1. Animal models of central post-stroke pain

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Abstract. Stroke is a leading cause of death and disability in industrialized countries. Evidence shows that approximately 8-14% of stroke survivors suffer from central post-stroke pain (CPSP) when hemorrhagic or ischemic stroke occurs in lateral thalamic regions, which severely affects patients’ quality of life. The mechanisms of CPSP are not well understood, and effective treatments have not yet been developed. In the present article, we discuss the immunohistochemical and electrophysiological results of a rat model that is based on thalamic hemorrhage in vivo. This model is characterized by thermal and mechanical allodynia/hyperalgesia that develops in the subacute to chronic phases of CPSP. We review models of both thalamic hemorrhage and ischemic stroke that are useful for studying the neuropathology and physiology of the thalamic syndrome, with the aim of developing potential therapeutic strategies for CPSP that may be beneficial for the recovery of patients from stroke.
1. Introduction

Pain is a physiological condition that reflects local tissue aberrations. Pain is essential for maintaining the basic functional status of living animals. Ordinary pain is not often associated with disease, but central pain syndrome is recognized as a physiological disorder. According to the definition by the National Institute of Neurological Disorders and Stroke, central pain syndrome is a neurological condition caused by damage to or dysfunction of the central nervous system (CNS), which includes the brain, brainstem, and spinal cord. This syndrome can be caused by stroke, multiple sclerosis, tumors, epilepsy, brain or spinal cord trauma, and Parkinson’s disease. Central pain syndrome is not a fatal disorder, but the syndrome causes disabling chronic pain and suffering among the majority of individuals who have it. Among various central pain syndromes, neuropathic central pain syndrome occurs after thalamic hemorrhage or ischemic stroke and was first described in 1906 by Dejerine and Roussy (Jensen and Lenz, 1995). The particular thalamic syndrome called central post-stroke pain (CPSP) occurs in up to 8% of stroke patients (Andersen et al., 1995). Although the prevalence of CPSP among stroke patients is relatively low, the persistent, often treatment-refractory painful sensations can be a major problem and decrease the patient’s quality of life (Hanada et al., 2014).

CPSP is usually accompanied by permanent damage that results in spontaneous pain and often consequently paralysis and depression (Kumar et al., 2009; Hackett and Anderson, 2005; Klehmet et al., 2009). Hyperalgesia and allodynia attacks have been shown to be induced by thalamic stroke and can be caused by even innocuous stimuli (Boivie et al., 1989). This intractable secondary disease is associated with various symptoms, such as a burning sensation and lancinating pain.

Several neural substrates, including cerebral activity, might be involved in the pathophysiology of CPSP (Craig et al., 1994; Greenspan et al., 2004; Wang and Thompson, 2008). A review article reported that the descending pain modulation system, including the dorsolateral prefrontal cortex, rostral anterior cingulate cortex (ACC), amygdala, hippocampus, periaqueductal gray (PAG), and rostral ventromedial medulla, comprises a network that regulates nociceptive processing (Denk et al., 2014). The lateral and medial pain pathways have been shown to govern the homeostasis of nociception processing in coding the intensity of pain (Wang and Thompson, 2008; Su et al., 2012; Zhang et al., 2011; Martin et al., 1990). Stroke patients with dysfunction in the lateral thalamus exhibit a disruption of inhibition of signaling to the medial thalamus (MT), resulting in mechanical allodynia and thermal hyperalgesia (Craig et al., 1994; Greenspan et al., 2004). CPSP of
Animal models of central post-stroke pain can be viewed as a disinhibition disorder associated with thermoregulatory integration. Additionally, the ventral posteromedial thalamic nucleus (VPM)-dorsal posterior insular pathway has been shown to inhibit pain processing in limbic networks that consist of the MT, ACC, and PAG, suggesting that the occurrence of CPSP may be attributable to the loss of the aforementioned inhibition (Craig, 2007; Sprenger et al., 2012).

Human functional magnetic resonance imaging (fMRI) studies have provided evidence that the spinothalamic tract (STT) and MT-ACC pathway might be involved in CPSP (Sprenger et al., 2012; Kalita et al., 2011; Krause et al., 2012; Symonds et al., 2006). For example, brain lesions of the lateral and posterior thalamus and ventral nucleus-pulvinar border zone have been shown to be associated with a higher risk of developing CPSP after thalamic insult (Sprenger et al., 2012). A recent correlational study used clinical quantitative sensory testing (QST), MRI, and single-photon emission computed tomography (SPECT) and found that MRI and SPECT images of thalamic and parietal cortex lesions were correlated with CPSP-related allodynia symptoms in QST tests (Kalita et al., 2011). A recent human fMRI study dissociated differences in thalamic subregions (e.g., ventral posterolateral [VPL] and posterior portion [VMpo] of the ventral medial nucleus) in CPSP, indicating that the VPL but not VMpo plays a crucial role in CPSP (Krause et al., 2012). Furthermore, another fMRI study indicated that the contralateral somatosensory cortex and bilateral mid/posterior insula, anterior insula, and posterior cingulate were activated during exposure to acute pain stimulation (Symonds et al., 2006). Therefore, the STT and MT-ACC pathways may be critically involved in CPSP symptoms.

Brain responses in CPSP patients have been investigated using functional brain mapping (Sprenger et al., 2012; Kalita et al., 2011; Seghier et al., 2005). Functional magnetic resonance imaging measures global neuronal activity in response to specific stimuli. Positron emission tomography (PET) is another brain mapping technique that determines active brain localization using a radioactive substance. Functional magnetic resonance imaging and PET are powerful tools with high spatial resolution for evaluating brain activity.

The exacerbation of stroke-related sensory symptoms decreases patients’ quality of life. Developing and characterizing animal models that mimic thalamic syndromes are the first steps to discovering the underlying mechanisms of this disease and possible therapeutics. In this article, we discuss our observations with rat and mouse models of thalamic hemorrhage and review studies that utilized models of both thalamic hemorrhage and ischemic stroke that are useful for studying the neuropathology and physiology of CPSP and developing potential therapeutics.
Experimental procedures and methods

Preparation of CPSP animal model

Male Sprague Dawley rats (300-400 g) and BALB/cB6 mice (25-30 g) were housed in an air-conditioned room with free access to food and water. All of the experiments were performed in accordance with the guidelines of the Academia Sinica Institutional Animal Care and Utilization Committee. The experimental animals were maintained under anesthesia with 1% isoflurane during surgery. Body temperature was maintained at 36.5-37.5°C with a homeothermic blanket system (Model 50-7079, Harvard Apparatus, Holliston, MA, USA). The experimental animals were injected with type IV collagenase (in 0.125U/0.5 μl saline for rats and 0.008U/0.5μl saline for mice; Sigma-Aldrich, Oakville, ON, Canada) into the right ventral posterior medial nucleus (VPM)/ventral posterior lateral nucleus (VPL) of the thalamus. Control animals were injected with 0.5 μl sterile saline only.

Behavioral tests

To measure mechanical and thermal nociceptive responses, von Frey and plantar tests were conducted on the bilateral hindpaws 1 week before the lesion procedure and 7, 14, 21, 28, and 35 days after the collagenase injection.

von Frey test. The animals were placed on an elevated mesh platform for 30 min before testing, and filaments were gradually applied with ascending, graded force to determine the minimal force that would elicit a response. The threshold was defined as the average of three minimal forces measured in consecutive trials, each separated by 5 min.

Plantar test. The plantar test was performed by placing the rats into a transparent Plexiglas box for 30 min before testing, and a radiant heat light (IITC 390G Plantar Test, IITC Inc. Life Science, Woodland Hills, CA, USA) was delivered through the glass floor. The hind paw was directly stimulated by the infrared source to assess withdrawal responses. The paw-withdrawal latency in response to thermal stimulation was measured. The latency of the withdrawal response was the time that elapsed between pressing and releasing the button for infrared stimulation. Each rat or mouse was tested in three trials with the right and left hindpaws, respectively. The intertrial interval was 5 min.

Rotarod. This test utilizes rodents’ natural fear of falling, and locomotor coordination was measured and recorded. A specialized apparatus (San Diego Instruments, San Diego, CA, USA) was used that is capable of rotating at speeds between 4 and 60 rotations per minute (rpm). During the training process, experimental rats or mice were trained to remain on the rod.
as it rotated at 4 rpm for at least 60 s. After this level of proficiency was achieved, the rotarod was set to accelerate from 4 to 40 rpm over 5 min. When the experimental animals fell off, the time that elapsed and speed of the rotarod were recorded. The rotarod tests were performed once per week 1 week pre-lesion and 5 weeks post-lesion.

Open field test. The open field test was performed 5 weeks after surgery. Exploratory movements, hyperactivity, and exploratory behavior were monitored. The apparatus consisted of a square arena (80 cm length × 80 cm width × 40 cm height) with a 6400 cm$^2$ inner area for rats, 20 cm length × 20 cm width × 25 cm height) with a 400 cm$^2$ inner area for mice. When the experimental animal was placed in one corner of the outer area, it was allowed to explore the arena for 10 min. In the open field test, the total observation time was 10 min. The total distance traveled and time and distance traveled in the inner area during the 10 min period were plotted and calculated.

**Immunofluorescent staining and cell counts**

The animals were sacrificed and perfused with 4% paraformaldehyde in phosphate-buffered saline, and the brains were removed. The brains were incubated with a 30% sucrose solution prior to cryosectioning (30-40 μm sections). The ACC, primary somatosensory cortex (S1), MT, and VPL/VPM were dissected and incubated with primary antibodies (mouse anti-CD11b [1:100, MCA275R, Serotec], mouse anti-NeuN [1:100, MAB377, Millipore], and rabbit anti-GFAP [1:200, GTX108711, Genetex]), followed by a secondary antibody (Alexa Fluor-488 goat anti-mouse IgG [H+L; 1:400, A11001, Invitrogen]) and DAPI staining (D21490, Invitrogen). After immunohistochemistry, four sections with visible lesions from the center were chosen for image analysis. Stacks of images at 2 μm depth intervals were collected using a confocal microscope (LSM780, Zeiss) with Zen software (Zeiss) and either a 20× air objective (NA 0.7) for automatic full-section scans or 40× oil objective (NA 1.3-1.4) for small-field single-cell distinguishable images. The continual disruption of tissue organization and/or the loss of staining were identified as the lesion area. The edges of the lesion were marked in individual sections, and 200 μm distances from the edges of the lesion were chosen as the distant field, the area of which (μm$^2$) was the region of interest (ROI), measured using ImageJ software with calibrated parameters from the image acquisition. Any positive signal < 5 μm or not accompanied by a DAPI signal was rejected as a false signal. The cell counts were manually performed within the distant field using ImageJ 1.47 software (National Institutes of Health, Washington DC, USA).
Gene transcript analysis: Reverse-transcription polymerase chain reaction for cytokines

RNA samples from sham control and CPSP rat brain tissues from peri-lesion sites were collected and processed with designed probes that flanked rat tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), IL-1β, IL-10, and brain-derived neurotrophic factor (BDNF) for the reverse-transcription polymerase chain reaction (RT-PCR) assay. Crude extracts of total RNA were obtained from each experimental animal using Trizol reagent (15596-026, Invitrogen). Reverse transcription was performed with 0.5 μg total RNA using designed probes and Superscript III (12574-018, Invitrogen) in a 20 μl reaction mixture. Quantitative PCR amplification was performed for all of the samples in a reaction volume of 50 μl that contained 1× PCR buffer, 2 mM MgCl₂, 200 M dNTPs, and 200 nM each of forward and reverse primers and 1 U Platinum Taq DNA polymerase (12574-018, Invitrogen). The PCR amplification included denaturation at 96°C for 5 min, followed by 48 cycles of 94°C for 45 s, 58°C for 45 s, and 72°C for 90 s, with final extension at 72°C for 10 min. The quantitative PCR product samples were analyzed using agarose gel electrophoresis. Each experiment consisted of TNF-α, IL-6, IL-1β, IL-10, and BDNF targets and was performed in triplicate. The internal sham controls consisted of GAPDH, and negative sham controls consisted of the omission of the reverse transcriptase reaction or no cDNA template.

Electrophysiological recordings

Electrophysiological recordings were performed 5 weeks after CPSP induction. Experimental animals were maintained under anesthesia with 1.5% isoflurane. Sixteen-channel probes (NeuroNexus, Ann Arbor, MI, USA) were used to record extracellular field potentials in the right ACC (2.5 mm anterior, 1 mm lateral to bregma for rats; 1.0 mm anterior, 0.5 mm lateral to bregma for mice) and right MT (2.2-3.5 mm posterior, 0.5-1.0 mm lateral to bregma for rats; 0.8-1.0 mm posterior, 0.5-0.7 mm lateral to bregma for mice). The left sciatic nerve was isolated and implanted with a cuffed electrode to deliver constant-current pulses (Model 2100, A-M Systems, Carlsborg, WA, USA). The sampling rate of recorded analog signals was 6 kHz for the field potential data and 24 kHz for the unit data. All of the data were processed using a multichannel data acquisition system (Tucker-Davis Technologies, Alachua, FL, USA).

Data processing

All of the electrophysiology data were transformed and processed with MatLab (MathWorks, Natick, MA, USA). Unit activity recorded from the
ACC and MT was digitally filtered to obtain high-frequency spike activity in response to sciatic nerve stimulation (SNS). The statistical data were analyzed using unpaired Student’s \( t \)-tests, one-way analysis of variance (ANOVA), and two-way ANOVA using SPSS software (SPSS, Chicago, IL, USA). Values of \( p < 0.05 \) were considered statistically significant.

**Results and discussion**

In our experiments, we observed the same tendency of phenomena among rat or mouse corresponding groups, in the following contents, rat experimental groups were selected to represent our results.

**CPSP rats exhibit allodynia and hyperalgesia in the von Frey and plantar tests**

In 2009, Wasserman and Koeberle published the first report of an induced lateral thalamic hemorrhage in rat brain that mimics the most common form of human CPSP (Wasserman and Koeberle, 2009). We thus sought to generate the same hemorrhage CPSP in rats. Clear tissue damage was seen directly in cryosections from CPSP rat brains (Fig. 1A, upper panel). Hand drawings of the lesion edges from different experimental animals were stacked and superimposed onto sections from a rat brain atlas (Paxinos and Watson, 2007) to verify accurate hemorrhage locations in the VPM/VPL (Fig. 1A, lower panel). The experimental animals that exhibited the occurrence of CPSP were first examined in behavioral tests. We observed nociceptive characteristics in CPSP rats, reflected by allodynia in response to mechanical and thermal pain stimulation beginning 1 week after surgical CPSP induction. These sensitized behaviors persisted for at least 5-8 weeks compared with the control group (Fig. 1; \( p < 0.05 \)).

In Wasserman and Koeberle (2009), the animals exhibited hyperesthesia in response to mechanical pinch stimulation, with sensitivity localized to the hindlimb. Such responses appeared within 7 days after the intra-thalamic hemorrhage. Their CPSP rats also exhibited an increase in thermal sensitivity in the contralateral hindlimb.

Tamiya et al. (2013) reported a mouse model of CPSP generated by bilateral carotid artery occlusion (BCAO), which resulted in random ischemic hemorrhage in the brain. This model of ischemia generated mechanical and thermal hypersensitivity in both hindpaws, and the authors suggested the involvement of alterations in C- and \( A\beta \)-fibers. Unlike focal cerebral ischemia models, their BCAO model caused thermal hypersensitivity and different alterations in primary afferent neurons. Their results suggested
Figure 1. CPSP rats exhibit allodynia and hyperalgesia in the von Frey and plantar tests. (A, B) One month post-thalamic hemorrhage induction, lesion scars or hollow spaces remained. (B, C) One week after the lesion, mechanical (B) and thermal (C) allodynia were observed, which were still observable 1 month later. We continued to maintain some of the lesion animals for more than 2 months, and these features of CPSP were still evident.

that the pathogenic mechanisms that underlie pain differ depending on the site and degree of cerebral ischemia.

Hanada et al. (2014) studied mice with lateral thalamic hemorrhage as an animal model of human CPSP. Their behavioral analysis demonstrated that the animals displayed diclofenac-, morphine-, and pregabalin-resistant mechanical allodynia and thermal hyperalgesia in the hindpaw contralateral to the lesion site for over 112 days. They also reported that the microglial inhibitor minocycline significantly ameliorated mechanical allodynia and thermal hyperalgesia.

**CPSP rats exhibit normal locomotor function but exploratory deficits in the open field test**

To ensure that general locomotor function was not influenced by surgery itself, rotarod tests were performed (Fig. 2A). No significant difference was found between CPSP rats and the sham control group. Rats with lateral
Figure 2. CPSP rats exhibit normal locomotor function but exploratory deficits in the open field test. (A) Rotarod tests revealed that locomotor function in CPSP rats was normal compared with the sham control group. (B) Significant decreases in locomotion (average/min) and exploratory behavior (max/min) were observed in CPSP rats \((n = 25)\) in the open field test compared with the sham control group \((n = 16)\). \(*p < 0.05, \text{Tukey post hoc test.*}\)

thalamic lesions exhibited a significant reduction of the average distance and maximum distance traveled per minute in the novel open field environment (Fig. 2B), indicating that the CPSP animals exhibited less “willingness” to explore the new environment, which was presumably attributable to their suffering from aberrant pain sensations under CPSP conditions.

CPSP rat brains exhibit enhanced astrocyte and microglia signals but decreased neuron marker signals and elevated inflammatory cytokine levels

In Wasserman and Koeberle (2009), histopathology indicated the presence of activated microglia adjacent to the core of hemorrhagic lesions in the thalamus. Neutrophils were confined to the hemorrhage core, indicating that they entered in the initial bleed. By day 7, bands of activated microglia and astrocytes separated the hematoma from surviving neurons at the edge of the lesion. Our study found enhanced astrocyte (GFAP) and reactive microglia (CD11b) marker immunoreactivity in the peri-lesion sites (Fig. 3, solid square) in CPSP rats, whereas both molecules remained at basal levels in the contralateral sites (Fig. 3, dashed square) and brains of control rats (data not shown). Neurons that were stained with NeuN exhibited a less positive signal compared with the contralateral unaffected site (Fig. 3A-C). Tissue damage may directly initiate the downstream secretion of inflammatory cytokines (TNF-\(\alpha\), IL-6, IL-1\(\beta\), and IL-10; Seil et al., 2008; Qu et al., 2007; Colomar et al., 2002; Brough et al., 2002).
Figure 3. CPSP rat brains exhibit enhanced astrocyte and microglia signals but decreased neuron marker signals and elevated inflammatory cytokine levels. (A-C) Enhanced astrocyte marker GFAP and reactive microglia marker CD11b immunoreactivity was localized to peri-lesion sites (solid square) in CPSP rats, whereas the immunoreactivity of both markers remained at basal levels in the contralateral sites (dashed square). (D) Tissue samples from the peri-lesion sites were screened using quantitative RT-PCR. TNF-α, IL-6, IL-1β, IL-10, and BDNF were significantly increased in peri-lesion tissues compared with the contralateral unaffected sites. The proinflammatory cytokine IL-1β exhibited a prominent elevation. *p < 0.05, **p < 0.01, Tukey post hoc test.

We thus tested various cytokines to evaluate potential links with such conditions of brain tissue inflammation. Tissue samples from para-lesion sites were collected, homogenized, and screened with quantitative RT-PCR. The analysis revealed that nitric oxide synthase (NOS), TNF-α, IL-6, IL-10, BDNF, and particularly IL-1β were strongly increased in para-lesion tissues compared with contralateral unaffected sites (Fig. 3D).

In Hanada et al. (2014), histopathology indicated that the thalamic hemorrhage produced a relatively confined lesion that destroyed the tissue within the initial bleed and also showed the presence of activated microglia adjacent to the core of the hemorrhagic lesions.
Aberrations of cell counts in proximal lesion areas and correlation with behavioral assessment

Wadderman and Koeberle (2009) reported that the cellular composition of the thalamus changed 1-7 days after the lesion. They stated that the thalamic hemorrhage produced a confined lesion that destroyed the tissue within the initial bleed, with little or no neuronal death beyond the hemorrhage core. Surviving neurons that were surrounded by activated glial cells likely contributed to neuropathic pain in this model. Our observations

Figure 4. Aberrations of cell counts and correlations with von Frey test results. Wadderman and Koeberle (2009) reported that the cell composition of the thalamus changed 1-7 days after the lesion. (A) Representative images of immunofluorescent-labeled cryosections with neuron marker NeuN, astrocyte marker GFAP, and reactive microglia marker CD11b in the ACC, MT nucleus, and VPM/VPL near the lesion area. (B) Cell count analysis in the ACC functional cortex area and MT relay nucleus in the parallel noxious pathway (i.e., distant from the lesion area) did not reveal changes in cell composition post-lesion (two upper panels). In the area proximal to the VPM/VPL lesion site, we observed increases in GFAP- and CD11b-positive cells and a decrease in NeuN-positive cells (lower panel). Aberrations of the cell composition in the lateral thalamus may suggest a possible mechanism of CPSP.
were based on their findings. The tissue composition of the lesion site changed, reflected by immunofluorescent staining (Fig. 4A) that was used to verify and group the cell counts 35-38 days after CPSP induction. A significant increase in GFAP- and CD11b-positive cell counts in the selected ROIs (Fig. 3, solid square) was observed ($p < 0.05$, Tukey post hoc test). A significant decrease in NeuN-positive cell counts in the ROIs in the VPM/VPL was also found. The cell count analysis in the ACC functional cortex area and MT relay nucleus in the parallel noxious pathway (i.e., distant from the lesion area) did not change post-lesion (Fig. 4B).

Wadderman and Koeberle (2009) used the terminal deoxynucleotidyl transferase (dUTP) nick-end labeling (TUNEL) test and found that the majority of NeuN-positive cells within the lesion core showed evidence of cell shrinkage and were TUNEL-positive, indicating DNA fragmentation accompanied by cell death. In contrast, no TUNEL-positive neurons were observed in the surrounding intact parenchyma. At the edge of the lesion core, they observed a sharp decrease in the number of neurons, although very few TUNEL-positive cells were present within this region. Both activated microglia and astrocytes were present in the parenchyma that surrounded the hematoma, but the majority of activated microglia were present at the edge of the lesion core. Interestingly, no microglia or astrocytes were observed inside the lesion core 1 day after the hemorrhage. The authors claimed that this was most likely attributable to massive tissue necrosis within the central infarct zone. Their results demonstrated that neuron death after hemorrhage is limited to the hematoma and a small band of tissue at the edge of the hematoma 1 day after stroke onset. Neurons beyond this central zone appeared intact and spared by the injury, with no observable TUNEL-positive cells in this region. Neuronal cell death at the edge of the lesion site was most progressive during the first 7 days after thalamic hemorrhage, and some further death complete following the wound’s nature. Neuronal cell death was found outside the hematoma, with corresponding increases in OX-42/CD11b and GFAP immunoreactivity. The number of OX-42/CD11b-positive microglia/ macrophages and GFAP-positive astroglia significantly increased 1-7 days after injury compared with the contralateral side. No significant changes were observed at 6 h. Therefore, glial reactivity and hypertrophy associated with thalamic hemorrhage were thought occurred 6h post thalamic hemorrhage until 24 h after injury, and gradually increased thereafter.
CPSP animals exhibit hyperexcitability along thalamocingulate pathways

Thalamocingulate circuitry in the CNS is known as the medial pain processing pathway. Changes of individual pain signaling likely results from aberrant neuron activity along this pathway (Shyu and Vogt, 2009; Walton and Llinás, 2010; Llinás et al., 1999). Although behavioral test results and changes in basic cell composition have been well described, neural activity features in the CNS in CPSP have not been reported. Thus, electrophysiological approaches that utilize multichannel electrodes to record neuronal activity in the ACC and MT in response to SNS were implemented and analyzed. The location of the recording and injection probes was verified in MT and ACC cryosections using Cresyl violet counterstaining with reference to a rat brain atlas (see Fig. 1A). Neuronal activity in the ACC and MT was recorded in response to graded SNS, and multi-unit activity in the ACC and MT was greatly enhanced in CPSP rats compared with control rats. Electrophysiological recordings verified minimal evoked neuronal response thresholds to SNS. Increasing the SNS strength 20-fold dramatically increased ACC and MT unit activity in CPSP rats (Fig. 5A-D). Total temporal unit activity in the ACC and MT in different groups of CPSP rats was statistically analyzed and compared (Fig. 5E, F). Significant changes in spontaneous electroencephalographic activity in CPSP rats strongly indicated the manifestation of persistent pain-related behavior.

Electrophysiological assessment along the thalamocingulate nociceptive pathway provided neurological evidence that neuronal hyperexcitability in the MT and enhanced responses in the ACC may underlie hyperalgesia and the reduction of exploratory movements in nociceptive pain-related behavior. Abnormal thalamic bursting activity was observed in patients who suffer from central pain, and an imbalanced lateral and medial thalamic interaction has been proposed (Lenz et al., 1989; Jeanmonod et al., 1993). The present study observed profound changes in the threshold and sensitivity of thalamic and cortical responses and spontaneous cortical electroencephalographic (EEG) activity, strongly supporting the hypothesis that deafferentation resulted from lateral thalamic lesions that altered medial thalamic neuronal excitability (Jeanmonod et al., 1993; Sarnthein et al., 2006). The altered spontaneous EEG patterns under CPSP conditions suggest that both cingulate cortical and thalamic neurons in the medial pain pathway may contribute to persistent pain-related behavior, manifested as alterations in locomotor activity and neurogenic central pain that results from thalamocortical dysrhythmia, as proposed by Llinás et al. (1999).
Figure 5. CPSP animals exhibit hyper-neuronal excitability along the thalamocingulate pathway. Noxious electrical stimulation of the sciatic nerve and the basal strength of electric stimuli were based on the minimal current that elicited twisting of the hindlimb (one-fold). Representative 20-sweep summation of multiunit activity indicated that a noxious response of the ACC and MT was evoked with a 20-fold increase in basal stimulation strength. The latency between stimuli and response of the ACC and MT were approximately 150 and 80 ms (A, D). Compared with control animals, the evoked responses in the ACC and MT were enhanced and lengthened (B, E). The total summation after 1000 ms stimulation of the ACC and MT in the CPSP group was much higher than in the control group (C, F). This experiment provided evidence of unusual noxious neuronal activity that may be related to the progression of neuropathic pain, but the detailed mechanism remains unclear.

Conclusion

The present article describes the development of rodent models of thalamic syndrome, based on thalamic and ischemic hemorrhage in vivo. Rodent CPSP models consist of brain tissue damage, and wound progression causes innate inflammatory responses that reflect the initiation of persistent pain. The development of CPSP may be accelerated in these rodent models relative to human CPSP, which typically develops months after thalamic stroke (Jensen and Lenz, 1995). Our findings are similar to those of Wasserman and Koeberle (2009), which showed that hypersensitivity to mechanical and thermal stimuli developed within 1 week after thalamic hemorrhage. Hemorrhagic stroke was chosen
because this insult causes thalamic syndrome (Boivie et al., 1989; Vestergaard et al., 1995; Bowsher et al., 1998; Bowsher, 2005; Greenspan et al., 2004; Frese et al., 2006), and the thalamus is one of the most common sites of hemorrhagic stroke in humans (Fewel et al., 2003; Frese et al., 2006).

Mechanical and thermal hypersensitivity could be observed within 48 h after kainate lesions (LaBuda et al., 2000) and within 1 week after electrolytic lesions of the thalamus (Saadé et al., 1999) in rats with lateral thalamic hemorrhage. Symptoms after hemorrhage were persistent for at least 3 weeks in the study by Wasserman Koeberle (2009) and the present study and 6 weeks following electrolytic lesions (Saadé et al., 1999). Altogether, these studies show that the development of hypersensitivity in rodent models of CPSP is greatly accelerated compared with humans. Still unclear, however, is why this occurs. However, the rapid and consistent development of symptoms in rodent models is beneficial in terms of studying the mechanisms that underlie these processes.

Strategies for treating CPSP have included the administration of lidocaine, opiates, tricyclic antidepressants, and antiepileptic drugs, but these have had minimal success and are associated with significant negative side effects. Other therapeutics, such as amitriptyline, gabapentin, and lamotrigine, have shown clinical efficacy (Nicholson, 2004). Tissue damage may directly initiate the downstream secretion of inflammatory cytokines (TNF-α, IL-6, IL-1β, and IL-10; Seil et al., 2008; Qu et al., 2007; Colomar et al., 2002; Brough et al., 2002). Our observations corroborate these previous findings, but further investigations are needed to elucidate the particular role of each inflammatory cytokine in the pathogenesis of CPSP.

Despite widespread research on peripheral pain, much less is known about the pathophysiology of central pain syndromes. The accelerated development of nociceptive hypersensitivity after thalamic or ischemic hemorrhage in rodent models allows investigations of the processes that underlie thalamic syndrome and CPSP. The aforementioned models may prove useful for elucidating the underlying causes and neuropathology of CPSP.

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