



Pharmacological modulation of brain activation to non-noxious stimulation in a cynomolgus macaque model of peripheral nerve injury

Molecular Pain
Volume 17: 1–19
© The Author(s) 2021
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/17448069211008697
journals.sagepub.com/home/mpx


Aldric Hama¹ , Mizuho Yano¹, Wakana Sotogawa¹, Rintaro Fujii¹, Yuji Awaga¹, Takahiro Natsume¹, Ikuo Hayashi², and Hiroyuki Takamatsu¹

Abstract

In vivo neuroimaging could be utilized as a noninvasive tool for elaborating the CNS mechanism of chronic pain and for elaborating mechanisms of potential analgesic therapeutics. A model of unilateral peripheral neuropathy was developed in the cynomolgus macaque, a species that is phylogenetically close to humans. Nerve entrapment was induced by placing a 4 mm length of polyvinyl cuff around the left common sciatic nerve. Prior to nerve injury, stimulation of the foot with a range of non-noxious von Frey filaments (1, 4, 8, 15, and 26 g) did not evoke brain activation as observed with functional magnetic resonance imaging (fMRI). Two weeks after injury, stimulation of the ipsilateral foot with non-noxious filaments activated the contralateral insula/secondary somatosensory cortex (Ins/SII) and anterior cingulate cortex (ACC). By contrast, no activation was observed with stimulation of the contralateral foot. Robust bilateral activation of thalamus was observed three to five weeks after nerve injury. Treatment with the clinical analgesic pregabalin reduced evoked activation of Ins/SII, thalamus and ACC whereas treatment with the NK1 receptor antagonist aprepitant reduced activation of the ipsilateral (left) thalamus. Twelve to 13 weeks after nerve injury, treatment with pregabalin reduced evoked activation of all regions of interest (ROI). By contrast, brain activation persisted in most ROI, except the ACC, following aprepitant treatment. Activation of the contralateral Ins/SII and bilateral thalamus was observed six months after nerve injury and pregabalin treatment suppressed activation of these nuclei. The current findings demonstrated persistent changes in CNS neurons following nerve injury as suggested by activation with non-painful mechanical stimulation. Furthermore, it was possible to functionally distinguish between a clinically efficacious analgesic drug, pregabalin, from a drug that has not demonstrated significant clinical analgesic efficacy, aprepitant. In vivo neuroimaging in the current nonhuman model could enhance translatability.

Keywords

Neuropathic pain, brain activation, insula cortex/secondary somatosensory cortex, thalamus, cingulate cortex, non-noxious stimulation

Date Received: 27 January 2021; Revised 27 January 2021; accepted: 27 February 2021

Introduction

A number of pharmacotherapeutic options exist for the management of neuropathic pain but overall efficacy has been described as modest.^{1,2} In addition, while some therapeutics may give significant pain relief, side effects at therapeutic doses may be intolerable. Finally, efficacy to a given therapeutic, such as opioids, may diminish over time, necessitating increasing amounts of analgesic or changing to other analgesics without any clear

¹Hamamatsu Pharma Research Inc., Hamamatsu, Japan

²Hamamatsu Pharma Research USA, Inc., San Diego, CA, USA

Corresponding Author:

Aldric Hama, Hamamatsu Pharma Research, Inc., 1-3-7, Shinmiyakoda, Kita-ku, Hamamatsu, Shizuoka 431-2103, Japan.
Email: aldrich-hama@hpharma.jp



indication as to whether or not the new treatment will be an improvement.² The clinical findings underscore the need for greater mechanistic understanding of neuropathic pain for the development of treatments with greater efficacy than that obtained with currently available treatments.

Most of the current understanding of CNS mechanisms of nociceptive processing is based on preclinical modeling of acute and neuropathic pain in rodents.^{3,4} Robust neurophysiological changes in nociceptive CNS neurons in preclinical neuropathic pain models have been observed, including increased resting-state activity, responding to previously non-noxious stimuli as painful and greatly enhanced responding to noxious stimuli.^{3,4} Increased excitability of nociceptive neurons, “sensitization,” has been suggested as the neural basis of neuropathic pain symptoms such as allodynia (pain due to a stimulus that does not normally provoke pain) and hyperalgesia (increased pain from a stimulus that normally provokes pain).^{5–7} Reduction of CNS neural sensitization with drugs is paralleled by reduced allodynia and hyperalgesia in rodent models of nerve injury.^{8–10} A drawback of preclinical in vivo CNS neural recordings is that the procedure is invasive and limits animal usage.

In chronic pain patients, electrophysiological methods have demonstrated sensitized CNS neurons but in-depth pharmacological assessments of these neurons have yet to be performed.^{11–13} Also, whether these neurons respond to cutaneous stimuli has yet to be documented. A larger issue is whether these neurons are in fact involved in pain perception.¹⁴

As a noninvasive alternative, in vivo neuroimaging, such as blood oxygen level-dependent (BOLD)-based functional magnetic resonance imaging (fMRI), could be utilized to identify sensitized brain areas in the neuropathic state. Increased oxygenated blood in tissues suggests increased neural activity and decreased oxygenated blood in tissues suggests decreased neural activity. Both clinical and preclinical studies have demonstrated activation and deactivation patterns in brain areas involved in nociceptive processing using fMRI.^{15–18} Furthermore, analgesic drugs reduced stimulus-evoked brain activation whereas drugs that are not analgesic did not.^{19,20} Thus, brain fMRI could be utilized to further elaborate mechanisms of novel analgesics and potentially used to “quantify” analgesia in vivo.

While rodent models have greatly contributed to understanding CNS processes related to pain and analgesia, there is a marked lack of translation of findings from these models to clinical application. There are likely a number of reasons for the less-than-seamless transition, but one that has yet to be thoroughly addressed is the reliance on rodents, a species that is phylogenetically distinct from humans.²¹ Some rodent

and human tissues show conserved genes, but a number of molecular targets related to pain modulation differ between humans and rodents, with respect to function as well as to tissue distribution.^{22–26} Nonhuman primates (NHP) have been used in other areas of neuroscience research, as their genetics, brain structure and social behavior are closer to humans than that of rodents and therefore could be used to reduce the translational gap.^{27,28}

The current report describes a NHP model of unilateral peripheral neuropathy. Using fMRI, non-noxious mechanical stimulus-evoked activation of brain nuclei was examined over time following nerve injury. As pharmacological validation, the effects of pregabalin, a gabapentinoid approved for the management of neuropathic pain symptoms, and the anti-emetic drug aprepitant, a potent neurokinin 1 (NK1) receptor antagonist, on stimulus-evoked brain activation were examined.^{29,30} The current findings suggest that evoked brain activation visualized with in vivo neuroimaging could be a useful method of elaborating the CNS mechanism of neuropathic pain and to examine potential treatments for efficacy.

Materials and methods

Animals

Six male *Macaca fascicularis* (4–9 y.o.; EveBioscience Co., Ltd., Wakayama, Japan) were used in the current study. Housing and environmental conditions were as per the *Guide for the Care and Use of Laboratory Animals: Eighth ed.*³¹ Macaques were individually housed in adjoining primate cages, maintaining olfactory, auditory and visual contact but limited tactile interaction. Each macaque was fed standard nonhuman primate chow (Oriental Yeast, Tokyo, Japan), about 100 g/day, and had free access to water. As part of their enrichment, macaques were provided with treats twice per week by research staff or animal care staff. Also as part of their enrichment, macaques were given enrichment devices. The current study was reviewed and approved by the Hamamatsu Pharma Research Animal Care and Use Committee. At the end of the study, macaques were returned to group housing.

Before the 12–13 week MRI scan, two macaques were removed from the current study for use in an unrelated study.

Study schedule

Prior to surgery, macaques were assessed for responsiveness to von Frey filaments in the awake state and then during fMRI at intervals indicated in Figure 1.

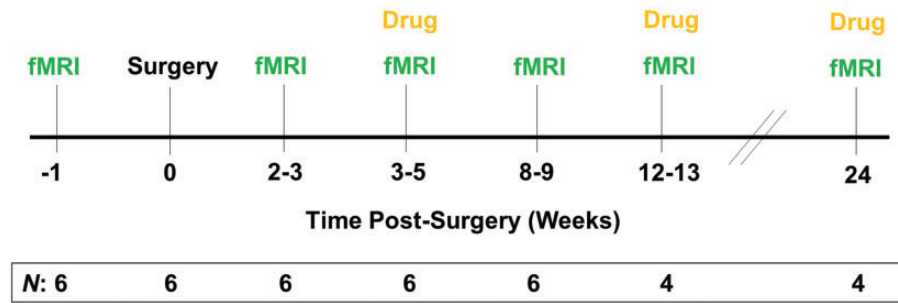


Figure 1. Study timeline and number (N) of macaques used.

Surgery

The current macaque unilateral nerve injury model was partially based on a rat unilateral nerve injury model of a “chronic constriction injury”, wherein a polyethylene cuff was applied to the common sciatic nerve.³² Surgery was performed in anesthetized macaques using aseptic technique. Under ketamine anesthesia (10–30 mg/kg, i. m.; Daiichi Sankyo, Tokyo, Japan), the skin of the left posterior-lateral upper thigh was shaved and cleaned with povidone-iodine. A skin incision of about 3 cm was made to expose the lateral aspect of the biceps femoris. The middle portion of the sciatic nerve was exposed via blunt dissection. A 1 cm length of PVC tubing, cut from a Shiley endotracheal tube (internal diameter about 3.5–4.0 mm; Mallinckrodt Medical, Dublin, Ireland) was split lengthwise on one side and placed around the sciatic nerve. To keep the tube closed, a length of silk suture was tied around the tube. The muscle and skin were closed with silk sutures. Immediately following surgery, macaques were treated once with buprenorphine (0.03 mg/kg, i.m.; Otsuka Pharmaceutical Co., Tokyo, Japan) for post-operative pain and enrofloxacin (5 mg/kg, i.m.; Bayer, Tokyo, Japan), once daily for at least three days to prevent infection.

Behavioral assessment

Prior to nerve injury surgery, macaques were habituated to restraint in a monkey chair and von Frey filament probing of both feet for no more than an hour a day, for two weeks, no more than five days per week. With feet resting on the chair’s lower cross bar, von Frey filaments (1, 4, 8, 15 and 26 g; Stoelting, Wood Dale, IL, US) were applied to the plantar surface of the foot. Each filament was applied to the mid-plantar foot, until there was a slight bend, for about 2–3 seconds, six times to the same spot.³³ A score was assigned based on the foot response: 0, no response; 1, mild response, consisting of shifting of the foot without lifting of the foot from the crossbar; 2, moderate response, consisting of lifting of the foot following application of the stimulus with replacement of the foot onto the crossbar; 3, robust

response, vigorous lifting of the foot away from the stimulus and avoidance of replacing the foot back on the crossbar.³³ The left foot was tested with all five filaments and then the right foot was tested with all five filaments.

Macaques that showed restlessness during training or testing were returned to their home cage and tested the following day. All six macaques habituated to restraint and filament testing; there were no exclusions. Testing was performed before nerve injury surgery and 6 mos. after nerve injury.

Functional magnetic resonance imaging

Stimulus-evoked brain activation was visualized using a Signa HDxt 3.0 T MRI system (GE Healthcare, Milwaukee, WI, US). Macaques were sedated by continuous intravenous infusion of propofol (0.2 mg/kg/min; Maruishi Pharmaceutical Co., Osaka, Japan) and heads were fixed within an MR compatible acrylic head holder (Matsui Co., Aichi, Japan). Anesthesia was used to minimize movement during scanning and the dose of propofol used has little, if any, antinociceptive effect.^{34,35} During scans, animals were kept warm with heating pads and blankets.

The anatomical MRI protocol consisted of a T1-weighted fast spoiled gradient-recalled (FSPGR) sequence (repetition time (TR)/echo time (TE), 15.8/7.0 ms; number of averages, 1; flip angle, 12°; field of view, 150 mm × 150 mm; matrix, 256 × 224; slice thickness/interval, 1.0/0.5 mm; number of slices, 168). Functional scan sequences consisted of field-echo, echo-planar imaging (TR/TE, 3000/35 ms; flip angle, 90°; field of view, 140 mm × 140 mm; matrix, 64 × 64; slice thickness, 2.4 mm; number of slices, 30).

During one fMRI scan, animals underwent a block design stimulation protocol: 10 sets of mechanical stimulations using the 4, 8, 15 and 26 g von Frey filaments (Figure 2). One stimulation set consisted of 30 sec. of an “OFF” stimulus, 1 g von Frey filament applied by hand to rest perpendicularly on the center of the plantar foot, followed by 30 sec. of an “ON” stimulus, a 4, 8, 15 or 26 g von Frey filament. For each set, 10 frames were acquired, for a total of 100 frames per functional scan.

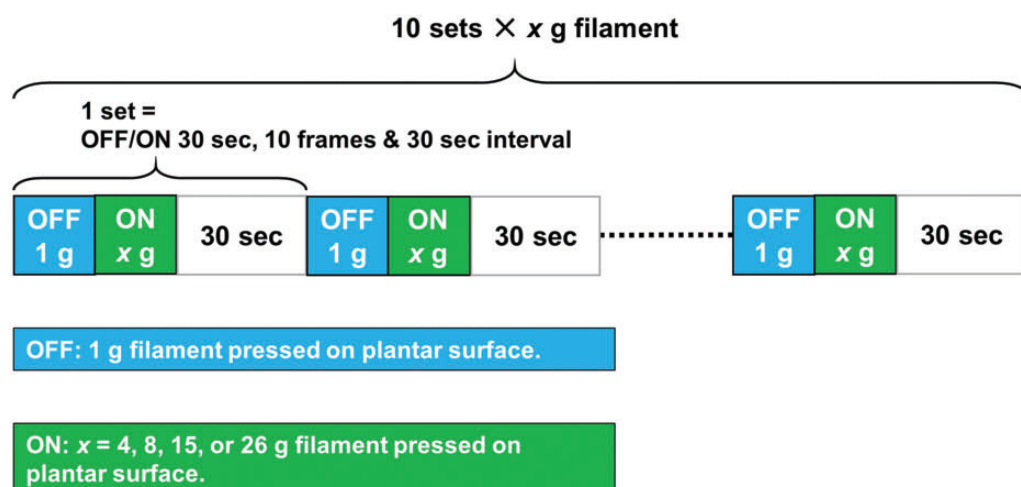


Figure 2. Stimulation pattern for fMRI. A 1 g filament (“OFF”) was pressed on the plantar surface for 30 sec., followed by a 4 g filament (“ON”) pressed on the plantar surface for 30 sec. and followed by no stimulation for 30 sec. This set was repeated ten times for the 4 g filament. In ascending order, ten sets of stimulation with the next higher force filament was performed. Following completion of one foot, the other foot underwent the same stimulation process.

A 30 sec. interval without stimulation separated each set. The filaments were tested in ascending order. Both ipsilateral and contralateral feet were tested. One fMRI scan was about 2 hrs. in duration.

Drug testing

On the day of MRI scanning, to examine the effects of drugs on stimulus-evoked brain activation, macaques underwent a MRI scan, as described previously, before and after drug treatment. Following the pre-drug scan, macaques received either vehicle (sterile water, 2 ml/kg, p.o.; Otsuka Pharmaceutical, Tokyo, Japan), pregabalin (30 mg/kg, p.o.; Kemprotec, Ltd., Cumbria, UK) or aprepitant (10 mg/kg, p.o.; Ono Pharmaceutical Co., Tokyo, Japan). One hour after dosing, a post-drug MRI scan was performed. Drugs were prepared on the day of MRI scanning.

For pregabalin, 30 mg/kg was utilized. A clinical study examined the acute efficacy of 150 mg pregabalin in patients with painful herpes zoster and found significant pain reduction beginning 1.5 hrs. after dosing, lasting for at least 5 hrs. after dosing.³⁶ According to the pregabalin package insert (Pfizer Inc., NY, NY) the dose range for pregabalin for use in managing neuropathic pain is 150–600 mg/day. The macaque equivalent dose of 600 mg/day would be about 30 mg/kg.³⁷

The dose of aprepitant used in the current study was extrapolated from the clinical pharmacokinetics of aprepitant in rhesus macaques and from a positron emission tomography study in which aprepitant blocked greater than 90% of striatal NK-1 receptors in rhesus macaques.^{30,38}

At week 3, two macaques each were assigned to either vehicle, pregabalin or aprepitant and at weeks 4 and 5, macaques were randomly assigned to a new treatment; by week 5 each macaque received all three treatments. Randomization was based on body weight and peak voxels obtained from pre-dosing MRI scans of the insula/secondary somatosensory cortex (Ins/SII) contralateral to the ligated sciatic nerve. At week 12, two macaques were assigned to either vehicle, pregabalin or aprepitant and randomized to a different treatment on week 13; macaques received a total of two out of the three treatments. Twenty-four weeks after nerve injury, four macaques were randomized to either vehicle or pregabalin and were crossed-over to the other treatment the following week. Each macaque received both treatments.

MRI data analysis

All subsequent image analyses were conducted with SPM12 software (Wellcome Trust Centre for Neuroimaging, London, UK). The images were realigned and resliced on to the mean echo-planar imaging (EPI) image to correct for head motion. The EPI images were co-registered to the corresponding T1-weighted anatomical image, and normalized to a macaque brain template³⁹. (Stereotaxic coordinates according to Horsley-Clarke’s stereotaxic coordinates.) The resulting image was smoothed with a 4 mm \times 4 mm \times 4 mm full-width at half-maximum Gaussian kernel. Voxel-wise statistical analysis was based on a general linear model. A fixed-effect model was used for group analysis of data from four to six macaques with a peripheral nerve injury.

Contrast (subtraction) was defined to isolate regions responsive to von Frey stimulation-related signals in the entire brain. Group mean contrasts were calculated between 1 g and the 8, 10, 15, 26 g filaments ($1\text{ g} - x\text{ g}$), as the 1 g filament did not evoke significant activation ($z\text{ score} < 1.96$). For drug treatment, group mean contrasts were defined as (vehicle - post-treatment) to determine decreases in activation following drug treatments. Peak voxels were considered significant at a z -score greater than 1.96 ($P < 0.05$, uncorrected for multiple comparisons, one-tailed t -test).

Statistics

No statistical method was used to determine sample sizes prior to the start of the current study. The fewest number of animals was used on the basis of ethical considerations and on the basis of animal availability. Group sizes were similar to those reported in previous publications.^{40–42} Unless otherwise indicated, data are presented as mean \pm SEM. Minimum statistical significance was set at $P < 0.05$.

Results

Response to von Frey filaments

Probing of the left plantar foot before nerve injury surgery with increasing force von Frey filaments evoked increasing responses as reflected by increasing mean total scores (Table 1). Six months following nerve injury, the mean scores of the ipsilateral left foot to filament probing tended to be lower compared to the ipsilateral foot before surgery (Mann Whitney test, $P > 0.05$).

Brain activation before and after nerve injury

While foot responses were observed in awake macaques prior to nerve injury, there was no significant brain activation following stimulation with any of the von Frey filaments (Table 2; Figure 3).

Beginning two weeks after nerve injury, significant activation of the anterior cingulate cortex (ACC) and the contralateral insular/secondary somatosensory cortex (Ins/SII) was observed following stimulation of the ipsilateral foot with the 15 and 26 g filaments (Table 3). No significant activation was observed in these areas with the 4 and 8 g filaments (Figure 4, vehicle). No stimulus evoked activation was observed in the left, ipsilateral Ins/SII. No significant activation was observed on stimulation of the contralateral unligated foot (data not shown).

Eight and nine weeks after nerve injury, in addition to the ACC and the contralateral Ins/SII, robust activation was observed bilaterally in thalamus with the 15 and 26 g

filaments (Table 4). No activation was observed with the 4 and 8 g filaments. No stimulus-evoked activation was observed of the left, ipsilateral Ins/SII. No activation was observed with stimulation of the contralateral unligated foot (data not shown).

Effect of drug treatment on evoked brain activation

Between three to five weeks following nerve injury, vehicle-treated nerve-injured macaques demonstrated robust activation of the contralateral Ins/SII, bilateral thalamus and ACC following ipsilateral foot stimulation with 15 and 26 g von Frey filaments (Table 5). No significant activation was observed with either 4 or 8 g filaments. Vehicle treatment did not significantly alter 26 g filament-evoked activation in nerve-injured macaques (Figure 5).

Treatment with aprepitant reduced 15 g filament-evoked activation of the left, ipsilateral thalamus (Table 5). No other brain nuclei (contralateral Ins/SII, ACC or the right thalamus) were affected. Aprepitant did not significantly reduce 26 g filament-evoked activation (Figure 6).

In contrast to aprepitant and vehicle treatment, pregabalin treatment significantly suppressed 15 g filament-evoked activation. In addition, pregabalin treatment suppressed 26 g filament-evoked activation except for the right (contralateral) thalamus (Table 5; Figure 7).

At 12 to 13 weeks after nerve injury, both the 15 g and 26 g filaments evoked activation of the ACC, contralateral Ins/SII and bilateral thalamus in vehicle-treated macaques (Table 6). Treatment with aprepitant reduced 15 g filament-evoked activation of the ACC alone. However, 26 g filament-evoked activation of the ACC was not reduced with aprepitant treatment—no other brain nuclei were affected by aprepitant treatment.

In contrast to aprepitant and vehicle treatment, pregabalin treatment reduced both 15 g and 26 g filament-evoked activation of the ACC, contralateral Ins/SII and bilateral thalamus (Table 6).

Six months after nerve injury, both 15 g and 26 g filament-evoked activation were observed in the contralateral Ins/SII and bilateral thalamus in vehicle-treated macaques (Table 7). Unlike previous time points after nerve injury, filament-evoked activation of the ACC was not observed. Pregabalin treatment suppressed activation of bilateral thalamus and the contralateral Ins/SII.

Comparisons of the effects of drug treatments vs. vehicle with 26 g filament-evoked activation are shown in Table 8. At three to five weeks, ACC activation was greater in vehicle-treated macaques compared to that of aprepitant-treated macaques (*i.e.* aprepitant reduced stimulus-evoked ACC activation). Contralateral Ins/SII, bilateral thalamus and ACC activation was greater in vehicle-treated macaques compared to that of

Table 1. Responses to von Frey filaments.

Total score	Filament (g)				
	1	4	8	15	26
Pre-surgery	4.8 ± 0.2	7.2 ± 0.5	8.4 ± 0.4	11.4 ± 0.3	13.8 ± 0.2
6 mos.	4.0 ± 0.5	5.8 ± 0.7	6.8 ± 0.7	9.2 ± 0.5	9.6 ± 0.7
P	0.6905	0.5476	0.5476	0.2222	0.0556

Responses of the foot ipsilateral to nerve injury to von Frey filaments before nerve injury surgery and 6 mos. after nerve injury surgery. The foot was probed six times with each filament. The response to one filament probing was scored (0–3) and all six responses per filament were totaled. Thus, the maximum score for each filament is 18. The mean ± SEM total scores from 6 macaques before nerve and 4 macaques 6 mos. after nerve injury are shown. Total scores at Pre-surgery and 6 mos. after surgery were not significantly different (Mann Whitney test.).

Table 2. Lack of evoked brain activation in macaques prior to nerve injury of the left sciatic nerve.

Area	Hemisphere	Z score	Coordinates (mm)			ROI volume (cm ³)
			x	y	z	
Pre-injury, 1 g - 4 g vF						
Insular Cortex	Left	0.68	16	20	8	0.232
	Right	0.79	−12	18	4	0.229
Thalamus	Left	0.75	−2	−10	0	0.942
	Right	0.69	6	8	2	0.935
ACC		0.91	0	−20	−12	0.461
Pre-injury, 1 g - 8 g vF						
Insular Cortex	Left	0.71	16	18	8	
	Right	0.83	−12	18	6	
Thalamus	Left	0.66	−2	−10	0	
	Right	0.57	4	−2	2	
ACC		0.93	0	−18	−12	
Pre-injury, 1 g - 15 g vF						
Insular Cortex	Left	0.84	16	16	8	
	Right	0.97	−14	18	6	
Thalamus	Left	0.66	−2	−10	0	
	Right	0.81	4	0	2	
ACC		0.83	0	−18	−12	
Pre-injury, 1 g - 26 g vF						
Insular Cortex	Left	0.79	16	16	8	
	Right	1.18	−16	18	6	
Thalamus	Left	0.71	−2	−10	0	
	Right	0.73	4	4	2	
ACC		0.89	0	−18	−12	

Group mean peak voxel z score and coordinates from each region of interest following stimulation with von Frey filaments. Mean contrasts obtained from six macaques. Prior to nerve injury ("Pre-CCI"), mean z scores were less than 1.96. Stereotaxic coordinates (x, y, z) are according to Horsely-Clarke's stereotaxic coordinates.

pregabalin-treated macaques (*i.e.* pregabalin reduced stimulus-evoked contralateral Ins/SII, bilateral thalamus and ACC activation).

At 12 to 13 weeks, there was no difference between vehicle and aprepitant treatment in terms of 26g stimulus-evoked activation. By contrast, stimulus-evoked activation of the ACC, contralateral Ins/SII and thalamus following pregabalin treatment was reduced compared to that of vehicle treatment (Table 8).

Similarly, at six months, stimulus-evoked activation of the contralateral Ins/SII and thalamus was greater in vehicle-treated macaques than in pregabalin-treated macaques (Table 8).

Discussion

The dearth of analgesics with mechanisms other than opioid or anti-inflammatory could be in part attributed to reliance on rodents, a species that is phylogenetically

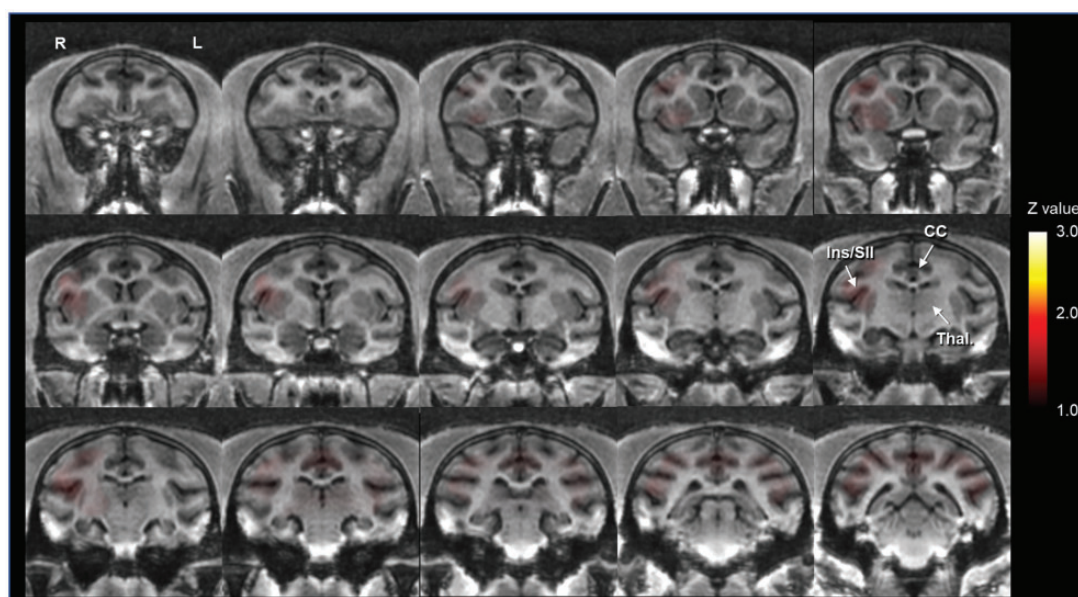


Figure 3. Contrast group average brain activation maps of uninjured macaques, before nerve injury. The left foot was stimulated with a 26 g von Frey filament. Ins/SII, insular/secondary somatosensory cortex; Thal., thalamic nuclei; ACC, anterior cingulate cortex. Serial coronal sections, from rostral to caudal (upper left to lower right). Contrast group average from 6 macaques.

Table 3. Evoked brain activation in macaques two weeks after left sciatic nerve injury.

Area	Hemisphere	Z score	Coordinates (mm)		
			x	y	z
Vehicle, 1 g - 4g vF					
Insular Cortex	Left	0.69	18	18	6
	Right	0.96	-16	18	6
Thalamus	Left	0.93	-2	-10	0
	Right	0.97	6	8	2
ACC		1.24	0	-20	-12
Vehicle, 1 g - 8g vF					
Insular Cortex	Left	0.71	16	16	4
	Right	1.49	-14	18	4
Thalamus	Left	1.13	-2	10	0
	Right	1.21	6	10	2
ACC		1.69	0	-20	-12
Vehicle, 1 g - 15g vF					
Insular Cortex	Left	0.76	18	16	4
	Right	2.04	-14	18	4
Thalamus	Left	1.35	-2	10	0
	Right	1.69	6	10	2
ACC		2.01	0	-20	-12
Vehicle, 1 g - 26g vF					
Insular Cortex	Left	0.99	18	16	4
	Right	2.53	-14	18	4
Thalamus	Left	1.69	-2	10	0
	Right	1.81	6	10	2
ACC		2.73	0	-20	-12

Group mean peak voxel z score and coordinates from each region of interest following stimulation with von Frey filaments. Mean contrasts obtained from six macaques. Ipsilateral stimulation evoked anterior cingulate cortex (ACC) and contralateral insular/secondary somatosensory cortex (Ins/SII) activation. Stereotaxic coordinates (x, y, z) are according to Horsely-Clarke's stereotaxic coordinates. Z score > 1.96, $P < 0.05$. Z score > 2.58, $P < 0.01$.

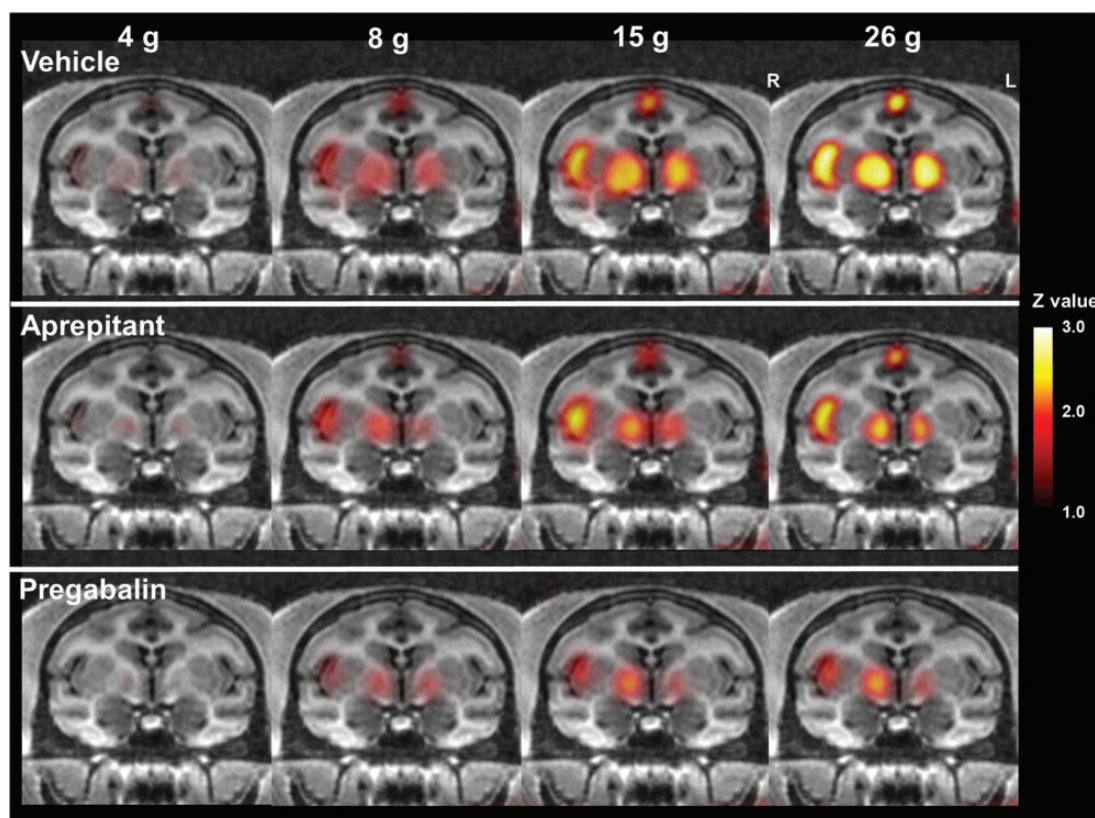


Figure 4. Contrast group average brain activation maps of vehicle, aprepitant and pregabalin-treated macaques three to five weeks after nerve injury. The left foot was stimulated with 4, 8, 15 and 26 g von Frey filaments. Coronal sections at the same level across treatments and across filaments. Contrast group average from 6 macaques.

and physiologically distant to humans, as the primary preclinical model species. Rodent-based pain modeling has yet to yield novel therapeutics that successfully completed Phase III clinical trials.^{43,44} Furthermore, an objective marker of pain, which is a subjective affective as well as somatosensory experience, has yet to be defined. The current study attempted to address both issues, in modeling a chronic pain state in a species that is phylogenetically close to humans and to pharmacologically validate stimulus-evoked brain activation as a marker of chronic neuropathic pain.

In the current study, 15 g and 26 g von Frey filament stimulation evoked activation of brain nuclei associated with pain perception as early as two weeks after unilateral sciatic nerve injury in macaques. While the von Frey Filaments (1–26 g) used in the current study evoked responses in awake, uninjured macaques, none of the von Frey filaments, including 26 g, elicited significant brain activation in propofol-anesthetized macaques before nerve injury. A previous study demonstrated that applying a grater with dentate projections attached to 1 kg in weight to the dorsum of the foot evoked an ipsilateral pain-related response in awake macaques.³⁵ Under propofol anesthesia, contralateral Ins/SII

activation was observed with fMRI.³⁵ Thus, the von Frey filaments used in the current study, even though they evoked responses in awake uninjured macaques, can be considered non-noxious. Extracellular recordings of dorsal horn spinothalamic tract (STT) neurons have shown responding to a similar range of von Frey filaments when applied to the macaque foot, but these were not as robust to in response to either pressure or pinch.⁴⁵ The current findings suggest persistent functional changes in brain neurons following a unilateral nerve injury, as reflected in robust responses in brain nuclei previously unresponsive to innocuous mechanical stimulation.

Unilateral nerve injury leads to significant ipsilateral hypersensitivity to non-noxious and noxious stimuli.⁴⁶ In addition to well-documented genomic and physiological changes in STT neurons following unilateral nerve injury, hypersensitivity to cutaneous stimuli is thought to be due to changes in neurons within the ventroposterior lateral (VPL) thalamus, a key relay center between spinal dorsal horn neurons and cortical areas involved in somatosensation and affect-motivation.³ Terminating within the VPL thalamus are STT neurons which transmit noxious information. Also within VPL thalamus are

Table 4. Evoked brain activation in macaques eight and nine weeks after left sciatic nerve injury.

Area	Hemisphere	Z score	Coordinates (mm)		
			x	y	z
Ig > 4g vF					
Insular Cortex	Left	0.66	18	18	2
	Right	1.01	−16	18	4
Thalamus	Left	1.23	−2	−10	0
	Right	1.39	6	8	2
ACC		1.05	0	−20	−12
Ig > 8g vF					
Insular Cortex	Left	0.72	16	16	2
	Right	1.61	−14	18	2
Thalamus	Left	1.64	−2	10	0
	Right	1.78	6	10	2
ACC		1.52	0	−20	−12
Ig > 15g vF					
Insular Cortex	Left	0.84	18	16	2
	Right	2.49	−14	18	2
Thalamus	Left	2.53	−2	10	0
	Right	2.61	6	10	2
ACC		2.19	0	−20	−12
Ig > 26g vF					
Insular Cortex	Left	0.92	18	16	2
	Right	2.96	−14	18	2
Thalamus	Left	3.04	−2	10	0
	Right	3.18	6	10	2
ACC		2.88	0	−20	−12

Group mean peak voxel z score and coordinates from each region of interest following stimulation with von Frey filaments. Mean contrasts obtained from six macaques. Ipsilateral stimulation evoked anterior cingulate cortex (ACC) and contralateral insular/secondary somatosensory cortex (Ins/SII) activation. Stereotaxic coordinates (x, y, z) are according to Horsely-Clarke's stereotaxic coordinates. Z score > 1.96, $P < 0.05$. Z score > 2.58, $P < 0.01$.

terminals of second order neurons of the dorsal column-medial lemniscus pathway, which are postsynaptic to large diameter primary afferents that respond to non-noxious cutaneous stimuli. Within the thalamus itself are neurons that respond to either noxious or non-noxious stimuli.^{47,48} Within two weeks of a unilateral nerve injury, rat thalamic neurons contralateral to nerve injury demonstrated altered responding to non-noxious and noxious cutaneous mechanical stimuli, including increased activation and persistent activation well after stimulus termination.^{46,49,50} Additionally, significant expansion of the cutaneous receptive field was observed following a nerve injury. While thought to be primarily due to changes in spinal dorsal horn neurons, thalamic neurons have also been implicated in receptive field changes.^{49,51,52} Along with the VPL thalamus, primary somatosensory cortex (SI), SII, and posterior Ins comprise the lateral pain system and mediate the sensory-discriminative aspects of pain.⁵³

Other key areas of the brain involved in pain perception include the ACC, anterior Ins, and medial thalamic nuclei—these nuclei comprise the medial pain system which mediates the affective-cognitive dimensions of

pain.⁵³ Lesions to the CC in humans appear to reduce the unpleasant aspect of pain but not entirely its perception.⁵⁴ Activation of the anterior Ins in humans evokes pain “with a strong affective component”.⁵⁵ In macaques, CC neurons are active during “pain avoidance behavior”.⁵⁶

Studies in rodents have found degrees of activation of the medial pain system, particularly the CC following nerve injury. For example, decreased excitatory postsynaptic potentials (EPSP) within the CC are observed five days after a unilateral partial sciatic nerve ligation.⁵⁷ Tachibana et al. directly stimulated thalamic neurons, rather than with cutaneous stimuli, to evoke CC neuron EPSP. In contrast, eight to nine weeks after unilateral chronic constriction injury (CCI) of the sciatic nerve in mice, increased spontaneous activity was observed in CC neurons.⁵⁸ Sellmeijer et al. also did not examine the effect of a cutaneous stimulus on CC neurons. In yet another study, no change in spontaneous activity of CC neurons was observed in rats with a spared nerve injury (SNI).¹⁶ Furthermore, decreased activation of the contralateral Ins following cold stimulation was observed in SNI rats.¹⁵ Perhaps in general

Table 5. Effect of drug treatment on stimulus-evoked brain activation in macaques three to five weeks after left sciatic nerve injury.

Area	Coordinates (mm)				Coordinates (mm)				Coordinates (mm)			
	Hemisphere	Z score	x	y	z	Area	Hemisphere	Z score	x	y	z	Area
Vehicle, 1 g - 8 g vF Ins/SII Thalamus ACC	Left	0.81	16	16	4	Aprepitant, 1 g - 8 g vF Ins/SII Thalamus ACC	Left	0.82	16	16	4	Pregabalin, 1 g - 8 g vF Ins/SII Thalamus ACC
	Right	1.63	-12	18	4		Right	1.66	-12	18	6	
	Left	1.55	-4	10	0		Left	1.23	-4	10	0	
	Right	1.58	6	10	2		Right	1.62	6	10	2	
Vehicle, 1 g - 15 g vF Ins/SII Thalamus ACC	Left	1.52	0	-18	-12	Aprepitant, 1 g - 15 g vF Ins/SII Thalamus ACC	Left	1.48	0	-18	-12	Pregabalin, 1 g - 15 g vF Ins/SII Thalamus ACC
	Right	0.91	18	16	4		Right	0.83	16	16	4	
	Left	2.61	-12	18	4		Left	2.52	-12	18	6	
	Right	2.49	-4	10	0		Right	1.91	-4	10	0	
Vehicle, 1 g - 26g vF Ins/SII Thalamus ACC	Left	2.56	6	10	2	Aprepitant, 1 g - 26g vF Ins/SII Thalamus ACC	Left	2.11	6	10	2	Pregabalin, 1 g - 26g vF Ins/SII Thalamus ACC
	Right	2.36	0	-18	-12		Right	1.97	0	-18	-12	
	Left	0.99	18	16	4		Left	0.96	16	16	4	
	Right	3.07	-12	18	4		Right	2.82	-12	18	6	
Vehicle, 1 g - 26g vF Ins/SII Thalamus ACC	Left	2.97	-4	10	0	Aprepitant, 1 g - 26g vF Ins/SII Thalamus ACC	Left	2.11	-4	10	0	Pregabalin, 1 g - 26g vF Ins/SII Thalamus ACC
	Right	2.98	6	10	2		Right	2.29	6	10	2	
	Left	3.02	0	-18	-12		Left	2.33	0	-18	-12	
	Right	3.02	0	-18	-12		Right	2.33	0	-18	-12	

Macaques were dosed (p.o.) 1 hr prior to brain imaging with either vehicle, aprepitant (10 mg/kg) or pregabalin (30 mg/kg). Group mean peak voxel z score and coordinates from each region of interest following stimulation with von Frey filaments. Mean contrasts obtained from six macaques. Evoked activation persisted following either vehicle and aprepitant treatment. Most evoked activation was eliminated following pregabalin treatment. Stereotaxic coordinates (x, y, z) are according to Horsely-Clarke's stereotaxic coordinates. Z score > 1.96, $P < 0.05$. Z score > 2.58, $P < 0.01$.

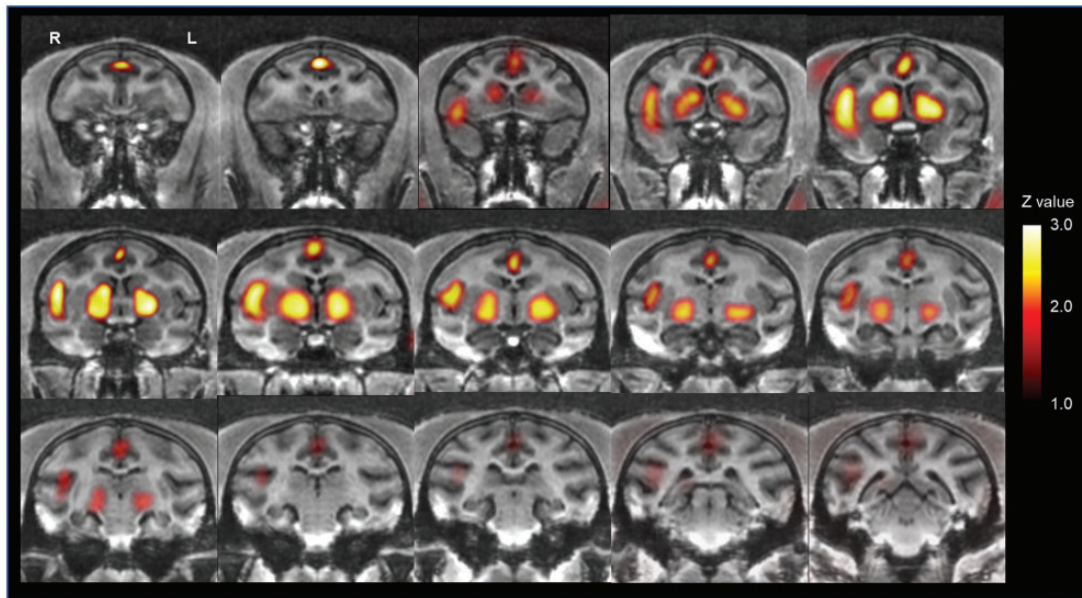


Figure 5. Contrast group average brain activation maps of vehicle-treated macaques three to five weeks after nerve injury. The left foot was stimulated with a 26 g von Frey filament. Activation of the contralateral insular/secondary somatosensory cortex (Ins/SII), bilateral thalamus (Thal.) and anterior cingulate cortex (ACC). Serial coronal sections, from rostral to caudal (upper left to lower right). Contrast group average from 6 macaques.

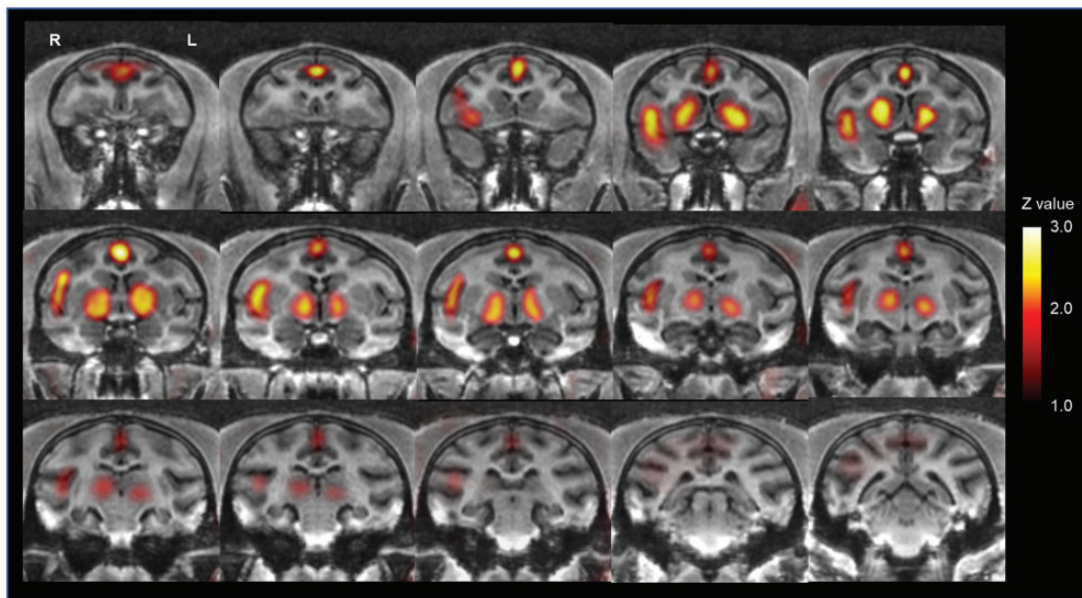


Figure 6. Contrast group average brain activation maps of aprepitant-treated macaques three to five weeks after nerve injury. The left foot was stimulated with a 26 g von Frey filament. Activation of the contralateral insular/secondary somatosensory cortex (Ins/SII), bilateral thalamus (Thal.) and anterior cingulate cortex (ACC). Serial coronal sections, from rostral to caudal (upper left to lower right). Contrast group average from 6 macaques.

agreement with the electrophysiological findings, in the case of a CCI, rats displayed anxiety-like behavior in the elevated plus maze.⁵⁹ Perhaps, again, in agreement with the electrophysiological findings, rats with a SNI did not display anxiety-like behavior.¹⁵

A limitation of *in vivo* extracellular recordings is its invasiveness, which restricts examination, for example, to one brain nucleus at one time-point for each animal. As a noninvasive alternative, BOLD fMRI infers *in vivo* neural activation through changes in tissue perfusion of

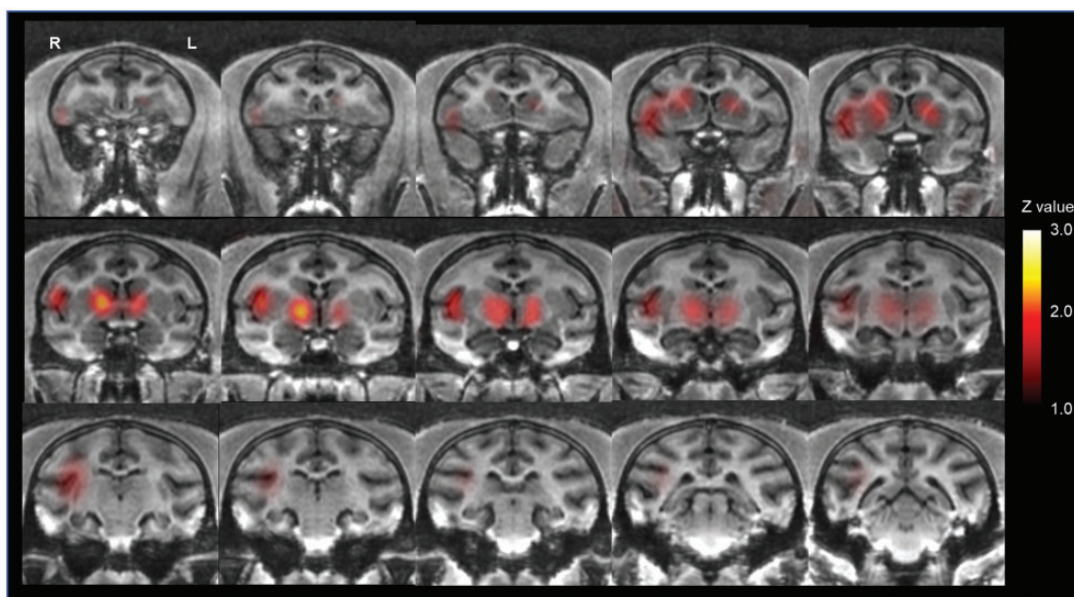


Figure 7. Contrast group average brain activation maps of pregabalin-treated macaques three to five weeks after nerve injury. The left foot was stimulated with a 26 g von Frey filament. Activation of the contralateral insular/secondary somatosensory cortex (Ins/SII), bilateral thalamus (Thal.) and anterior cingulate cortex (ACC). Serial coronal sections, from rostral to caudal (upper left to lower right). Contrast group average from 6 macaques.

paramagnetic hemoglobin.⁶⁰ With neuroimaging, multiple pain-associated brain nuclei at a time can be examined and the same subject may be repeatedly examined over time, allowing for the tracking of disease progression and observing the effects of therapeutics over time within the same subject.

Cold stimulation-evoked brain activation was visualized with fMRI in anesthetized rats with a SNI.¹⁵ Four weeks after SNI, increased activation to acetone-induced cooling was observed in the contralateral ventroposterior thalamus compared to rats that underwent a sham surgery.¹⁵ At the same time, decreased activation was also observed in the contralateral medial thalamus and insula.¹⁵ On the side ipsilateral to the injury, decreased activation, relative to sham-operated rats, was observed in the ACC, mid-cingulate cortex (MCC) and thalamus. At 20 weeks after nerve injury, rats demonstrated bilaterally increased SII activation. Additionally, nerve-injured rats at 20 weeks showed increased ipsilateral ACC and contralateral MCC activation and decreased bilateral thalamic activation.¹⁵

The effects of cutaneous tactile stimuli on brain activation was assessed in awake rats with a SNI.¹⁷ Five days after nerve injury, no change in evoked activation, relative to activation reported before injury, was noted. Twenty-eight days after nerve injury, decreased activation was observed in mesolimbic nuclei such as ipsilateral nucleus accumbens, contralateral prefrontal cortex and bilateral Ins and caudate putamen.¹⁷

The findings in total do not strongly support the notion that tactile stimulation in neuropathic rats evokes “unpleasantness”. The differences in activation between Hubbard et al. and Chang et al. could be due to the stimulus (cooling vs. air puff, respectively) or possibly due to the sex of the animals (female vs. male rats, respectively). In the case of Hubbard et al., anesthetic could have impinged upon stimulus-evoked brain activation, but low concentrations of isoflurane do not appear to dampen activation in pain-related brain nuclei and functional connectivity, or the connectivity between nuclei with shared functions.¹⁸ While rats with an SNI demonstrated robust ipsilateral hypersensitivity to nonnoxious cutaneous stimuli, as noted earlier, they do not display robust anxiety-like behavior.

In the current macaque model, progressive activation of both the medial and lateral pain systems is observed following nerve injury. Non-noxious mechanical stimuli evoked activation of the ACC and the contralateral Ins/SII. Over time, bilateral activation of the thalamus is also observed. At six months after injury, bilateral activation of thalamus and contralateral activation of Ins/SII are still present but ACC activation is no longer present. Similarly, in awake patients with a painful peripheral neuropathy, brush-evoked activation of bilateral thalamus, Ins/SII and ACC were observed.¹⁹ In contrast to findings in the macaque, bilateral, rather than contralateral, activation of Ins/SII was observed in patients. Other areas evoked in awake patients following

Table 6. Effect of drug treatment on stimulus-evoked brain activation in macaques 12 to 13 weeks after left sciatic nerve injury.

Area	Hemisphere	Coordinates (mm)			Area	Hemisphere	Coordinates (mm)			Area	Hemisphere	Coordinates (mm)					
		Z score	x	y			Z score	x	y			Z score	x	y	Z score	x	y
Vehicle, 1g - 4g vF Insula Cortex	Left	0.71	16	18	6	Aprepitant, 1g - 4g vF Insula Cortex	Left	0.69	16	18	6	Pregabalin, 1g - 4g vF Insula Cortex	Left	0.71	16	18	4
	Right	1.03	-12	18	4		Right	1.01	-12	16	4		Right	1.00	-14	16	4
	Left	1.01	-2	-10	0		Left	1.02	-4	-10	0		Left	1.00	-4	-10	0
	Right	1.03	6	8	2		Right	1.03	6	8	2		Right	1.05	6	8	2
ACC		1.05	0	-20	-12	ACC		1.02	0	-20	-12	ACC		0.74	0	-18	-12
Vehicle, 1g - 8g vF Insula Cortex	Left	0.72	16	16	4	Aprepitant, 1g - 8g vF Insula Cortex	Left	0.71	16	16	4	Pregabalin, 1g - 8g vF Insula Cortex	Left	0.79	16	18	4
	Right	1.58	-12	18	4		Right	1.51	-12	18	4		Right	1.37	-12	16	4
	Left	1.51	-4	8	0		Left	1.43	-4	10	0		Left	1.39	-4	10	0
	Right	1.54	6	10	2		Right	1.56	6	10	2		Right	1.42	6	8	2
ACC		1.21	0	-18	-12	ACC		1.18	0	-18	-12	ACC		0.83	0	-18	-12
Vehicle, 1g - 15g vF Insula Cortex	Left	0.81	16	16	4	Aprepitant, 1g - 15g vF Insula Cortex	Left	0.81	18	16	4	Pregabalin, 1g - 15g vF Insula Cortex	Left	0.81	18	18	4
	Right	2.34	-12	18	4		Right	2.46	-14	18	4		Right	1.62	-12	16	4
	Left	2.21	-4	10	0		Left	1.99	-4	8	0		Left	1.67	-4	10	0
	Right	2.36	6	10	2		Right	2.04	6	10	2		Right	1.71	6	8	2
ACC		2.19	0	-18	-12	ACC		1.57	0	-18	-12	ACC		0.91	0	-18	-12
Vehicle, 1g - 26g vF Insula Cortex	Left	0.93	16	16	4	Aprepitant, 1g - 26g vF Insula Cortex	Left	0.91	18	16	4	Pregabalin, 1g - 26g vF Insula Cortex	Left	0.93	18	16	4
	Right	2.67	-12	18	4		Right	2.79	-14	18	4		Right	1.84	-12	16	4
	Left	2.69	-4	10	0		Left	2.11	-4	8	0		Left	1.88	-4	10	0
	Right	2.74	6	10	2		Right	2.14	4	10	2		Right	1.94	6	8	2
ACC		2.58	0	-18	-12	ACC		2.21	0	-18	-12	ACC		1.00	0	-18	-12

Macaques were dosed (p.o.) 1 hr prior to brain imaging with either vehicle, aprepitant (10 mg/kg) or pregabalin (30 mg/kg). Group mean peak voxel z score and coordinates from each region of interest following stimulation with von Frey filaments. Mean contrasts obtained from six macaques. Evoked activation persisted following either vehicle and aprepitant treatment. No evoked activation was observed following pregabalin treatment. Stereotaxic coordinates (x, y, z) are according to Horsely-Clarke's stereotaxic coordinates. Z score > 1.96, $P < 0.05$. Z score > 2.58, $P < 0.01$.

Table 7. Effect of drug treatment on stimulus-evoked brain activation in macaques 24 weeks after left sciatic nerve injury.

Vehicle 1g - 4g vF											
Coordinates (mm)					Pregabalin 1g - 4g vF						
Area	Hemisphere	Z score	x	y	z	Area	Hemisphere	Z score	x	y	z
Insula Cortex	Left	0.74	16	18	6	Insula Cortex	Left	0.77	16	18	4
	Right	1.02	-14	18	4		Right	1.03	-14	16	4
Thalamus	Left	1.02	-2	-10	0	Thalamus	Left	1.00	-4	-10	0
	Right	1.03	6	8	2		Right	1.04	6	8	2
ACC		0.98	0	-20	-12	ACC		0.64	0	-18	-12
Vehicle, 1 g - 8 g vF											
Insular Cortex	Left	0.79	16	16	2	Insular Cortex	Left	0.81	16	18	2
	Right	1.49	-14	18	2		Right	1.41	-14	16	2
Thalamus	Left	1.38	-4	8	0	Thalamus	Left	1.31	-4	10	0
	Right	1.59	6	10	2		Right	1.41	6	8	2
ACC		1.11	0	-18	-12	ACC		0.77	0	-18	-12
Vehicle, 1 g - 15 g vF											
Insular Cortex	Left	0.85	16	16	2	Insular Cortex	Left	0.85	18	18	2
	Right	2.09	-14	18	2		Right	1.54	-14	16	2
Thalamus	Left	2.28	-4	10	0	Thalamus	Left	1.61	-4	10	0
	Right	2.47	6	10	2		Right	1.74	6	8	2
ACC		1.27	0	-18	-12	ACC		0.81	0	-18	-12
Vehicle, 1 g - 26 g vF											
Insular Cortex	Left	0.89	16	16	2	Insular Cortex	Left	0.87	18	16	2
	Right	2.78	-14	18	2		Right	1.88	-14	16	2
Thalamus	Left	2.71	-4	10	0	Thalamus	Left	1.83	-4	10	0
	Right	2.88	6	10	2		Right	1.93	6	8	2
ACC		1.51	0	-18	-12	ACC		0.88	0	-18	-12

Macaques were dosed (p.o.) 1 hr prior to brain imaging with either vehicle or pregabalin (30 mg/kg). Group mean peak voxel z score and coordinates from each region of interest following stimulation with von Frey filaments. Mean contrast obtained from four macaques. No evoked activation was observed following pregabalin treatment. Stereotaxic coordinates (x, y, z) are according to Horsely-Clarke's stereotaxic coordinates. Z score > 1.96, $P < 0.05$. Z score > 2.58, $P < 0.01$.

Table 8. Group mean contrasts following drug treatment over time in macaques with a nerve injury.

Area	Hemisphere	Z score	Coordinates (mm)			Area	Hemisphere	Z score	Coordinates (mm)		
			x	y	z				x	y	z
Weeks 3–5											
Vehicle > Aprepitant, Ig - 26g vF						Vehicle > Pregabalin, Ig - 26g vF					
Ins/SII	Left	0.07	16	16	4	Ins/SII	Left	0.11	16	16	6
	Right	1.32	−12	18	6		Right	2.94	−12	18	6
Thalamus	Left	1.81	−4	10	0	Thalamus	Left	3.21	−4	8	0
	Right	1.67	6	10	2		Right	2.78	6	10	2
ACC		2.07	0	−18	−12	ACC		3.57	0	−18	−12
Weeks 12–13											
Vehicle > Aprepitant, Ig - 26g vF						Vehicle > Pregabalin, Ig - 26g vF					
Ins/SII	Left	0.24	16	16	4	Ins/SII	Left	0.07	18	16	4
	Right	1.41	−14	18	4		Right	2.51	−12	16	4
Thalamus	Left	1.57	−4	8	0	Thalamus	Left	2.54	−4	10	0
	Right	1.64	4	10	2		Right	2.74	6	10	2
ACC		1.02	0	−18	−12	ACC		3.24	0	−18	−12
Week 24											
Vehicle > Pregabalin, Ig - 26g vF											
Ins/SII	Left	0.32	18	16	2						
	Right	2.19	−14	16	2						
Thalamus	Left	2.39	−4	10	0						
	Right	2.68	6	8	2						
ACC		1.74	0	−18	−12						

Differences in brain activation with 26 g filament stimulation between vehicle treatment drug treatment (vehicle > drug). Mean contrasts were obtained from four macaques. Compared to vehicle treatment, at three to five weeks, aprepitant reduced ACC activation. Pregabalin treatment, compared to vehicle treatment, suppressed Ins/SII and thalamic activation at all time points. (At week 24, no activation of the ACC was observed in vehicle treated nerve-injured macaques.) Stereotaxic coordinates (x, y, z) are according to Horsely-Clarke's stereotaxic coordinates. Z score > 1.96, $P < 0.05$. Z score > 2.58, $P < 0.01$.

ipsilateral brushing include the prefrontal cortex and supplemental motor area.¹⁹ It is possible that propofol anesthesia used in the current study suppressed activation of nuclei associated with movement and executive functioning. The lack of ipsilateral Ins/SII activation in the macaque may be due to anesthesia or perhaps could have been seen at longer time points after nerve injury—neuropathic pain patients who underwent imaging had neuropathic pain for at least one year.¹⁹

If neuropathic pain is mediated by the brain nuclei activated in the current study, then gabapentinoid treatment should reduce activation. Antinociceptive doses of pregabalin reduced stimulus-evoked activation of thalamic neurons in nerve-injured rats but not stimulus-evoked activation of thalamic neurons in uninjured rats.⁴⁶ A systemic dose of antinociceptive gabapentin reduced evoked and spontaneous activation of several brain nuclei including thalamic nuclei and the ACC, in both rodent neuropathic pain and in clinical neuropathic pain.^{18,19,61,62} The findings in rat neuropathic pain models suggest a neural basis by which gabapentinoids could have effects other than antinociception. Microinjection of gabapentin into the ACC in rats with a spinal nerve ligation did not alleviate hind paw hypersensitivity to non-noxious mechanical stimulation

but it did reduce pain-associated anxiety.⁹ In neuropathic pain patients, pregabalin treatment decreased evoked activation of the CC and Ins.¹⁹ It is possible that the analgesic effect of gabapentinoids is in part due to reduction of pain-associated anxiety as well as pain itself.

The current study in nonhuman primates also showed reductions in evoked activation following pregabalin dosing in the ACC, contralateral Ins and ipsilateral thalamus, three to five weeks after nerve injury. At 12 to 13 weeks after injury, bilateral thalamus, along with contralateral Ins and ACC were suppressed with pregabalin. A similar reduction in thalamic and Ins activation (but without the presence of evoked ACC activation) was observed 24 weeks after nerve injury. Thus, pregabalin treatment, whether early or late after nerve injury, appears to have a similar level of efficacy over time in the macaque model. Interestingly, pregabalin treatment did not reduce bilateral thalamic activation in patients with neuropathic pain—and such treated patients did not report pain relief.¹⁹ The findings suggest reduction of thalamic activation as a crucial CNS mechanism of action of pregabalin. The differential response to pregabalin, between the macaque model and neuropathic pain patients, could be due to the type of pain and its duration. Pregabalin's site of action will need further

elucidation, as pregabalin could be acting directly on thalamic neurons or in other CNS nuclei which send terminals to the thalamus.

Few *in vivo* fMRI studies have tested negative control drugs, that is, treatments based on a pain-related mechanism that demonstrated no significant clinical pain relief. While block of CNS NK1 receptors demonstrated robust antinociception in rodent models of neuropathic pain, NK1 receptor antagonists did not demonstrate significant clinical efficacy.^{63,64} Aprepitant was ineffective in both reducing noxious heat pain and noxious heat evoked activation of pain-related brain nuclei—in fact aprepitant tended to potentiate heat activation of limbic nuclei.⁶⁵ The current study in nonhuman primates demonstrated limited CNS effects of NK1 receptor block. At three to five weeks after nerve injury, evoked activation of the ipsilateral thalamus was reduced following aprepitant treatment. At 12 to 13 weeks after nerve injury, aprepitant reduced activation (15 g filament) of the ACC. With a higher stimulus (26 g), however aprepitant did not reduce ACC activation. It is possible that reduced evoked activation of the ACC by aprepitant suggests a degree of pain relief, but given the role of the thalamic nuclei in pain perception, it is not clear if reduction of ACC activation is sufficient for significant pain relief.

A number preclinical nonhuman animal models of neuropathic pain have been developed with a range of evoked, learned and spontaneous behavioral outcome measures.²⁸ Whether these actually reflect neuropathic pain as observed in humans is not entirely clear.²¹ The current study attempted to develop a method of quantitative assessment of pain following nerve injury using von Frey filaments as used in rodent neuropathic pain models. By six months, however, there was no increased sensitivity to von Frey filament probing of the ipsilateral foot—instead, there was a trend of decreased responding to the filaments. Whether it is possible to obtain robust behaviors indicating unilateral neuropathic pain in the nonhuman primate is not very clear.³³ One of the aims of the current study was to tie a behavioral response to a neurophysiological response following nerve injury. The current study nonetheless uncovered a neurophysiological response to a unilateral nerve injury to non-noxious stimulation following a unilateral nerve injury that evolves over time.

Insight from studies at the synaptic and cellular levels could explain the presence and persistence of evoked activation of brain nuclei observed in the current study. It has been hypothesized that spontaneous activation and altered responses of CNS neurons to nonnoxious stimuli following peripheral nerve injury are due to neural plasticity, highlighted by the phenomenon of long-term potentiation (LTP).^{7,66} In short, brief, high intensity presynaptic stimulation can lead to a persistent

postsynaptic activation, persisting long after termination of the initiating stimulus. In the context of pain, LTP has been observed in spinal dorsal horn neurons following peripheral nerve stimulation and tissue injury.⁶⁷ However, LTP has also been described in cortical regions, including the ACC and Ins.⁶⁶ In addition to increased synaptic activity mediated through increased excitatory neurotransmission and upregulation of cation channels that increase EPSP, intracellular signaling, between receptors and cation channels is also elevated.⁶⁷ Thus, while blocking excitatory glutamate neurotransmission in established LTP may reduce it, LTP is not entirely suppressed, as a number non-glutamatergic neurotransmitters and intracellular processes have forced the neuron into a highly activated state.

Furthermore, the increased activation of intracellular second messengers and protein kinases leads to changes in gene expression.⁶⁶ Interestingly, immediate early gene expression was observed in cortical regions such as the CC and Ins following ipsilateral non-noxious stimulation in rats following unilateral tissue injury.^{68,69} Thus, nonnoxious stimulation which does not normally evoke immediate early gene expression, could lead to molecular, physiological and structural changes to the synapse and postsynaptic cell. These cellular processes have been suggested to exist in clinical chronic pain, but confirmation in humans remains challenging. However, processes that have been well delineated in rodent models could be further explored in a preclinical non-rodent species that is phylogenetically close to humans.

The current macaque model of neuropathic pain demonstrated robust brain activation to non-noxious stimulation and the evolution of activation over time. While the current study examined discrete nuclei, further examination of functional connectivity could uncover changes in physiological properties of brain networks involved in pain perception and the possibility of modulating these networks for pain relief. Pharmacologically challenging the discrete brain nuclei observed in the current preclinical model could lead to greater understanding of drug mechanism and possibly boost the likelihood of successful clinical translation.

Acknowledgements

We gratefully acknowledge the HPR Animal Care Group for expert care of the animals during the course of the study.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: All authors are current or former employees of Hamamatsu Pharma Research, Inc.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Aldric Hama  <https://orcid.org/0000-0001-7372-979X>

References

1. Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH, Gilron I, Haanpaa M, Hansson P, Jensen TS, Kamerman PR, Lund K, Moore A, Raja SN, Rice AS, Rowbotham M, Sena E, Siddall P, Smith BH and Wallace M. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol* 2015; 14: 162–173.
2. Borsook D, Moulton EA, Schmidt KF and Becerra LR. Neuroimaging revolutionizes therapeutic approaches to chronic pain. *Mol Pain* 2007; 3: 25.
3. Millan MJ. The induction of pain: an integrative review. *Prog Neurobiol* 1999; 57: 1–164.
4. Sandkuhler J. Models and mechanisms of hyperalgesia and allodynia. *Physiol Rev* 2009; 89: 707–758.
5. IASP Task Force on Taxonomy. Part III: pain terms, a current list with definitions and notes on usage. In: H Merskey and N Bogduk (eds) *Classification of chronic pain*. Seattle, WA: IASP Press, 1994, pp.209–214.
- 6.Coderre TJ and Melzack R. The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. *J Neurosci* 1992; 12: 3665–3670.
7. Zhuo M. Cortical plasticity as synaptic mechanism for chronic pain. *J Neural Transm (Vienna)* 2020; 127: 567–573.
8. Omori Y, Kagaya K, Enomoto R, Sasaki A, Andoh T, Nojima H, Takahata H and Kuraishi Y. A mouse model of sural nerve injury-induced neuropathy: gabapentin inhibits pain-related behaviors and the hyperactivity of wide-dynamic range neurons in the dorsal horn. *J Pharmacol Sci* 2009; 109: 532–539.
9. Bannister K, Sikandar S, Bauer CS, Dolphin AC, Porreca F and Dickenson AH. Pregabalin suppresses spinal neuronal hyperexcitability and visceral hypersensitivity in the absence of peripheral pathophysiology. *Anesthesiology* 2011; 115: 144–152.
10. Chu KL, Xu J, Frost J, Li L, Gomez E, Dart MJ, Jarvis MF, Meyer MD and McGaraughty S. A selective alpha2 B adrenoceptor agonist (A-1262543) and duloxetine modulate nociceptive neurones in the medial prefrontal cortex, but not in the spinal cord of neuropathic rats. *Eur J Pain* 2015; 19: 649–660.
11. Davis KD, Kiss ZH, Tasker RR and Dostrovsky JO. Thalamic stimulation-evoked sensations in chronic pain patients and in nonpain (movement disorder) patients. *J Neurophysiol* 1996; 75: 1026–1037.
12. Gorecki J, Hirayama T, Dostrovsky JO, Tasker RR and Lenz FA. Thalamic stimulation and recording in patients with deafferentation and central pain. *Stereotact Funct Neurosurg* 1989; 52: 219–226.
13. Guenot M, Bullier J, Rospars JP, Lansky P, Mertens P and Sindou M. Single-unit analysis of the spinal dorsal horn in patients with neuropathic pain. *J Clin Neurophysiol* 2003; 20: 143–150.
14. Radhakrishnan V, Tsoukatos J, Davis KD, Tasker RR, Lozano AM and Dostrovsky JO. A comparison of the burst activity of lateral thalamic neurons in chronic pain and non-pain patients. *Pain* 1999; 80: 567–575.
15. Hubbard CS, Khan SA, Xu S, Cha M, Masri R and Seminowicz DA. Behavioral, metabolic and functional brain changes in a rat model of chronic neuropathic pain: a longitudinal MRI study. *NeuroImage* 2015; 107: 333–344.
16. Chao TH, Chen JH and Yen CT. Plasticity changes in forebrain activity and functional connectivity during neuropathic pain development in rats with sciatic spared nerve injury. *Mol Brain* 2018; 11: 55.
17. Chang PC, Centeno MV, Prociassi D, Baria A and Apkarian AV. Brain activity for tactile allodynia: a longitudinal awake rat functional magnetic resonance imaging study tracking emergence of neuropathic pain. *Pain* 2017; 158: 488–497.
18. Hooker BA, Tobon G, Baker SJ, Zhu C, Hesterman J, Schmidt K, Rajagovindan R, Chandran P, Joshi SK, Bannon AW, Hoppin J, Beaver J, Fox GB, Day M and Upadhyay J. Gabapentin-induced pharmacodynamic effects in the spinal nerve ligation model of neuropathic pain. *Eur J Pain* 2014; 18: 223–237.
19. Wanigasekera V, Wartolowska K, Huggins JP, Duff EP, Vennart W, Whitlock M, Massat N, Pauer L, Rogers P, Hoggart B and Tracey I. Disambiguating pharmacological mechanisms from placebo in neuropathic pain using functional neuroimaging. *Br J Anaesth* 2018; 120: 299–307.
20. Shidahara Y, Natsume T, Awaga Y, Ogawa S, Yamoto K, Okamoto S, Hama A, Hayashi I, Takamatsu H and Magata Y. Distinguishing analgesic drugs from non-analgesic drugs based on brain activation in macaques with oxaliplatin-induced neuropathic pain. *Neuropharmacology* 2019; 149: 204–211.
21. Blackburn-Munro G. Pain-like behaviours in animals – how human are they? *Trends Pharmacol Sci* 2004; 25: 299–305.
22. Chen J, Kang D, Xu J, Lake M, Hogan JO, Sun C, Walter K, Yao B and Kim D. Species differences and molecular determinant of TRPA1 cold sensitivity. *Nat Commun* 2013; 4: 2501.
23. Serrano A, Mo G, Grant R, Pare M, O'Donnell D, Yu XH, Tomaszewski MJ, Perkins MN, Seguela P and Cao CQ. Differential expression and pharmacology of native P2X receptors in rat and primate sensory neurons. *J Neurosci* 2012; 32: 11890–11896.
24. Zhang X, Priest BT, Belfer I and Gold MS. Voltage-gated Na(+) currents in human dorsal root ganglion neurons. *eLife* 2017; 6: e23235.
25. Ray P, Torck A, Quigley L, Wangzhou A, Neiman M, Rao C, Lam T, Kim JY, Kim TH, Zhang MQ, Dussor G and Price TJ. Comparative transcriptome profiling of the human and mouse dorsal root ganglia: an

- RNA-seq-based resource for pain and sensory neuroscience research. *Pain* 2018; 159: 1325–1345.
26. Gharib WH and Robinson-Rechavi M. When orthologs diverge between human and mouse. *Brief Bioinform* 2011; 12: 436–441.
 27. Phillips KA, Bales KL, Capitanio JP, Conley A, Czoty PW, Hart BA, Hopkins WD, Hu SL, Miller LA, Nader MA, Nathanielsz PW, Rogers J, Shively CA and Voytko ML. Why primate models matter. *Am J Primatol* 2014; 76: 801–827.
 28. Calvo M, Davies AJ, Hebert HL, Weir GA, Chesler EJ, Finnerup NB, Levitt RC, Smith BH, Neely GG, Costigan M and Bennett DL. The genetics of neuropathic pain from model organisms to clinical application. *Neuron* 2019; 104: 637–653.
 29. Tuchman M, Barrett JA, Donevan S, Hedberg TG and Taylor CP. Central sensitization and Ca(V) α (2)Delta ligands in chronic pain syndromes: pathologic processes and pharmacologic effect. *J Pain* 2010; 11: 1241–1249.
 30. Jiang J, Bunda JL, Doss GA, Chicchi GG, Kurtz MM, Tsao KL, Tong X, Zheng S, Uthagrove A, Samuel K, Tschirret-Guth R, Kumar S, Wheeldon A, Carlson EJ, Hargreaves R, Burns D, Hamill T, Ryan C, Krause SM, Eng W, DeVita RJ and Mills SG. Potent, brain-penetrant, hydroisindoline-based human neurokinin-1 receptor antagonists. *J Med Chem* 2009; 52: 3039–3046.
 31. Institute for Laboratory Animal Research. *Guide for the care and use of laboratory animals: eighth edition*. Washington, DC: The National Academies Press, 2011.
 32. Mosconi T and Kruger L. Fixed-diameter polyethylene cuffs applied to the rat sciatic nerve induce a painful neuropathy: ultrastructural morphometric analysis of axonal alterations. *Pain* 1996; 64: 37–57.
 33. Carlton SM, Lekan HA, Kim SH and Chung JM. Behavioral manifestations of an experimental model for peripheral neuropathy produced by spinal nerve ligation in the primate. *Pain* 1994; 56: 155–166.
 34. Steinbacher DM. Propofol: a sedative-hypnotic anesthetic agent for use in ambulatory procedures. *Anesth Prog* 2001; 48: 66–71.
 35. Shirai T, Yano M, Natsume T, Awaga Y, Itani Y, Hama A, Matsuda A and Takamatsu H. Pharmacologic modulation of noxious stimulus-evoked brain activation in cynomolgus macaques observed with functional neuroimaging. *J Am Assoc Lab Anim Sci* 2020; 59: 94–103.
 36. Jensen-Dahm C, Rowbotham MC, Reda H and Petersen KL. Effect of a single dose of pregabalin on herpes zoster pain. *Trials* 2011; 12: 55.
 37. Food and Drug Administration. *Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers*. Rockville, MD: US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2005.
 38. Wu D, Paul DJ, Zhao X, Douglas SD and Barrett JS. A sensitive and rapid liquid chromatography-tandem mass spectrometry method for the quantification of the novel neurokinin-1 receptor antagonist aprepitant in rhesus macaque plasma, and cerebral spinal fluid, and human plasma with application in translational NeuroAIDS research. *J Pharm Biomed Anal* 2009; 49: 739–745.
 39. Black KJ, Koller JM, Snyder AZ and Perlmuter JS. Atlas template images for nonhuman primate neuroimaging: baboon and macaque. *Methods Enzymol* 2004; 385: 91–102.
 40. Nagasaka K, Yamanaka K, Ogawa S, Takamatsu H and Higo N. Brain activity changes in a macaque model of oxaliplatin-induced neuropathic cold hypersensitivity. *Sci Rep* 2017; 7: 4305.
 41. Asad AB, Seah S, Baumgartner R, Feng D, Jensen A, Manigbas E, Henry B, Houghton A, Evelhoch JL, Derbyshire SW and Chin CL. Distinct BOLD fMRI responses of capsaicin-induced thermal sensation reveal pain-related brain activation in nonhuman primates. *PLoS One* 2016; 11: e0156805.
 42. Seah S, Asad AB, Baumgartner R, Feng D, Williams DS, Manigbas E, Beaver JD, Reese T, Henry B, Evelhoch JL and Chin CL. Investigation of cross-species translatability of pharmacological MRI in awake nonhuman primate – a buprenorphine challenge study. *PLoS One* 2014; 9: e110432.
 43. Rowbotham MC, Arslanian A, Nothaft W, Duan WR, Best AE, Pritchett Y, Zhou Q and Stacey BR. Efficacy and safety of the α 4 β 2 neuronal nicotinic receptor agonist ABT-894 in patients with diabetic peripheral neuropathic pain. *Pain* 2012; 153: 862–868.
 44. Huggins JP, Smart TS, Langman S, Taylor L and Young T. An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of the knee. *Pain* 2012; 153: 1837–1846.
 45. Carlton SM, Rees H, Gondesens K and Willis WD. Dextrorphan attenuates responses of spinothalamic tract cells in normal and nerve-injured monkeys. *Neurosci Lett* 1997; 229: 169–172.
 46. Patel R and Dickenson AH. Neuronal hyperexcitability in the ventral posterior thalamus of neuropathic rats: modality selective effects of pregabalin. *J Neurophysiol* 2016; 116: 159–170.
 47. Chung JM, Lee KH, Surmeier DJ, Sorkin LS, Kim J and Willis WD. Response characteristics of neurons in the ventral posterior lateral nucleus of the monkey thalamus. *J Neurophysiol* 1986; 56: 370–390.
 48. Kenshalo DR, Giesler GJ, Leonard RB and Willis WD. Of neurons in primate ventral posterior lateral nucleus to noxious stimuli. *J Neurophysiol* 1980; 43: 1594–1614.
 49. Guilbaud G, Benoist JM, Jazat F and Gautron M. Neuronal responsiveness in the ventrobasal thalamic complex of rats with an experimental peripheral mononeuropathy. *J Neurophysiol* 1990; 64: 1537–1554.
 50. Bordini F and Quartaroli M. Modulation of nociceptive transmission by NMDA/glycine site receptor in the ventro-posterolateral nucleus of the thalamus. *Pain* 2000; 84: 213–224.
 51. Suzuki R, Kontinen VK, Matthews E, Williams E and Dickenson AH. Enlargement of the receptive field size to low intensity mechanical stimulation in the rat spinal nerve

- ligation model of neuropathy. *Exp Neurol* 2000; 163: 408–413.
52. Lenz FA, Lee JJ, Garonzik IM, Rowland LH, Dougherty PM and Hua SE. Plasticity of pain-related neuronal activity in the human thalamus. *Prog Brain Res* 2000; 129: 259–273.
 53. Moisset X and Bouhassira D. Brain imaging of neuropathic pain. *NeuroImage* 2007; 37 Suppl 1: S80–S88.
 54. Brotis AG, Kapsalaki EZ, Paterakis K, Smith JR and Fountas KN. Historic evolution of open cinglectomy and stereotactic cingulotomy in the management of medically intractable psychiatric disorders, pain and drug addiction. *Stereotact Funct Neurosurg* 2009; 87: 271–291.
 55. Treede R-D, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, Hansson P, Hughes R, Nurmikko T and Serra J. Pain: redefinition and a grading system for clinical and research purposes. *Neurology* 2008; 70: 1630–1635.
 56. Koyama T, Kato K and Mikami A. During pain-avoidance neurons activated in the macaque anterior cingulate and caudate. *Neurosci Lett* 2000; 283: 17–20.
 57. Tachibana K, Kato R, Tsuruga K, Takita K, Hashimoto T and Morimoto Y. Altered synaptic transmission in rat anterior cingulate cortex following peripheral nerve injury. *Brain Res* 2008; 1238: 53–58.
 58. Sellmeijer J, Mathis V, Hugel S, Li XH, Song Q, Chen QY, Barthas F, Lutz PE, Karatas M, Luthi A, Veinante P, Aertsen A, Barrot M, Zhuo M and Yalcin I. Hyperactivity of anterior cingulate cortex areas 24a/24b drives chronic pain-induced anxiodepressive-like consequences. *J Neurosci* 2018; 38: 3102–3115.
 59. Roeska K, Doods H, Arndt K, Treede RD and Ceci A. Anxiety-like behaviour in rats with mononeuropathy is reduced by the analgesic drugs morphine and gabapentin. *Pain* 2008; 139: 349–357.
 60. He B, Yang L, Wilke C and Yuan H. Electrophysiological imaging of brain activity and connectivity – challenges and opportunities. *IEEE Trans Biomed Eng* 2011; 58: 1918–1931.
 61. Jeong KY and Kang JH. Investigation of spinal nerve ligation-mediated functional activation of the rat brain using manganese-enhanced MRI. *Exp Anim* 2018; 67: 23–29.
 62. Lin HC, Huang YH, Chao TH, Lin WY, Sun WZ and Yen CT. Gabapentin reverses Central hypersensitivity and suppresses medial prefrontal cortical glucose metabolism in rats with neuropathic pain. *Mol Pain* 2014; 10: 63.
 63. Goldstein DJ, Wang O, Gitter BD and Iyengar S. Dose-response study of the analgesic effect of lanepitant in patients with painful diabetic neuropathy. *Clin Neuropharmacol* 2001; 24: 16–22.
 64. Goldstein DJ, Wang O, Todd LE, Gitter BD, DeBrotta DJ and Iyengar S. Study of the analgesic effect of lanepitant in patients with osteoarthritis pain. *Clin Pharmacol Ther* 2000; 67: 419–426.
 65. Upadhyay J, Anderson J, Schwarz AJ, Coimbra A, Baumgartner R, Pendse G, George E, Nutile L, Wallin D, Bishop J, Neni S, Maier G, Iyengar S, Evelhoch JL, Bleakman D, Hargreaves R, Becerra L and Borsook D. Imaging drugs with and without clinical analgesic efficacy. *Neuropsychopharmacology* 2011; 36: 2659–2673.
 66. Zhuo M. Cortical excitation and chronic pain. *Trends Neurosci* 2008; 31: 199–207.
 67. Bliss TV, Collingridge GL, Kaang BK and Zhuo M. Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain. *Nat Rev Neurosci* 2016; 17: 485–496.
 68. Lin HC and Yen CT. Differential expression of phosphorylated ERK and c-Fos of limbic cortices activities in response to tactile allodynia of neuropathic rats. *Chin J Physiol* 2018; 61: 240–251.
 69. Wei F and Zhuo M. Activation of Erk in the anterior cingulate cortex during the induction and expression of chronic pain. *Mol Pain* 2008; 4: 28.