulations (in a session lasting 3 min; Fig. 2), there was a pause of 1 min before the next session. MRI scans were acquired throughout the 36-min period. The subjects were asked to rate, using NRS, the overall pain at the end of each series of three stimulations (mean of three periods of 20-s stimulation). Before the

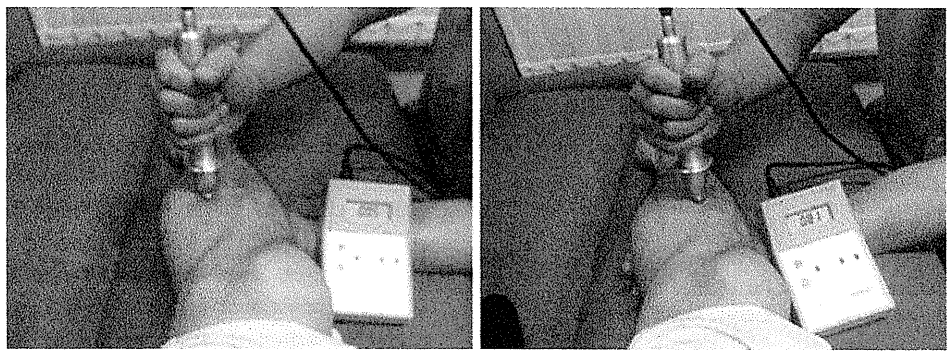

Fig. 1 Photograph showing how a digital algometer was used to apply stimulation to muscle (*left*) and bone (*right*). Bone stimulation was applied to the surface (skin) at the midpoint of tibia. Muscle stimulation was applied to the surface of gastrocnemius 3–5 cm posterior from bone stimulation point. Intensities of stimulation (muscle vs. bone, strong vs. weak) were decided for each subject by averaging the values of the median of three of five trials

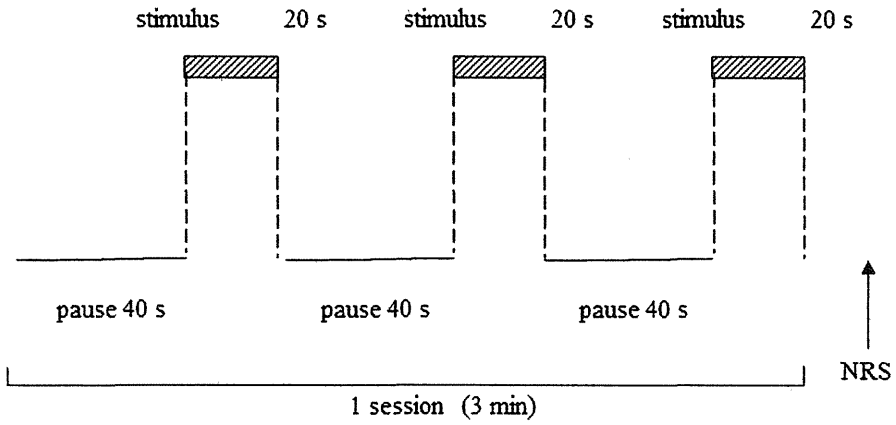

Fig. 2 Schematic representation of the experiment. Each subject underwent one trial comprising 12 sessions with a 1-min interval between sessions. Each session, lasting a total of 3 min, started with 40-s pause, and then a 20-s strong or weak stimulation to either muscle or bone. Weak and strong stimulations for each volunteer were decided in the preliminary testing. The order of stimulation was pseudo randomized. After each session, volunteers were asked to score the perceived pain. *NRS* numerical rating scale

commencement of the protocol, each volunteer was informed that the site (muscle or bone) and intensity (weak or strong) of the forthcoming series of stimulation would be random.

MRI

Imaging was performed with a 1.5-T MRI scanner (Signa EXCITE XI 11.0; GE Healthcare, Milwaukee, WI, USA). Functional MR images were obtained using a multislice echo planar imaging technique (EPI) based on 30 oblique slices (repetition time, 3,000 ms; time to echo, 60 ms; flip angle, 90°; slice thickness, 5 mm; gap, 0 mm; field of view, 300 mm × 300 mm; in-plane resolution, 4.69 mm × 4.69 mm). All the subjects were positioned in the scanner with a foam rubber pad to minimize head movement and instructed simply to lie with their eyes closed without moving or speaking. Their heads were placed so that the uppermost superior aspect of brain was within the field of view. High-resolution T_1-weighted anatomic images with the same orientation as the EPI slices were collected from each subject. In the subsequent analysis, these images were used for coregistration of functional and anatomic data.

Data analysis

Psychophysics

Using paired *t* testing, we compared the stimulation intensity and the average subjective NRS scores for muscle (weak and strong) and bone (weak and strong), respectively. Effects of order of stimulus application on NRS were evaluated with two-way analysis of variance (ANOVA); $P < 0.05$ was regarded as significant. Values are given as mean ± SD.

fMRI data analysis

The fMRI data were analyzed with Statistical Parametric Mapping software (SPM99; Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 6.1 (Mathworks, Sherborn, MA, USA). The functional images were realigned to correct for head movements, coregistered with each subject's anatomic MRI, and transformed to the format of the standard brain according to Talairach coordinates [13]. The functional images were spatially smoothed with an 8-mm full-width half-maximum Gaussian kernel. We fitted a linear regression model (fixed effects within subjects). Each condition was modeled with a boxcar function and convolved with a hemodynamic response function. Temporally, the voxel time series were high-pass filtered (124-s cutoff periods) to remove slow trends in the data, and low-pass filtered with a hemodynamic response filter.

We compared image data for each group—weak muscle stimulation, strong muscle stimulation; weak bone stimulation, strong bone stimulation—against the data from each resting condition, respectively. All the data were pooled for group statistical comparisons. Across the subjects, random effect analysis was performed to determine the significant activation associated with different sites and intensities of stimulation ($P < 0.001$ uncorrected; minimum cluster size, 20 voxels). To investigate the brain network related to painful stimulation intensity, we calculated the difference in the areas activated by strong stimulation minus weak stimulation (paired *t* test; $P < 0.005$ uncorrected; minimum cluster size, 20 voxels). To identify the brain areas that are differently activated by muscle and bone stimulation, we also analyzed the data separately (strong muscle minus strong bone; weak muscle minus weak bone; and all

together) (paired t test; $P < 0.005$ uncorrected; minimum cluster size, 20 voxels).

Results

Stimulation and pain intensity

The mean intensity of muscle stimulation was 21.1 ± 8.4 N for weak stimulation and 40.7 ± 7.8 N for strong stimulation; the mean intensity of bone stimulation was 14.2 ± 5.7 N for weak stimulation and 30.1 ± 7.9 N for strong stimulation. The NRS scores with strong muscle and strong bone stimulation were significantly higher than those with weak muscle and weak bone stimulation, respectively ($P < 0.05$ for both) (Table 1). All the volunteers clearly distinguished the two intensities of stimulation because the weaker stimulation was always scored lower. Although the volunteers found weak muscle stimulation trials more painful ($P < 0.05$) than weak bone stimulation, no similar difference was found for strong stimulations. Comparing the data among the three trials of the same intensity at the same site, no differences in NRS scores were found; this indicates that repeated mechanical stimulation did not sensitize or desensitize the volunteers ($P > 0.05$).

fMRI

In response to painful muscle stimulation, brain activation was apparent within the bilateral anterior cingulate cortex (ACC), insula cortex, the secondary somatosensory cortex (S2), the inferior parietal lobule (IPL), the posterior cingulate cortex (PCC), putamen, the ipsilateral dorsolateral prefrontal cortex (DLPFC), thalamus, caudate, and the contralateral claustrum (Fig. 3a,b; Table 2). In response to painful bone stimulation, brain activation was also apparent within the bilateral ACC, the IPL, the S2, the PCC, the ipsilateral DLPFC, and the contralateral claustrum (Fig. 4a,b; Table 2). Peak coordinates (x, y, z) in Montreal Neurological Institute (MNI) space and Z-scores (>6.00) for the activated brain regions by muscle and bone pain are shown in Table 2. Differences in the areas activated by strong versus weak muscle stimulation were mainly found in the contralateral S2 and the bilateral thalamus (Fig. 3c). For strong versus weak bone stimulation, the difference was found in the contralateral S2 (Fig. 4c). With weak stimulations, the activation differences between muscle and bone stimulation were apparent in the bilateral caudate nucleus and contralateral Brodmann areas 22 and 45 (Fig. 5a). With strong stimulations, the activation differences were apparent in the bilateral putamen, the ipsilateral ACC, and the contralateral claustrum (Fig. 5b). Analyzing the sum of the differences between muscle- and bone-related pain revealed that activation was different in the bilateral caudate and contralateral putamen (Fig. 5c; Table 3).

Discussion

In this study, as an initial step toward elucidating the brain activation associated with mechanically induced deep tissue pain, we found that the contralateral S2 was more activated by stronger stimulations to muscle or bone. We also found that parts of the basal ganglia (putamen and caudate nucleus) were more activated by muscle stimulation than by bone stimulation.

Psychophysics

The NRS scores measured during fMRI were higher than those determined in the preliminary testing. Although the reason is not clear, the difference of the rate of pressure increase between preliminary testing and the fMRI session may be a cause.

On the other hand, the NRS scores for the two sets of three stimulations at each site did not change during the protocol (see Table 1), suggesting that no further change in sensitivity occurred. Nie et al. [14] have reported that after

Table 1 Numerical rating scale (0–10 NRS) by mechanical stimulation

	1st stimulation	2nd stimulation	3rd stimulation	mean
weak muscle stimulation	4.83±1.99	4.67±1.88	4.5±1.98	4.67±1.90
strong muscle stimulation	8.17±1.34	8.5±1.17	8.25±1.42	8.31±1.28
weak bone stimulation	4.25 ±2.14	3.25 ±1.14	3.92 ±1.78	3.81±1.74
strong bone stimulation	8.83 ±1.03	8.25 ±1.36	8.17 ±1.70	8.42 ±1.38

NRS with strong muscle and strong bone stimulation were significantly higher than those with weak muscle and weak bone stimulation (*$P < 0.05$). In weak stimulations, NRS with muscle stimulation was higher than with bone stimulation (**$P < 0.05$). There were no differences between strong muscle and bone stimulations among NRS with 1st, 2nd, and 3rd stimulation in the same study groups

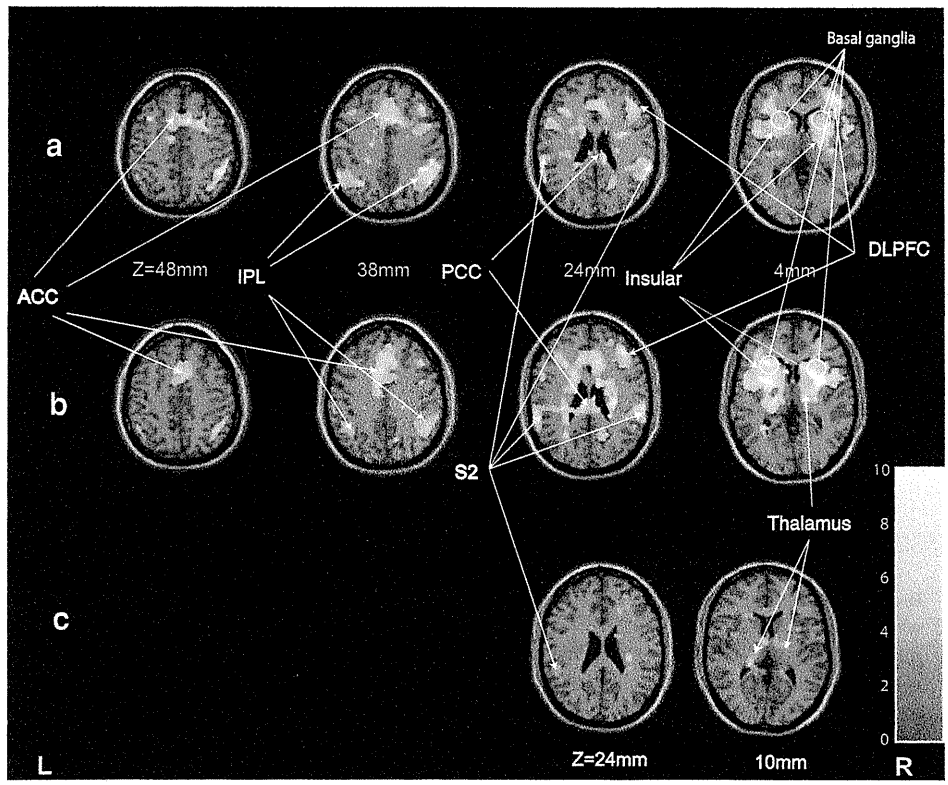

Fig. 3 Brain activation induced by muscle stimulation: weak stimulation (**a**), strong stimulation (**b**), and contrast of strong and weak stimulation (**c**). In millimeter elevations relative to a line through the anterior–posterior commissure (AC–PC line), brain slices are shown in superior–inferior sequence. The axial slices are arranged from dorsal (*left*) to ventral (*right*). Statistical map thresholds are $P < 0.001$ (**a**, **b**, uncorrected), and $P < 0.005$ (**c**, uncorrected, paired test). Minimum cluster size is 20 voxels. Right (*R*) and left (*L*) sides are indicated. *ACC* anterior cingulate cortex; *IPL* inferior parietal lobule; *S2* secondary sensory cortex; *DLPFC* dorsolateral prefrontal cortex; *PCC* posterior cingulate cortex

ten stimulations with 30-s intervals between stimulations, subjects gave higher VAS scores, increasing to 192% ± 71% for stimulation of the tibia and to 117% ± 42% for stimulation of the tibia anterior muscle. In the present study, the protocol specified a 60-s interval during all the sessions. This interval was apparently long enough to prevent temporal sensitization. To evoke equivalent pain in muscle and bone, greater stimulation had to be applied largely to the muscle; this may be because nociceptive nerve density is greater in the periosteum than in muscle [15].

Imaging

A previous positron emission tomography (PET) study and event-related fMRI study using noxious electronic stimulation of muscle showed activation in the ACC, S2, and anterior insula [8, 10]. Also, PET and fMRI study using injection of hypertonic saline into muscle evoked the activation in contralateral insula and putamen [9, 16]. In our study, the results for mechanical stimulation are consistent with the previous muscle pain studies, which have shown that the different types of stimulation (hypertonic saline, electrical stimulation, and mechanical stimulation) applied to muscle induce equivalent activation patterns [8, 10, 16]. We found that stronger muscle stimulation resulted in greater activation in the contralateral S2, the bilateral thalamus (contralateral > ipsilateral), and that stronger bone stimulation caused greater activation in the contralateral S2. In each case, the contralateral S2 was more activated by strong stimulation. However, caution is needed in interpreting these results because greater application of the force to skin is also involved during strong stimulation. The activation of the contralateral S2 may also come from greater cutaneous stimulation. In a PET study investigating thermal stimulation to skin, Coghill et al. [17] have shown that the ipsilateral cerebellum, the contralateral S1, the supplementary motor area, the bilateral S2, the lentiform nucleus, the insular cortex, and the thalamus and ACC are more activated depending on perceived pain intensity. In an fMRI study, using mechanical phasic stimulation on the skin, Ringler et al. [18] have shown that the contralateral S2 is more activated by stronger stimulation. The contralateral S2 may take part in processing pain intensity derived from both skin and deep tissues.

Comparison of the activation associated with muscle and bone stimulation revealed that caudate nucleus and putamen were more activated by muscle than by bone stimulation. No activation existed that was evoked by (bone > muscle) stimulation (data not shown). The activation in these areas is supposed to be related to stimulated sites (muscle or bone), not to stimulation or pain intensity, because these areas were not included as a contrast when we stimulated the single target (muscle or bone) with the

Table 2 Peak coordinates (*x*, *y*, *z*) in Montreal Neurological Institute (MNI) space and Z-scores (>6.00) for the activated brain regions by muscle and bone pain

Region	BA	Muscle pain				BA	Bone pain			
		Peak voxel coordinate			Z-score		Peak voxel coordinate			Z-score
		x	*y*	*z*			*x*	*y*	*z*	
ACC	32	−8	12	44	7.07					
	32	−6	18	32	6.42					
	32	−12	26	30	6.42					
Insula right	13	28	26	2	6.33					
		34	4	8	6.09					
Insula left		−34	6	8	6.25	44	−48	10	6	6.27
	47	−28	24	−8	6.05					
S2 right	40	62	−32	30	6.32					
S2 left	40	−60	−32	28	6.39					
IPL left						40	−48	−52	40	6.57
	40	−48	−58	42	6.33	40	−48	−40	28	6.48
	40	−44	−48	42	6.13	40	−62	−36	32	6.27
Thalamus right		20	−18	10	6.04					
Putamen right		26	2	6	6.16					
		20	−4	8	6.10					
Putamen left		−32	6	6	6.54					
Claustrum left		−26	12	10	6.39		−30	10	6	6.18
Caudate right		16	12	8	6.13					

Brodmann areas are given where available

BA Broadmann area, *ACC* anterior cingulate cortex, *S2* secondary sensory cortex, *IPL* inferior parietal lobule

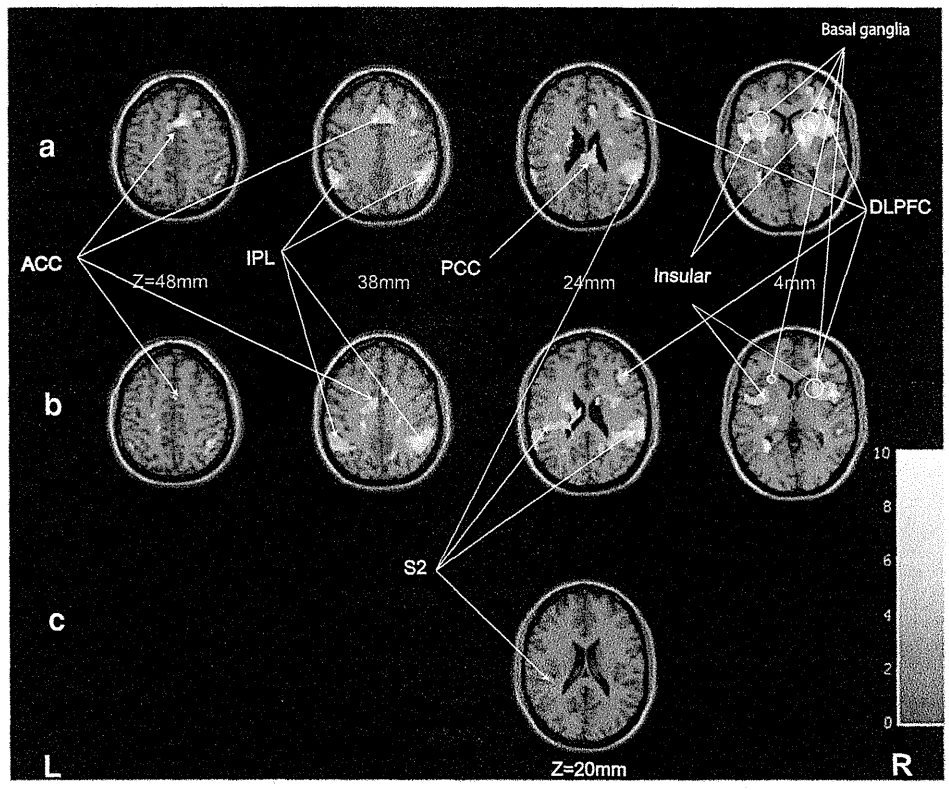

Fig. 4 Brain activation induced by bone stimulation: weak stimulation (**a**), strong stimulation (**b**), and contrast of strong and weak stimulation (**c**). In millimeter elevations relative to a line through the anterior–posterior commissure (AC–PC line), brain slices are shown in superior–inferior sequence. The axial slices are arranged from dorsal (*left*) to ventral (*right*). Statistical map thresholds are $P < 0.001$ (**a**, **b**, uncorrected), and $P < 0.005$ (**c**, uncorrected, paired test). Minimum cluster size is 20 voxels. Right (*R*) and left (*L*) sides are indicated. *ACC* anterior cingulate cortex; *IPL* inferior parietal lobule; *S2* secondary sensory cortex; *DLPFC* dorsolateral prefrontal cortex; *PCC* posterior cingulate cortex

different intensities. Additionally, pain intensities were identical between strong muscle and strong bone pain. Reports of the previous pain imaging studies have suggested that the putamen is activated by nociceptive stimulation [17, 19], including muscle pain with hypertonic saline [16]. Basal ganglia are reported to have a role in

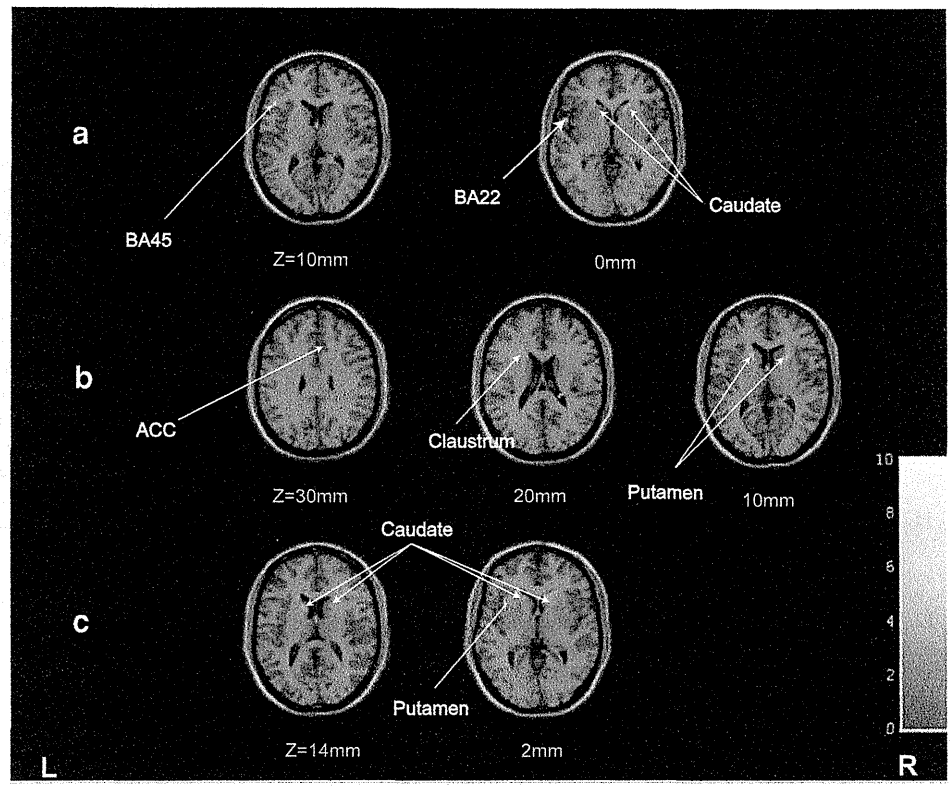

Fig. 5 Contrast of brain activation induced by muscle and bone stimulation: contrast of weak stimulation to muscle and bone (**a**), contrast of strong stimulation to muscle and bone (**b**), and contrast of all the stimulations to muscle and bone (**c**). In millimeter elevations relative to a line through the anterior–posterior commissure (AC–PC line), brain slices are shown in superior–inferior sequence. The axial slices are arranged from dorsal (*left*) to ventral (*right*). Statistical map thresholds are $P < 0.005$ (uncorrected, paired test). Minimum cluster size is 20 voxels. Right (*R*) and left (*L*) sides are indicated. *BA* Brodmann area; *ACC* anterior cingulate cortex

Table 3 Peak coordinates (*x*, *y*, *z*) in Montreal Neurological Institute (MNI) space and Z-scores for activated brain regions by muscle–bone pain

Region	(Muscle–bone) pain			
	Peak voxel coordinate			Z-score
	x	*y*	*z*	
Putamen left	−24	12	24	4.12
Caudate left	−18	16	8	4.01
Caudate right	16	22	0	3.95

motor preparation, movement control, and emotional, motivational, and cognitive function [20–22]. Processing in the putamen and caudate nucleus is also reported to be related to pain-avoidance behavior [23]. Our results suggest that the putamen and caudate nucleus may have a more significant role in the brain processing of muscle pain compared with bone pain.

Conclusion

In conclusion, the putamen and caudate nucleus may have a more significant role in the brain processing of muscle pain compared to bone pain.

Acknowledgment We thank Syoji Kobashi and Tamotsu Nomura for helping in imaging procedures, Tateo Iwakura for help with image analysis, and Sho Karl Shibata and David Eunice for editing. Scans were performed at Ishikawa Hospital. This work was supported by a Grant-in-aid for community health and medical care from the Ichou Association for Promotion of Medical Science.

Conflict of interest None.

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