Characterization of Neural Plasticity in Monkey Models of Neuropathic Pain – A Secondary Publication

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Abstract: Neuropathic pain can occur as a result of injuries and diseases of the nervous system. Animal models using rodents have been developed and characterized to reveal plastic changes underlying neuropathic pain. However, structures and functions of some brain areas that are associated with pain perception differ between rodents and primates. Therefore, animal models using non-human primates, such as the macaque monkey, with brain structures and functions closer to those of humans are important for elucidating the mechanisms underlying pain in human patients. Recently, we measured brain activity using functional magnetic resonance imaging (fMRI) in a macaque model of chemotherapy-induced neuropathic pain and reported abnormal activation of pain-related brain regions including insular and secondary somatosensory cortices. In the monkey model of central post-stroke pain, moreover, the increased activation of pain-related areas as seen in the patients was confirmed by fMRI. These results indicate that fMRI measurement of brain activity combined with behavioral outcomes in macaque models could be used not only to understand the pathogenetic mechanisms but also to test therapeutic interventions for neuropathic pain.

Keywords: Thalamic pain; Peripheral neuropathy; Chronic pain; Animal model; Allodynia

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1. Introduction

The International Association for the Study of Pain defines pain as an unpleasant sensation or emotional experience that accompanies or is described in terms of substantial or potential tissue damage. Pain is further divided into nociceptive pain, which serves as a defense signal for the body, and neuropathic pain, which is caused by damage to the somatosensory system of peripheral or central nerves. The former is an important biological response for self-defense and survival, while the latter is an abnormal condition in which abnormal pain is perceived and strong unpleasant emotions are perceived that are not related to physiological responses. Neuropathic pain is characterized by rest pain, allodynia, or hyperalgesia, in which the patient feels pain from tactile stimuli that are not normally painful, and hyperalgesia, in which the pain increases. In the clinical
setting of physical therapy, there is no specific approach to neuropathic pain, and even mild stimulation is often
accompanied by severe pain, which may impede rehabilitation intervention and significantly limit the patient’s
return to society. For these reasons, neuropathic pain is an important research topic not only in modern clinical
medicine and basic medicine but also in the field of rehabilitation.

In recent years, it has become clear that neuropathic pain is caused by abnormal plasticity of the central
nervous system, which is formed by damage to the nervous system responsible for somatosensory perception \[1\].
To clarify this reality, anatomical and functional investigations of various elements of the nervous system have
been conducted on pain patients and animal models that reproduce their pathological conditions. On the other
hand, rodents such as mice and rats, which are widely used for pain research, have a clear genetic background,
can be adapted to genetic modification techniques, and allow for robust population control, but their genetic
phylogenetic differences from humans are becoming increasingly apparent as a limitation. Therefore, we are
studying pain in macaque monkeys, a species that is relatively closely related to humans. As will be described
later, the creation of animal models for macaques can reproduce pathological conditions similar to human
clinical symptoms that cannot be reproduced in rodents. In this paper, we first discuss the reasons for using
monkeys in pain research, and then outline the results obtained from our monkey models of pain after peripheral
nerve injury and central nerve injury.

2. Necessity of using monkeys in neuropathic pain research

The Japanese macaque and rhesus macaque monkeys belonging to the genus *Cercopithecus* are widely used
for brain research because their genetic background and brain structure are known to be the second closest
to humans after apes such as gorillas and chimpanzees. In the somatosensory cortex and thalamus, which are
believed to be involved in pain perception, the anatomical structure and connections between these regions
are found to be more homologous to those of humans than to rodents \[2,3\]. The fact that the brain structures
of monkeys and humans are similar suggests that they share common functions in cognition and perception.
Therefore, if we can elucidate the phenomena occurring in the monkey brain using neuroscientific methods, we
may be able to use them as clues to explain the pathological mechanisms occurring in the human brain.

It is thought that pain is not caused by a single brain region but by a complex of neural circuits related
to perception and emotion, and that modulation of these circuits results in the onset and maintenance of
pathological pain \[1,4\]. The relatively large brain of monkeys is a suitable target for brain imaging techniques
such as functional magnetic resonance imaging (fMRI). In other words, it is possible to explore changes in brain
activity caused by circuit modulation at the whole-brain level. Furthermore, the abnormal brain activity induced
by circuit modulation can be used as a physiological index to develop new rehabilitation intervention methods
or to conduct basic research for clinical applications, such as neuromodulation using electrical brain stimulation
or brain-machine interface. For these reasons, our laboratory is conducting research on the mechanisms of
neuropathic pain in macaque monkeys, intending to develop therapeutic methods.

3. Evaluation of brain activity in a monkey model of peripheral nerve injury

We were the first in the world to successfully visualize brain activity changes in monkeys associated with
chemotherapy-induced neuropathic pain \[5\]. The study is described below.

Peripheral neuropathy induced by chemotherapy is one of the side effects frequently encountered in
clinical practice. Oxaliplatin, which is prescribed for advanced colorectal cancer, causes peripheral neuropathy
targeting the dorsal root ganglia of the spinal cord, resulting in neuropathic pain, or cold hypersensitivity, in
which pain is perceived in response to cold stimuli that are not painful under normal conditions. Today, there are no approved analgesics for this pain, and no effective treatment has been established \cite{6}. To solve this problem, Shidahara et al. established an oxaliplatin-induced peripheral nerve injury model of cold hypersensitivity in macaque monkeys and examined the effects of various analgesics \cite{7}. If we can identify the brain activity that contributes to this pathology, we can not only clarify the neural mechanism underlying the perception of cold hypersensitivity but also establish an objective pain assessment method using brain activity as an indicator. In this study, we aimed to identify brain activity contributing to oxaliplatin-induced cold hypersensitivity using fMRI.

To reproduce this condition in monkeys, we administered oxaliplatin intravenously and evaluated the appearance of cold hypersensitivity by performing a tail immersion test (Figure 1A). In this behavioral test, the monkeys were immersed in cold water (10°C) and control water (37°C) in an experimental environment that allowed them to freely avoid pain on their own, and the time it took them to escape was evaluated. On the day after oxaliplatin administration, the animals showed a significantly faster escape response to cold water than before, and this trend was sustained for 3 days (Figure 1B). On the other hand, there was no change in escape response after immersion in 37°C water. These results suggest that oxaliplatin treatment transiently induces a specific hypersensitivity to cold stimuli in animals. Next, we used fMRI to evaluate the brain activity involved in cold hypersensitivity and immobilized the animals by continuous administration of propofol, which has a very weak analgesic effect \cite{8,9}. We statistically calculated the activation areas related to pain by comparing the activation areas before and after oxaliplatin administration.

![Figure 1. Illustration of tail immersion test (A) and effect of oxaliplatin treatment on tail withdrawal latency over time (B). The withdrawal latency to cold water was significantly decreased compared with pre-infusion withdrawal latency. Values are expressed as mean ± SEM. *** \(P < 0.001\), compared with pre-infusion (one-way repeated measures analysis of variance followed by Dunnett’s post hoc test). Modified from Nagasaka et al. \cite{5}.

The results of this analysis showed that oxaliplatin treatment significantly increased activity in the bilateral insular cortex and adjacent secondary somatosensory cortex (Figure 2). The insular cortex and secondary somatosensory cortex have long been known as areas associated with pain perception \cite{10}, suggesting that they contribute to the mechanism of abnormal pain perception in this condition.
Figure 2. Brain activity changes associated with oxaliplatin-induced hypersensitivity. Increased activation in the secondary somatosensory cortex (SII) and insular cortex (Ins) in both hemispheres, compared with intact (pre-infusion), were associated with cold stimulation (i.e., activity in response to 10°C cold stimulation minus activity in response to 37°C control stimulation) at 3 days after oxaliplatin infusion ($P < 0.01$, uncorrected). Modified from Nagasaka et al. [5].

These results alone indicate that increased brain activity was observed in anesthetized animal models, and it is not clear whether there is a causal relationship between brain activity and pain perception. Therefore, we administered a small amount of muscimol, an agonist of gamma-aminobutyric acid (GABA) receptors, to the bilateral insular cortex of oxaliplatin-treated animals and evaluated the effects of transient inhibition of these regions by the tail immersion test during wakefulness. Muscimol significantly attenuated escape behavior to cold stimuli compared to the same conditions after administration of the vehicle’s solution. The abnormal activation in the insular cortex was causally related to pain perception in cold hypersensitivity.

This study is the first to measure brain activity in a monkey model of peripheral nerve injury and the first to report those pain-related brain regions common to humans show increased activity causally related to pain perception [5]. These results not only lead to the elucidation of the mechanism, but also demonstrate the usefulness of fMRI as a pain assessment method in monkeys, and establish a fundamental research technique. The evaluation of pain in animals using this method has already been reproducible in many studies, and the technique has been used for the evaluation of various analgesic drugs and in other pain disease models [11-13].

4. Establishment of a monkey model of post-stroke pain and changes in brain activity

Post-stroke pain is intractable chronic pain that occurs several weeks after a cerebrovascular accident [14] and is present in approximately 10% of stroke patients [15]. The number of patients with post-stroke pain is expected to increase in developed countries with super-aging societies. However, because the mechanism of post-stroke pain is not fully understood, no curative treatment has been established, causing great suffering to patients.

It has been shown that post-stroke pain is triggered by strokes in the posterior lateral ventral nucleus of the thalamus, the prethalamic nucleus, the thalamocortical interstriatal area, secondary somatosensory cortex, insular cortex, lateral medulla oblongata, and the bridge [15]. These brain regions correspond to the pathways from the lateral spinal thalamic tract, which is a neural projection pathway from peripheral nociceptors, to their cortex, suggesting that damage to the pathways that transmit pain information and subsequent plasticity may be
causing abnormal pain. The posterior lateral ventral part of the thalamus is relatively often caused by stroke \cite{16}, and rodent models of post-stroke pain have been established by artificially destroying this region \cite{17,18}.

To clarify the plasticity involved in post-stroke pain, we started by establishing a monkey model and succeeded \cite{19}. Macaque monkeys differ from rodents in the size and shape of their brains due to the lack of a unified experimental animal lineage. Therefore, it is difficult to induce localized damage to the target area by injecting drugs to the coordinates of the posterior lateral ventral nucleus of the thalamus using a brain atlas, as is conventionally done in rodent models. To solve this problem, we performed MRI imaging for each individual to obtain brain structure images. We then identified the posterior lateral ventral nucleus of the thalamus, a somatosensory relay nucleus, by inserting electrodes at the coordinates of the posterior lateral ventral nucleus estimated by MRI and using electrophysiological techniques to search for neurons that fire in response to sensory stimulation of the fingers. After identification, a small amount of collagenase type IV, a vascular wall degrading enzyme, was injected into the area to induce hemorrhage. MRI images taken over time were used to evaluate the site and magnitude of hemorrhage damage (Figure 3A and B). Hematomas and edema increased transiently from 3 days to 1 week after administration of collagenase type IV, but decreased after 2 weeks, indicating that they were localized in the posterolateral ventral part of the thalamus (Figure 3C and D). To evaluate the presence or absence of pain symptoms before and after hemorrhage injury to the posterolateral ventral nucleus, mechanical stimulation using a von Frey filament was applied and the escape threshold was measured. After 8 weeks following the hemorrhage, the threshold of the hand contralateral to the injury was significantly reduced compared with that of the hand before the injury and the hand ipsilateral to the injured hemisphere (Figure 4A). Similarly, even when thermal stimuli (50°C and 37°C) were applied to the hand to which no escape behavior was observed before the hemorrhage, escape behavior was significantly observed only for the 50°C stimulus after 4 weeks after the hemorrhage (Figure 4B). The behavioral changes exhibited by macaque monkeys after injury may be the result of hyperalgesia and hyperalgesia specific to post-stroke pain patients after the dynamics of edema and hematoma have stabilized. The most significant feature of the monkey model is the appearance of pain symptoms after 4 weeks post-injury, which contrasts with the results of many conventional rat and mouse models of thalamic hemorrhage, in which pain responses were observed within a week post-injury \cite{20}. Since the onset of pain often occurs 1 to 2 months after stroke in patients \cite{14,15}, we believe that this monkey model is a model animal that more closely reproduces human symptoms.

We have attempted to clarify changes in brain activity using fMRI in a post-stroke pain model as well as in a monkey model treated with oxaliplatin \cite{21}. When mechanical stimulation was applied to the fingers with a brush during fMRI imaging, significant activation was observed in the posterior insular cortex and anterior cingulate cortex of the injured hemisphere only when the fingers on the contralateral side of the injury were stimulated. Equivalent activation was not observed before injury or during stimulation of the finger contralateral to the injury when no behavioral changes were observed. Similar activation was observed in patients with post-stroke pain \cite{22-24}. We believe that the identification of cellular changes that underlie activation in the monkey model will help elucidate the mechanisms underlying the appearance of abnormal pain in the future.

In addition, the same experimental system may contribute to the establishment of treatment techniques for post-stroke pain. For example, repetitive transcranial magnetic stimulation (rTMS) with high frequency to the motor cortex of post-stroke pain patients has been shown to temporarily relieve pain \cite{15,25}, but the background mechanism is still unclear. In the future, if rTMS can be applied to a monkey model to identify optimal stimulation conditions and identify changes in brain activity that contribute to the pain relief mechanism, it may contribute to the development of intervention techniques for patients with intractable pain.
Figure 3. Time course of stroke after collagenase injection. (A) Coronal T2-weighted MRIs of a monkey showing the time course of stroke (yellow arrows) after the injection. (B) A higher magnification image shows the hypointense stroke cord (red arrow) and the surrounding hyperintense rim (blue arrow). (C, D) Temporal volume changes of the hypointense (C) and hyperintense areas (D). Modified from Nagasaka et al. [19].

Figure 4. Behavioral changes after collagenase injection. (A, B) Weekly changes in the withdrawal response to mechanical (A) and thermal stimulation (B). The withdrawal thresholds, shown as the pressure exerted (in grams) and the latencies, represented as the ratio of the withdrawal latency for thermal stimulation (50°C) to that for control stimulation (37°C), are shown for both hands, one contralateral (contra-lesional hand) and the other ipsilateral (ipsi-lesional hand) to the injected hemisphere. ** P < 0.01 and **** P < 0.0001, compared with pre-injection (Kruskal-Wallis one-way ANOVA followed by Dunn’s post hoc test). Modified by Nagasaka et al. [19].

5. Conclusion

In this paper, we have presented results obtained in monkey models of pathological pain after peripheral nerve injury and stroke. We focused on the insular cortex as an abnormally activated region commonly associated with pain in both monkey models and found that the stimuli presented during fMRI measurements were thermal
stimuli in the peripheral nerve injury model, whereas they were mechanical stimuli in the post-stroke pain model, and that the latency to onset of pain symptoms and the duration of pain were distinctly different between the two models. Because there are clear differences in the latency and duration of onset of pain symptoms between the two models, it may be difficult to integrate the results obtained from the two models. However, it is highly likely that the insular cortex, which has tight anatomical connections with areas of the somatosensory cortex involved in perception and with limbic systems such as the frontal association cortex and amygdala involved in emotion, functions as a hub of pain-related neural communication and induces pathophysiological plasticity.

Rodents are well suited for detailed investigations of mechanisms at the molecular and cellular levels using techniques such as genetic modification, while monkeys are suitable for brain activity measurements that are also used in humans. The complementary role of the monkey model in translating the results obtained in the rodent model to the patient will greatly contribute to the elucidation of the mechanisms of pathological pain and the development of treatment methods.

**Disclosure statement**

The authors declare no conflict of interest.

**References**


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