

Longitudinal FDG microPET imaging of neuropathic pain: does cerebellar activity correlate with neuropathic pain development in a rat model?

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Abstract

Background We used [F-18] FDG microPET imaging as part of a longitudinal study to investigate changes in the brain.

Methods Glucose metabolism during the development of neuropathic pain after tibial and sural nerve transection (TST) model rats. MicroPET images were obtained 1 week before operation and then weekly for 8 weeks post-operation.

Results The behavioral test was performed immediately after the every FDG administration. After TST modeling, neuropathic pain rats showed increased mechanical sensitivity of the injured hind paw. The withdrawal response to mechanical pain stimulation by von Frey filaments was observed within the first week (3.8 ± 0.73), and it rapidly increased in the third week (7.13 ± 0.82). This response reached a peak in the fourth week after surgery (9.0 ± 0.53), which persisted until the eighth week. In microPET scan imaging, cerebellum, which initially started from the ansiform lobule, was activated gradually to all part from the third week in all image acquisitions through the eighth week.

Conclusions The longitudinal microPET scan study of brains from neuropathic pain rat models showed sequential cerebellar activity that was in accordance with results from behavioral

test responses, thus supporting a role for the cerebellum in the development of neuropathic pain.

Keywords Neuropathic pain · microPET · FDG · Cerebellum · Neuromodulation

Introduction

Neuropathic pain is a neurodegenerative disease caused by lesions or dysfunction of the central or peripheral nervous system, and it is one of the most difficult pains to control because it is a multidimensional clinical entity mediated by many different pathophysiological mechanisms [2, 8, 15, 48].

Recently, there have been a number of studies evaluating the processing of pain in the brain by positron emission tomography (PET) or functional magnetic resonance imaging (fMRI), and these studies have shown changes in brain activity indicating the network of brain areas involved in forming a pain matrix [3, 27, 30, 31, 33, 41, 47]. The primary and secondary somatosensory cortices, insular cortex, anterior cingulate cortex, thalamus, and prefrontal cortex were the sites that were consistently activated in response to pain [1, 10, 32, 46]. In addition to these lateral and medial pain systems, the brainstem, amygdale, basal ganglia, and cerebellum are also related to the processing of nociceptive information and pain perception, but the details of their roles have not been clearly demonstrated yet [6]. Further, imaging studies for neuropathic pain have been reported using a resting state as well as stimulus-induced state, but these studies also only reflect the static states of the brain activities related to neuropathic pain. Although there have been few imaging studies based on the longitudinal acquisition of data on nociceptive pain processing, imaging studies during the development of neuropathic pain are lacking [35, 42]. Therefore, we performed a

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longitudinal study with [F-18] FDG microPET imaging to investigate changes in brain glucose metabolism during development of neuropathic pain.

Materials and methods

Animals and surgical procedures

A total of 15 male Sprague–Dawley rats (6 weeks old, weighing approximately 180–200 g) were used in this experiment. All 15 rats were operated on to induce neuropathic pain, but only 13 were confirmed to have neuropathic pain based on behavioral test results. Therefore, 13 rats were included for PET scans.

The rats were housed in groups of five per cage with food and water available ad libitum under a 12-h light/dark cycle. To induce peripheral neuropathic pain, we anesthetized the rats using pentobarbital sodium (50 mg/kg) and a segment of the left sciatic nerve was exposed. For the TST (tibial and sural transection) model, the left tibial and sural nerves were tightly ligated and completely transected, leaving the common peroneal nerve intact.

This study was conducted according to the guideline of the Ethical Committee of International Association for the Study of Pain [49] and the Institution Animal Care and Use Committee of Yonsei University. Every effort was made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques.

Mechanical hyperalgesia by von Frey test

To measure mechanical hyperalgesia, rats were placed on a metal mesh floor under a custom-made transparent plastic dome (8×8×18 cm) and innocuous mechanical stimuli were applied with a von Frey filament (8 mN bending force) ten times (once every 3–4 s) to the sensitive area of each hind paw. The frequency of foot withdrawal was expressed as a percentage that was used as the index of mechanical hyperalgesia.

[F-18] FDG microPET acquisition

[F-18] FDG was used to reveal brain glucose metabolism. One week before the induction of neuropathic pain, baseline [F-18] FDG PET scans were performed in all 13 rats as a control. Each rat was in a cage at 30 °C for 30 min before [F-18] FDG injection for adaptation. [F-18] FDG (0.5 mCi/100 g) was administered via the lateral vein of the tail. After administration, the rat was in the conscious state inside the cage for 60 min, and during that period, we performed a behavioral test to encourage glucose uptake. Following FDG uptake, the rat was initially anesthetized with 5 % isoflurane (O₂: 1.5 l/min, with vacuum). Then, we fixed the head to minimize

the motion artifacts. Under a maintenance dose of 2 % isoflurane (O₂: 1.0 l/min, with vacuum), the microPET scanning was performed for 60 min. While the scanning image was taken by using microPET, the body temperature was maintained with a heating pad using a warm sensor. Every week until the eighth week after the induction of neuropathic pain through operation, behavioral tests were performed and [F-18] FDG PET scans were done.

[F-18] FDG microPET data analysis

In order to improve the accuracy for anatomical locations, the MRI scan was co-registered on [F-18] FDG microPET scan. [F-18] FDG microPET images were reconstructed using the ordered subset expectation maximization (OSEM) algorithm with ten iterations. The nominal pixel size was 0.43×0.43×0.81 mm³. For statistical analysis, only the brain regions within the [F-18] FDG microPET images were manually extracted. To make the [F-18] FDG rat brain templates, baseline images ($n=12$) were coregistered to the respective images and resliced with trilinear interpolation (0.4×0.4×0.4 mm³) using SPM5 (<http://www.fil.ion.ucl.ac.uk/spm>). Individual images were averaged to create a [F-18] FDG rat brain template. Then, the template was normalized to the MRI template for accurate anatomical information, which was placed in stereotaxic space [40]. All individual images were normalized using the [F-18] FDG rat brain template. To increase the statistical power, all the individual normalized images were smoothed with an isotropic Gaussian kernel (2 mm FWHM). Voxel-based statistical analyses were carried out with statistical parametric mapping. Proportional scaling was used for global normalization. The statistical threshold was set at $p<0.05$ (family-wise error correction) with an extent threshold of 100 contiguous voxels. T-value maps of results were overlaid on transverse views of the MRI template to define voxels that showed significant change. For the analysis of correlations among the brain areas with significant metabolic changes, average glucose metabolism in each activated or deactivated brain region was calculated with all voxels within 1 mm at the Paxinos position of each region. Pearson's correlation coefficients among regions of interest were calculated ($p<0.01$, two-tailed).

The baseline images from before the induction of neuropathic pain operation and the images from each week after operation were compared.

Results

Behavioral test after TST model

After TST modeling, neuropathic pain rats showed increased mechanical sensitivity of the injured hind paw. The withdrawal

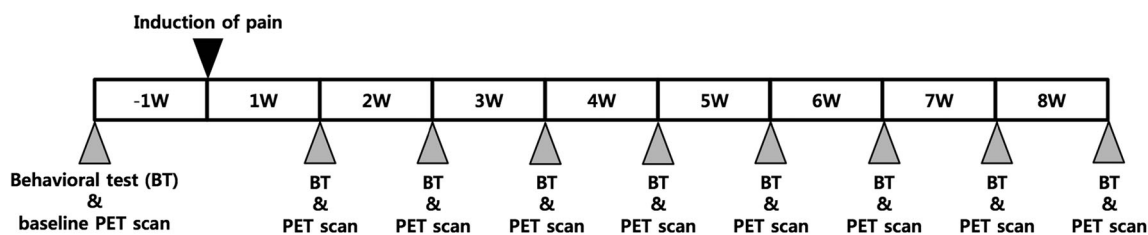


Fig. 1 Experimental design (uptake from stimulus-evoked activity) for the longitudinal study of neuropathic pain; behavioral tests and microPET scans were performed a total of nine times (1 week before induction of pain, and weeks 1 through 8 after induction of pain) on each subject

response to mechanical pain stimulation by von Frey filaments was observed within the before surgery to neuropathic pain (0.62 ± 0.14) in pain group, and it rapidly increased in the third week (5.24 ± 0.56). This response reached a peak in the seventh week after surgery (6.63 ± 0.64), and persisted until the eighth week. Conversely, in the normal group, the behavioral results from before surgery (0.61 ± 0.21), and it persisted until the eighth week (Figs. 1 and 2). The withdrawal responses confirmed the induction of neuropathic pain.

[F-18] FDG microPET scan

Primary motor cortex (AP: 0.4 mm; ML: 2.2 mm; DV: 2.0 mm) was revealed as an active area from the first to third week after operation, whereas the primary sensory cortex (AP: 1.0 mm; ML: -4.0 mm; DV: 2.0 mm) and whole cerebellum, which initially started from the ansiform lobule (AP: -13.6 mm; ML: 2.4 mm; DV: 3.2 mm), were activated in all the image acquisitions through the eighth week. The cerebellar uptake gradually increased in the third week, and after the fourth week, the whole cerebellum was activated. The inferior colliculus (AP: -9.0 mm; ML: ± 1.8 mm; DV: 4.0 mm) showed activation in the second week that was maintained into the eighth week. The ventral posteromedial thalamic nucleus (AP: -3.6 mm; ML: 2.6 mm; DV: 6.6 mm) was activated from the sixth to eighth week (Figs. 3a, c and 4).

Fig. 2 a Primary motor cortex was activated from the first to third week after operation, but primary sensory cortex and whole cerebellum, which initially started from the ansiform lobule, were activated in all the image acquisitions through the eighth week. The inferior colliculus showed activation in the second week that was maintained until the eighth week. The ventral posteromedial thalamic nucleus was activated from the sixth to the eighth week

The primary and secondary motor cortex of the contralateral side (AP: 4.2 mm; ML: ± 2.4 mm; DV: 2.0 mm) and substantia nigra (AP: -6.4 mm; ML: -2.0 mm; DV: 8.0 mm) were gradually deactivated from the first through the eighth week. The fornix (AP: -0.4 mm; ML: 0.6 mm; DV: 6.4 mm) and insular cortex (AP: 3.2 mm, ML: -3.8 mm, DV: 5.8 mm) began to deactivate from the third and fourth week, respectively. After the fifth week, the hippocampus (AP: -4.4 mm; ML: 0.6 mm; DV: 3.8) was deactivated, and after the sixth week, the nucleus accumbens (AP: 1.0 mm; ML: 0.8 mm; DV: 7.0 mm) and retrosplenial cortex (AP: -5.4 mm; ML: ± 0.4 mm; DV: 2.6 mm) were deactivated (Fig. 3b).

Discussion

In our microPET images acquired after stimulus, we observed longitudinal metabolic changes in the rat brain occurring in parallel with induced neuropathic pain for eight sequential weeks. Images from between the first and second week, compared with images of normal phase, showed that brain metabolic activity increased in the contralateral side of the primary motor cortex, primary sensory cortex, posterior thalamic nuclear group and crus II of the ansiform lobule of cerebellum. Cerebellar uptake is expected in the early phase because it is part of the pain matrix, including the thalamus, primary

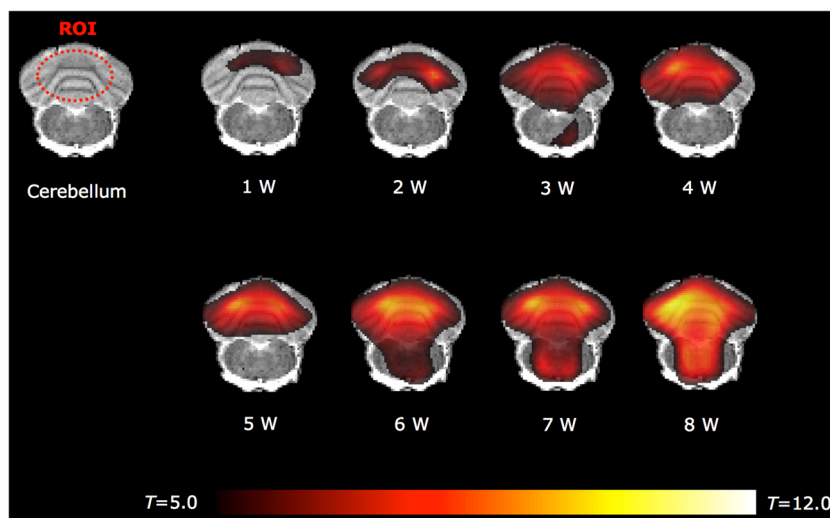
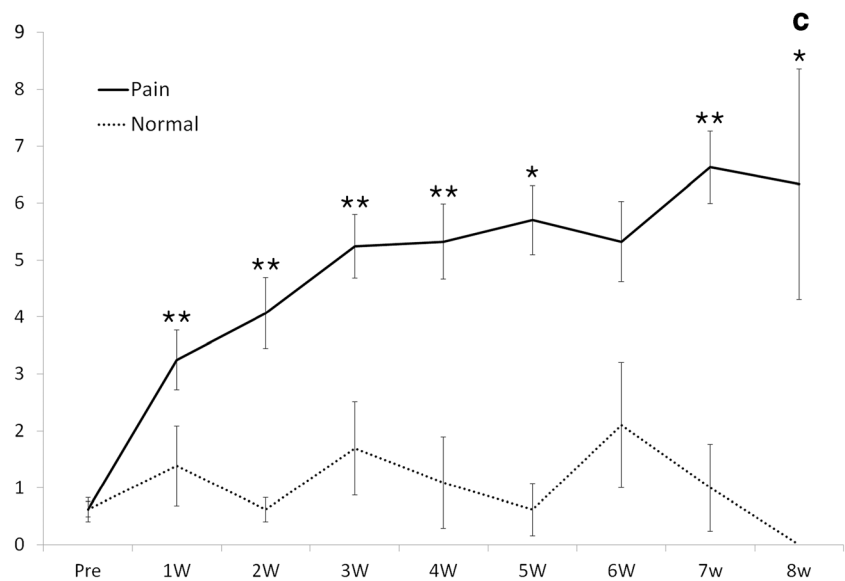
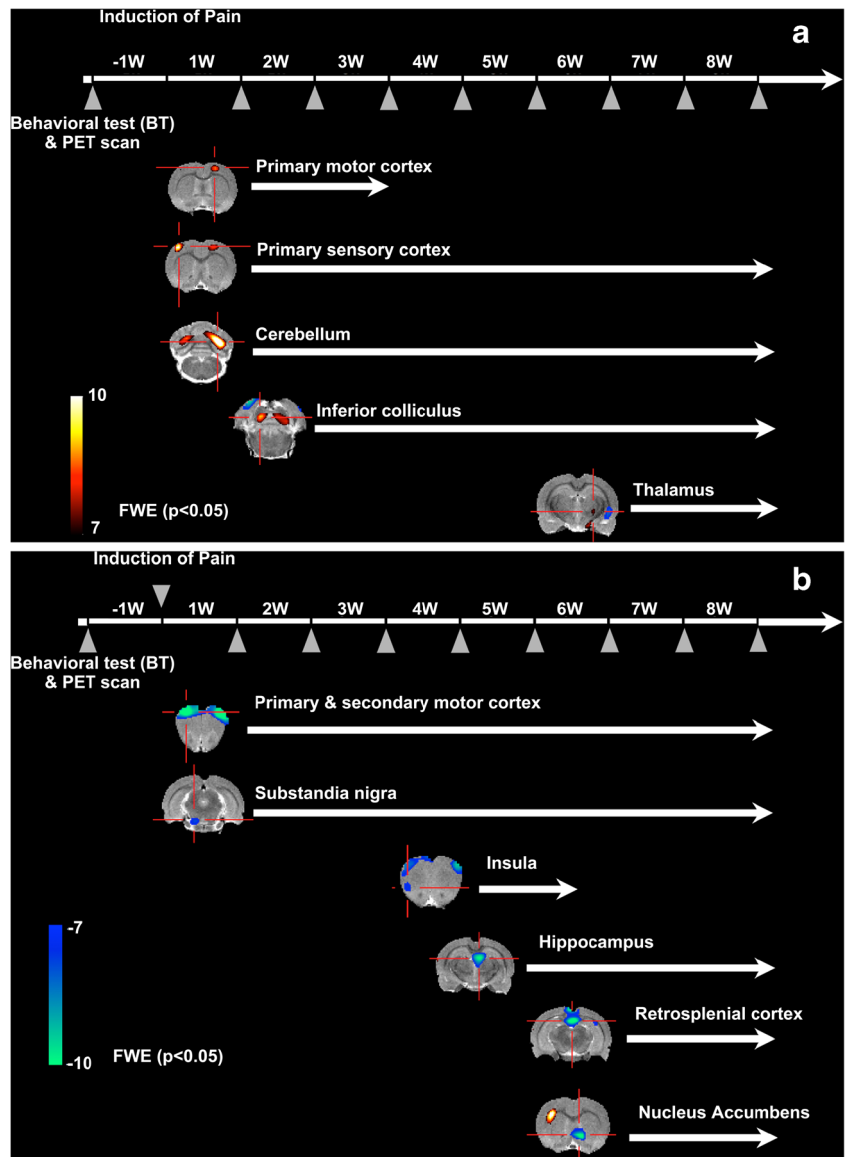


Fig. 3 **a** Primary motor cortex (AP: 0.4 mm; ML: 2.2 mm; DV: 2.0 mm) was active from the first to third week after operation, but primary sensory cortex (AP: 1.0 mm; ML: -4.0 mm; DV: 2.0 mm) and whole cerebellum, which initially started from the ansiform lobule (AP: -13.6 mm; ML: 2.4 mm; DV: 3.2 mm), were activated in all the image acquisitions through the eighth week. The inferior colliculus (AP: -9.0 mm; ML: ±1.8 mm; DV: 4.0 mm) showed activation in the second week that was maintained until the eighth week. The ventral posteromedial thalamic nucleus (AP: -3.6 mm; ML: 2.6 mm; DV: 6.6 mm) was activated from the sixth week to the eighth week. **b** Primary and secondary motor cortex of the contralateral side (AP: 4.2 mm; ML: ±2.4 mm; DV: 2.0 mm) and substantia nigra (AP: -6.4 mm; ML: -2.0 mm; DV: 8.0 mm) were gradually deactivated from the first through eighth week. The fornix (AP: -0.4 mm; ML: 0.6 mm; DV: 6.4 mm) and insula cortex (AP: 3.2 mm, ML: -3.8 mm, DV: 5.8 mm) were deactivated in the third and fourth weeks, respectively. After the fifth week, the hippocampus (AP: -4.4 mm; ML: 0.6 mm; DV: 3.8) was deactivated, and after the sixth week, the nucleus accumbens (AP: 1.0 mm; ML: 0.8 mm; DV: 7.0 mm) and retrosplenial cortex (AP: -5.4 mm; ML: ±0.4 mm; DV: 2.6 mm) were deactivated. **c** Glucose metabolism changes in the cerebellum of the neuropathic pain rat model. The cerebellum showed significantly progressive activation in glucose metabolism compared with baseline ($p < 0.001$)



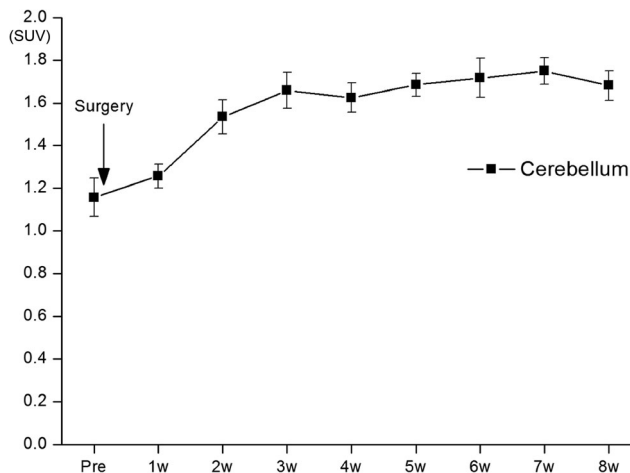


Fig. 4 Neural response changes in the cerebellum of the neuropathic pain rat model. The glucose metabolism (*SUV* standardized uptakes values) of the cerebellum increased until the third week and reached a steady state from fourth to eighth week

somatosensory cortex/primary motor cortex, secondary somatosensory cortex, midbrain, anterior insula, anterior cingulate cortex, and ventrolateral prefrontal cortex [10, 18, 32], and the uptakes in these sites, which are known as the lateral pain system, may be the result of nociceptive pain evoked by operation. From the third week, the central nucleus of the inferior colliculus and almost all of the cerebellum showed increased activity whereas the periaqueductal gray matter and primary and secondary motor cortex activity decreased. Interestingly, a good correlation was observed between the metabolic increase in the cerebellum on microPET and withdrawal rate of behavioral tests after peripheral nerve injury. The rate of the withdrawal response to mechanical allodynia of the ipsilateral hind paw slowly increased, and after the third week, the increment reached a maximal point that persisted for the length of the study (8 weeks). These findings suggest that changes in metabolic activity over time could reflect the development of surgery-induced neuroplastic adaptive modification, shifting to pain modulation of the cerebellum in response to nociceptive and neuropathic pain. From the sixth week, the nuclei of the brainstem also showed gradual uptake. The nuclei of the brainstem, such as the pons and the medulla oblongata, are known to participate in the descending modulation of nociception [25]. This finding indicates that the descending pain modulation process is also activated after neuropathic pain.

The spinothalamic system is known as the major route for transmitting nociceptive information to the cerebral cortex [9]. Still, the cortical targets of this system are unclear. A recent study showed that the spinothalamic system targets mainly the insula [13], and the appearance of deactivation in the insula on the fourth week of our stimulus-evoked study may be the result of the advent of neuropathic pain. Near completion of the

neuropathic pain model showed some changes from the sixth-week image studies. With regards to both affective and sensory aspects of altered pain perception, anatomical changes in the brain regions after chronic neuropathic pain have been reported [42]. The posterior cingulate cortex, which does not exist in rats, and retrosplenial cortex, showed important roles in pain processing in several studies, and the volume of the cortex decreased with chronic pain from other etiologies [4, 23]. Deactivation of the retrosplenial cortex after the sixth week in our study is in accordance with these results. The ascending nociceptive control pathway mediates pain-induced analgesia, and the nucleus accumbens is one of the structures that has specific mechanisms related to this pathway [37–39]. The role of the nucleus accumbens in the pain modulation pathway, such as antinociception or functional defensive behavior, has been studied [26, 44]. We observed deactivation of the nucleus accumbens and retrosplenial cortex in the sixth week after neuropathic pain induction. This deactivation is thought to be similar to the relative decrease in thalamic regional cerebral blood flow given the compensatory mechanism for inhibiting excessive nociceptive input after a behavioral test [17, 27].

The cerebellum is thought to be involved in a wide range of tasks, including sensorimotor control, language, spatial, and executive functions. Recent studies have consistently shown that the cerebellum may have a role in sensory processing including nociception [5, 28, 36] but its significance for pain processing largely remains unknown. From animal and human studies, it is known that peripheral afferent sensory inputs may activate the cerebellum. The input from stimulation of A-delta and C fiber nociceptors passes via climbing and mossy fibers to reach Purkinje cells in the cerebellum [14, 19]. Early cerebellar uptake in our images is due to the nociceptive signal from spontaneous pain induced by operation. With advances in technology, recent studies have revealed functional topography of the cerebellum as the presence of sensorimotor homunculi and association area projection in cerebellar lobules [16, 20, 43]. In the first and second weeks of the study, the crus II of the cerebellum was activated, which is in accordance with the sensory input pathway to the cerebellum, but after the third week, almost all cerebellar uptake was noted, which is in contrast to results from previous studies. It is well known that cerebellar activity from motor withdrawal and nociceptive sensory processing is indistinguishable by functional imaging [11, 24]. One of the different points of our study is that we acquired the images from the resting state, while previous studies obtained their results under noxious or non-noxious stimulation.

Romero et al. showed cerebellar activation in their microPET study of a rat model of surgery-induced latent pain sensitization, and it was nociceptive pain that reflected the slow development of surgery-induced neuroplastic adaptive modification [35]. However, our microPET study showed

activation of the cerebellum as time passed after nerve injury, and the uptake was correlated with the results of the behavioral test for neuropathic pain. The glucose metabolism of the cerebellum increased until 3 weeks and reached steady state from 4 to 8 weeks. These longitudinal metabolic changes without external stimulation could be the result of an adaptive or modulating process, especially in the cerebellum. Borsook et al. reported that painful external stimuli in chronic neuropathic pain patients activated cerebellar areas related to cognitive processing as well as sensory-motor integration and secondary sensory processing [7]. Our whole cerebellar uptake suggests that the cerebellar response to neuropathic pain is not only encoding pain modulation, but also cognitive modulation, and the whole cerebellar activation could be the result of rhythmic sensorimotor synchronization, although there is still no consensus on the cerebellar timekeeping function. After the cessation of neuropathic pain, the peripheral nerve-injured rat, in ordinary time, may experience pain and adapt to produce a sequential or coincidence motor response to such pain. Although resting state of our study, the cerebellum, as a pain modulation region, could be activated, and this finding persisted into the eighth week of our investigation. Though there has not been thorough research into the putative comparative role of the cerebellum in anticipation of pain, the difference in spatial locations of the anticipatory and pain activation, as reported by Restuccia et al., suggests that multiple parts of the cerebellum respond to pain in different ways [21, 29, 34, 45].

From our behavioral tests of mechanical stimulation, the withdrawal response rate manifested within with the first week and reached a maximum after 4 weeks, and this rapid peripheral nerve injury-specific remodeling of cortical circuit responses was verified as being associated with neuropathic pain development.

Our study has some limitations. Specifically, we used an anesthetic material that could affect the uptake of neurons, and we did not get any images after the eighth week. In animal models used for neuropathic pain study, neuropathic pain established by transection or partial crush of peripheral nerve maintained for over or less than 8 weeks [12]. It is different from neuropathic pain in human patients. Generally, patients with neuropathic pain suffer from pain for their entire lives, but animal models of neuropathic pain cannot mimic this long-lasting feature, even though those animal models are well mimicking algesia and allodynia [12, 22]. We suppose that this could have resulted from artificial manipulation of the animal's nervous system. Therefore, we performed our microPET study for 8 weeks.

Even with these limitations, the longitudinal microPET scans of peripheral nerve-injured rat brains showed sequential cerebellar uptake that was in accordance with behavioral test responses. In conclusion, the cerebellum, an integrator of multiple effector systems related to pain, is thought to be involved

not only in sensorimotor processing but also affective processing and pain modulation. Our longitudinal microPET scan study of peripheral nerve-injured rat brains showed sequential cerebellar activity that was in concordance with behavioral test responses, thus supporting a role of the cerebellum in the processing of neuropathic pain.

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Conflict of interest None.

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