DEVELOPMENT AND CHARACTERIZATION OF A HEMORRHAGIC RAT MODEL OF CENTRAL POST-STROKE PAIN

J. K. WASSERMAN AND P. D. KOEBERLE*

University of Toronto, Division of Anatomy, MSB 1186, 1 King’s College Circle, Toronto, Ontario, Canada M5S 1A8

Abstract—Stroke is the leading cause of disability in the industrialized world and it is estimated that up to 8% of stroke victims suffer from some form of central post-stroke pain (CPSP). Thalamic syndrome is form of central pain that typically results from stroke in the thalamus. In the present study, we describe the development and characterization of a rat model of thalamic CPSP. This model is based on a hemorrhagic stroke lesion in the ventral posterolateral nucleus of the thalamus, one of the reported causes of thalamic syndrome in humans. Behavioral analysis showed that animals displayed hyperesthesia in response to mechanical pinch stimulation, with sensitivity localized to the hind limb. This response appeared within 7 days of the intra-thalamic hemorrhage. Animals also showed increased thermal sensitivity in the contralateral hind limb. 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 7 days, bands of activated microglia and astrocytes separated the hematoma from surviving neurons at the edge of the lesion. We did not observe any terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) positive neurons beyond the immediate hematoma at 1, 3, or 7 days after hemorrhage. Surviving neurons were located in the vicinity of activated microglia and astrocytes at the outer edge of the hematoma. Thus, thalamic hemorrhage produces a confined lesion that destroys the tissue within the initial bleed, with little or no neuron death beyond the hemorrhage core. Surviving neurons surrounded by activated glial cells likely contribute to neuropathic pain in this model. This thalamic hemorrhage model is useful for studying the neuropathology and physiology of thalamic syndrome, and developing therapeutics for central post-stroke pain. Crown Copyright © 2009 Published by Elsevier Ltd on behalf of IBRO. All rights reserved.

Key words: thalamic syndrome, animal model, central post-stroke pain, intracerebral hemorrhage, thalamus.

Neuropathic pain arises from variety of different medical conditions or insults. In 1906, Dejerine and Roussy described a neuropathic central pain syndrome that occurred after thalamic stroke (Jensen and Lenz, 1995). Thalamic syndrome, a form of central post-stroke pain (CPSP) is characterized by spontaneous pain, attacks of allodynia, and dysesthesia. Stroke is the leading cause of disability in the industrialized world, and it has previously been reported that up to 8% of stroke patients suffer from central pain (Andersen et al., 1995). Stroke in the thalamus is one of the most common causes of neuropathic central pain (Boivie et al., 1989; Leijon et al., 1989; Vestergaard et al., 1995; Bowsher et al., 1998, 2005; Fewel et al., 2003; Greenspan et al., 2004; Frese et al., 2006).

Lesions in thalamic pathways may produce the conditions necessary to elicit spontaneous hyperactivity in thalamic neurons that modulate pain perception (Jensen and Lenz, 1995; Weng et al., 2003). In patients with neuropathic pain, spontaneous neuronal hyperactivity has been observed in the primary thalamic sensory nucleus, the nucleus ventralis caudalis (Lenz et al., 1994a,b). The involvement of these neurons in pain signaling pathways suggests that hypersensitivity in these cells may contribute to the pain observed in thalamic syndrome, however there is no direct experimental evidence to support this contention. The lack of suitable thalamic syndrome models has hampered research into the dysregulated perception of pain resulting from stroke in the thalamus. Therefore, the development and characterization of a model of thalamic syndrome is the first hurdle to discovering the underlying mechanisms of this disease, and possible therapeutics.

The present study describes the development of a rodent model of thalamic syndrome, based on thalamic hemorrhage in vivo. This model may prove useful for elucidating the underlying causes and neuropathology of thalamic syndrome. Hemorrhagic stroke was chosen because this insult in known to cause thalamic syndrome (Boivie et al., 1989; Leijon et al., 1989; Vestergaard et al., 1995; Bowsher et al., 1998, 2005; Fewel et al., 2003; 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).

EXPERIMENTAL PROCEDURES

Thalamic hemorrhage

All animal experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care, using aseptic technique. All experiments conformed to international guidelines for the ethical use of animals. Every attempt was made to minimize the number of animals used and prevent any suffering. No mortality or signs of illness were observed in experimental animals. Adult male Sprague-Dawley rats (250–300 g; Charles River, Wilmington, MA) were used for all experiments. Animals were anesthetized using vaporized isoflurane (4% induction, 2% maintenance) and placed in a stereotaxic frame. Temperature was maintained using a rectal thermometer coupled to a regulated heating blanket. Under stereotaxic guidance, a 0.25 μl volume of 0.05% heparinized saline was injected into the thalamus. Animals were allowed to recover for at least 1 week before use.

*Corresponding author. Tel: +1-416-978-6583; fax: +1-416-978-3844. E-mail address: paulo.koeberle@utoronto.ca (P. D. Koeberle).

Abbreviations: CPSP, central post-stroke pain; GFAP, glial fibrillary acidic protein; MPO, myeloperoxidase; NeuN, neuron nuclear antigen; PBS, phosphate-buffered saline; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.
Tissue processing

At 1, 3, or 7 days after thalamic hemorrhage, deeply anesthetized animals received an intracardial perfusion of phosphate-buffered saline (PBS, pH 7.5), followed by fixative (4% paraformaldehyde + 2% sucrose in PBS). The brains were rapidly removed and post-fixed in the same fixative overnight at 4 °C. The brains were then rinsed in PBS and placed in a 10% sucrose solution (in PBS) for 24 h followed by a 30% sucrose solution (in PBS) for 2–3 days. Brains were divided into coronal blocks using a rat brain matrix. Coronal sections (16 μm thick) were made using a Leica cryostat microtome, and sections were collected on APTEX (Sigma)-coated slides. Sections were taken from the entire rostral–caudal extent of the hematoma beginning 0.5 mm in front of the injury, and ending 0.5 mm posterior to the injury. Tissue sections were stored at −20 °C.

Immunohistochemistry and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining

Double-label immunohistochemistry was carried out on frozen brain sections in order to characterize neuronal degeneration and glial reactivity after thalamic hemorrhage. To identify neurons, astrocytes, and macrophages/microglia respectively, we used primary antibodies directed against neuron nuclear antigen (NeuN; mouse monoclonal, 1:100, Chemicon, Temecula, CA, USA), glial fibrillary acidic protein (GFAP; rabbit polyclonal, 1:200, Sigma, Oakville, ON) and complement receptor 3 (OX-42, mouse monoclonal, 1:500, Serotec, Raleigh, NC, USA). Primary antisera was diluted in PBS containing 3% normal goat serum and 0.3% Triton X-100. Sections were incubated in primary antibodies overnight, at 4 °C. After a 3×15 min PBS rinse, secondary antisera (Cy3 or Alexa 488 conjugated, Affinity Purified; Jackson ImmunoResearch, West Grove, PA, USA) were applied for 4 h at room temperature. Secondary antibodies were diluted in PBS + 3% normal goat serum + 0.3% Triton X-100. Afterward, sections were coverslipped in 50:50 glycerol:PBS following a final 3×15 min rinse in PBS. Confocal microscopy (Zeiss LSM 510) was used to image the fluorescently labeled cells.

TUNEL staining was used to identify DNA fragmentation, which is a hallmark of apoptotic cell death, and is also known to be displayed when cells undergo necrotic cell death. Frozen brain sections were incubated (45 min, 37 °C) in the TUNEL reaction solution, which contained biotin-labeled dUTPs, and was prepared according to the manufacturer’s instructions (Roche, Laval, PQ). The sections were washed in PBS (3×10 min) and incubated in PBS containing streptavidin-conjugated FITC (1:500). In order to identify dead or dying neurons, half of the TUNEL-stained sections were co-labeled with the neuron-specific NeuN antibody. Negative controls were processed in the same manner with the omission of the transferase enzyme from the reaction solution. Colocalization of staining in labeled sections was observed using laser scanning confocal microscopy (Zeiss LSM 510).

Immunoreactivity for NeuN, OX42 or GFAP, as well as TUNEL reactivity was quantified immediately outside the hematoma core at 6 h, 1 day, 3 days, or 7 days after thalamic hemorrhage. A total of six brains were examined at each time point. Frozen sections (16 μm thick) were processed for immunohistochemistry or TUNEL staining. The number of immunoreactive cells was counted in coronal sections of brains from six experimental animals. In each section, counts were made from four regions of interest around the circumference of the hematoma (each region of interest covering an area of 250×250 μm). A total of four sections representative of the anterior–posterior extent of the hematoma were quantified from each animal, and the results for the six total animals were averaged. The data were expressed as the number of cells/mm²±SEM. All confocal images were processed to remove background using automated software (ImageJ, ver 1.37c, NIH) and cell counts were performed manually after processing in the same program.

Nociceptive assessment

Injury to the thalamus produces contralateral mechanical hypersensitivity, typically localized to the limbs (Frese et al., 2006). In order to determine if thalamic hemorrhage results in altered pain perception, animals were assessed using standard nociceptive tests (Yashpal et al., 1998; Le Bars et al., 2001), at 7 days following hemorrhage. In the first test, animals were examined in a clear Plexiglas box with a mirrored bottom that permitted observation of the use of the forelimb and hind limb for weight bearing and vertical exploratory behavior. Changes in the contralateral affected limb, were assessed by comparing it to the ipsilateral unaffected limb. Animals were tested in a blinded manner and nociceptive scores assigned based on the following criteria: 0 = exploratory use and weight bearing was indistinguishable from the ipsilateral side, 1 = reduced weight bearing on the contralateral limb, 2 = no weight bearing on the contralateral limb, combined with elevation of the limb, 3 = extreme avoidance of limb contact with vocalization and “shielding” the limb from contact by researcher. The second test consisted of a series of toe pinches, delivered by a researcher blinded to the experimental treatments. Toe pinch (500 g force) was delivered using forceps with a built-in stopper to limit travel and prevent over-compression of the tissue. No evidence of lesions was apparent during testing or afterward. Reactions were compared between the contralateral (affected) and ipsilateral (unaffected) limbs. Normally, the toe pinch stimulus elicits little response from a rat that is regularly handled, however CPSP may result in this stimulus being perceived as painful. Reaction to mild toe pinch stimulus was graded as 0 = no reaction, 1 = limb withdrawal, 2 = limb withdrawal with vocalization. Trials were spaced at 30 s intervals and a score was assigned after four successive trials. Results were expressed as mean±SEM, and statistically significant differences between groups were calculated using the Mann–Whitney test for non-parametric data at P<0.05.

Thermal testing for hind limb sensitivity was performed at 7, 14, and 21 days after thalamic hemorrhage in a separate group of six animals. A feedback-regulated hotplate equipped with a surrounding enclosure 20 cm high was used to test thermal sensitivity. The hotplate surface was maintained at 50 °C in order to prevent possible tissue damage due to higher temperatures. Animals were initially placed on an identical hotplate set at 37 °C for a 2 min acclimation period in order to account for possible variance in the baseline hind limb temperature environment. Animals were then transferred to the 50 °C hotplate for a duration of 90 s. Withdrawal and licking of the hind limb was classified as a positive response to thermal stimulation. The time (in seconds) of withdrawal and the limb that was withdrawn (left or right) were recorded over the course of the 90 s test. Animals were returned to


...of storing at 4 °C to ensure the integrity of the tissue. Tissue sections were then subjected to various immunohistochemical techniques. Confocal microscopy was used to analyze the distribution of specific markers, such as NeuN, GFP, and OX-42, in order to investigate the presence of neuronal, glial, and macrophage/microglial cells, respectively. The data were analyzed using appropriate statistical methods, and the results were expressed as mean±SEM. The significance of differences between groups was determined using the Mann–Whitney test for non-parametric data at P<0.05.

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their cages and the test was repeated 15 min later. The latency until first withdrawal of each hind limb (contralateral=affected vs. ipsilateral=unaffected) was averaged from the results of the six experimental animals. The asymmetry of hind limb withdrawal was calculated as the percentage of total hind limb withdrawal of the contralateral or ipsilateral hind limb. Results were expressed as mean±SEM, and statistically significant differences between groups were calculated using Tukey’s post hoc test at P<0.05 and P<0.01.

Cold nociceptive testing was performed according to the procedure described by Flatters and Bennett (2004), at 7, 14, and 21 days after injury. With the animals standing on a wire mesh floor, a drop of acetone was placed against the center of the plantar surface of the hind limb. The response of the animal was monitored to 30 s. If the rat did not withdraw, flick or stamp its paw within this period then no response was recorded for that trial. However if the animal responded to the cooling effect of the acetone, the response was monitored for an additional 30 s in order to observe nociceptive related behavior separately from startle response, and to permit complete observation of the interrupted series of behavior associated with acetone cooling. Responses to acetone were graded on the following scale: 0=no response; 1=quick withdrawal, flick or stamp of the paw; 2=prolonged withdrawal or repeated flicking of the paw; 3= repeated flicking of the paw with licking directed at the ventral side of the paw. Acetone was applied alternately three times to each paw and the responses scored. The scores were then added for a cumulative response. Statistical analysis was carried out as described above.

RESULTS

Neuronal degeneration and glial reactivity after thalamic hemorrhage

Using TUNEL staining to identify dying cells and immunoreactivity to identify neurons (NeuN), astrocytes (GFAP), and microglia (OX42), we assessed the temporal and spatial relationship between neuronal death and gliosis after hemorrhage in the thalamus. Collagenase injection into the thalamus produced a small localized hemorrhagic stroke (Fig. 1A). The hemorrhage was localized to the ventral posterolateral nucleus of the thalamus and did not extend into the adjacent white matter of the internal capsule. Lesions did not extend vertically into the dorsolateral thalamus. A small amount of bleeding was observed in the hippocampal formation and overlying cortex, along the needle track in some animals. No bleeding into the lateral ventricle or midline third ventricle was observed following this procedure. The mean infarct volume (2.3 mm²±0.34) was calculated from serial cryostat sections of fixed brains stained with Cresyl Violet. These findings indicate that a small infarct localized to the thalamus is created by collagenase infusion. Hemorrhage in the thalamus was accompanied by the activation of microglia (Fig. 1C), relative to their resting ramified state in normal tissue (Fig. 1B). Myeloperoxidase (MPO) positive neutrophils (Fig. 1E) entered the brain tissue with the initial bleed and were only localized to the hemorrhagic core, and not found in the vicinity of surviving neurons (Fig. 1D). There was no additional neutrophil infiltration after 24 h.

Within 1 day of stroke onset, there was a striking difference in the staining pattern of the brain parenchyma in the vicinity of the hematoma. Most noticeably, the majority of NeuN positive cells inside the hematoma showed evidence of cell shrinkage and were TUNEL positive (Fig. 2), indicative of DNA fragmentation that accompanies cell death. In contrast, no TUNEL positive neurons were observed in the surrounding intact parenchyma. At the edge of the hematoma we observed a sharp decrease in the number of neurons although very few TUNEL positive cells were present within this region. While both activated microglia and astrocytes were present in the parenchyma surrounding the hematoma, the majority of activated microglia were present at the edge of the hematoma (Fig. 2). Interestingly, no microglia or astrocytes were observed inside the hematoma at 1 day after hemorrhage, most likely due to the massive tissue necrosis within the central infarct zone. These results demonstrate that neuron death after hemorrhage is limited to the hematoma and a small band of tissue at the edge of the hematoma at 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.

By 3 days after hemorrhage, the band of tissue at the edge of the hematoma was almost completely devoid of NeuN positive cells (Fig. 3). Similar to day 1, no TUNEL positive neurons were observed outside of the central hematoma (Fig. 3). In addition to the lack of NeuN immunoreactivity, the edge of the hematoma was now defined by a prominent band of activated microglia/macrophages. Although the accumulation of activated microglia/macrophages may contribute to the loss of neurons at the edge of the hematoma, these results suggest that the majority of these cells migrate into the area in response to neuronal injury. Interestingly, activated astrocytes in the surrounding parenchyma were separated from the hematoma by the band of activated microglia/macrophages (Fig. 3). Despite the lack of neuronal degeneration outside of the hematoma, there was a noticeable increase in the number of activated microglia and astrocytes in the parenchyma surrounding the hematoma, compared to day 1. These findings indicate that by 3 days after thalamic hemorrhage, a wall of activated microglia/macrophages develops around the central hematoma, surrounded by an external zone containing hypertrophied astrocytes. Neurons located beyond this region in the peripheral brain parenchyma, do not show any signs of degeneration at this time point.

At 7 days after thalamic hemorrhage, three distinct cellular boundaries were now clearly defined. Most prominently, a thick wall of macrophages was observed closest to the hematoma, forming what appeared to be a barrier between the necrotic lesion and the surrounding parenchyma (Fig. 4). Next, reactive astrocytes aligned their processes along the outer extent of the collection of microglia/macrophages, thereby creating a second barrier between the hematoma and the adjacent neurons (Fig. 4). Importantly, we did not observe any neuron death outside of the hematoma at 7 days (Fig. 4). These findings show that a glial limitans consisting first of activated microglia/macrophages and then astrocytes forms around the thalamic hemorrhage within 7 days. The glial barrier likely protects...
neurons in the surrounding parenchyma from toxic by-products of the hematoma.

We quantified NeuN, TUNEL, OX-42, and GFAP immunoreactivity at 6 h, 1 day, 3 days, and 7 days after injury.

Fig. 1. Thalamic hemorrhage in the adult rat. (A) A hemorrhage in the ventral posterolateral thalamus is apparent (black arrow), within 24 h of collagenase injection. The blood in the small localized hematoma has clotted by this time. (B) OX-42 immunostaining shows the normal resting appearance of brain microglia in the region of the thalamus identified by a box in (A). Resting microglia have small cell somata, and extensive ramified processes. (C) At 24 h after thalamic hemorrhage, activated microglia had a rounded or amoeboid morphology at the edge of the hemorrhage core (box in A). (D) NeuN immunoreactivity showed surviving neurons (right side of image) immediately adjacent to the hemorrhage that contains dead and degenerating neurons. (E) The same section in (D) was double-labeled for MPO. MPO positive neutrophils were apparent within the first 24 h after hemorrhage. Neutrophils were only observed in the hemorrhage core (left side of image), and no additional neutrophil infiltration was seen beyond the first 24 h post-hemorrhage. Scale bars= 25 μm (B, C), (D, E)= 200 μm.
Immunoreactivity for each marker was measured immediately beyond the outer perimeter of the hematoma, the region where neurons degenerate after hemorrhage. In comparison to the unlesioned contralateral thalamus the number of NeuN positive neurons significantly decreased as early as 6 h after injury (Fig. 5A). NeuN reactivity at the border of the hematoma continued to decrease until 7 days after injury, at which time no NeuN positive cells could be detected adjacent to the hematoma. Decreases in the number of NeuN positive cells were accompanied by increased TUNEL staining. The number of TUNEL positive cells outside the hematoma increased significantly between 6 h and 3 days after injury (Fig. 5B). No TUNEL positive nuclei were detected in the contralateral thalamus. Decreases in the number of NeuN positive cells were accompanied by increased TUNEL staining. The number of TUNEL positive cells outside the hematoma increased significantly between 6 h and 3 days after injury (Fig. 5B). No TUNEL positive nuclei were detected in the contralateral thalamus. The number of TUNEL positive cells declined significantly between 3 and 7 days after injury. Together with the decline in NeuN positive cells, these results demonstrate that neuronal cell death at the edge of the hematoma is confined to the first 7 days after thalamic hemorrhage, and complete in nature. As neuronal cell death was occurring outside the hematoma, there were corresponding increases in OX-42 and GFAP immunoreactivity. The number of OX-42 positive microglia/macrophages and GFAP positive astrocytes increased significantly between 1 and 7 days after injury relative to the contralateral side (Fig. 5C, D). No significant changes were observed at 6 h. Therefore, glial reactivity and hypertrophy that accompany thalamic hemorrhage are protracted until 24 h after injury and gradually increase thereafter.

**Mechanical nociceptive observations**

A behavioral assessment designed to detect changes in pain perception indicative of CPSP was carried out on animals that had received thalamic hemorrhages or sham (saline) injections at 3 and 7 days following surgery. Animals did not show signs of any behavioral alterations or mechanical hypersensitivity at 3 days following injury. At 7 days after thalamic hemorrhage however, animals began to show an avoidance to weight bearing and the use of the limbs on the contralateral side to the injury (Table 1). Animals also demonstrated hypersensitivity to mechanical...
stimulation (toe pinch), with a few of the animals “shielding” the affected limb and vocalizing in response to approach by the researchers. One animal in the experimental group did not appear to have an altered mechanical sensitivity. Saline-injected animals did not show a noticeable sensitivity in weight bearing or the mechanical toe pinch stimulus. When statistical analysis was performed on the averaged nociceptive scores (Fig. 6), statistically significant differences between control and experimental animals were present in the hind limb box exploration task (Mann–Whitney; \( P < 0.0301 \)) and in the hind limb toe pinch test (Mann–Whitney; \( P = 0.0120 \)). Although the forelimb tests showed an increased mean response by experimental animals in both tests, these results were not significant (Mann–Whitney; forelimb box \( P = 0.0502 \), forelimb pinch \( P = 0.1241 \)). These findings indicate that thalamic hemorrhage injury in rats induces various degrees of mechanical hypersensitivity, that is most evident in the hind limb. Thus, it will be important to study how the physiology of this injury correlates with the presence and severity of symptoms in this model.

**Thermal nociceptive testing**

Hypersensitivity to thermal stimuli is a common hallmark of thalamic syndrome. We therefore tested the effects of elevated temperature on hind limb sensitivity after thalamic hemorrhage. Temperature elevation (50 °C) under the hind limbs induced limb withdrawal and repeated licking of the limb. We quantified the latency until first withdrawal of the contralateral limb was significantly lower on average over the 3-week testing period. The latency for contralateral limb retraction ranged from 15 (1 week) to 28 (2 weeks) seconds, whereas ipsilateral withdrawal latency ranged from 50 (3 weeks) to 58 seconds.
Thus the contralateral limb was withdrawn two- to threefold earlier than the ipsilateral limb on average. In addition to latency, we observed significant differences in the asymmetry of limb withdrawal (Fig. 7). The contralateral limb was withdrawn a higher percentage of the time, over the 3-week testing period. Together these results show that nociceptive sensitivity in the contralateral limb is significantly elevated compared to the ipsilateral limb after thalamic hemorrhage.

We further tested the thermal effects of thalamic hemorrhage by rapidly cooling the ventral surface of the hind limb via acetone application (Flatters and Bennett, 2004). None of the animals in this study showed any reaction to cooling of the hind limbs, despite demonstrating altered nociceptive sensitivity to elevated temperature. These findings show that thalamic hemorrhage in this model does not produce a noticeable hypersensitivity to cold.

**DISCUSSION**

In this paper, we describe the development of a rodent model of thalamic syndrome, based on hemorrhagic injury; a known cause of thalamic syndrome in humans (Boivie et al., 1989; Leijon et al., 1989; Vestergaard et al., 1995; Bowsher et al., 1998, 2005; Fewel et al., 2003; Greenspan et al., 2004; Frese et al., 2006). This model of thalamic hemorrhage is easy to perform however it is essential that each batch of collagenase be titrated in order to find a concentration that produces a small hematoma, restricted to the ventral posterolateral nucleus of the thalamus. While the optimal concentration of collagenase may vary between batches, the total volume of collagenase solution injected into the brain should not change. Increased volumes will allow the collagenase to diffuse out of the thalamus while lower volumes will increase variability.
Neuron death was rapid and restricted to the hematoma immediately after thalamic hemorrhage, however there was a continued loss of neurons at the immediate edge of the hematoma for at least 3 days after onset. The apparent loss of NeuN reactivity in this zone most likely represents the rapid uptake of apoptotic cells by activated microglia migrating into the region. This is striking because in the brain parenchyma adjacent to the edge of the hematoma we did not observe any neuron death. Neurons appeared intact (NeuN positive) and there was no evidence of TUNEL positive cells throughout the time course of the study. Although there was no additional neuron death after day 3, animals developed signs of increased pain sensitivity within 7 days after stroke onset. Importantly, neuropathic pain also developed without any degeneration of neurons outside of the hematoma. Activated microglia/macrophages and astrocytes appeared to wall off the hematoma, which may prevent the loss of neurons outside of the hemorrhage core. Alterations in the physiology of the remaining neurons, that are surrounded by activated microglia and astrocytes, may contribute to the neuropathic pain observed in this model of CPSP.

Both microglia and astrocytes are believed to play an important role in neuropathic pain (Moalem and Tracey, 2006). Recent studies suggest that microglia are more important for the initiation of neuropathic pain, whereas astrocytes are involved in the maintenance of neuropathic pain (Colburn et al., 1999; Sweitzer et al., 1999; Raghavendra et al., 2003). Activated microglia outside of the hematoma may stimulate repair and reorganization through the production of neurotrophic factors (e.g. BDNF, IGF, GDNF) however the production of growth factors by microglia after spinal cord injury has been associated with neuropathic pain (Coull et al., 2005; Griffen et al., 2007). Indeed, the release of BDNF by activated microglia has been shown to shift neuronal anion gradients in the spinal cord, producing neuropathic pain (Coull et al., 2005). Furthermore, activation of the complement cascade in spinal microglia also induces neuropathic pain after injury (Griffen et al., 2007). It is possible that following thalamic hemorrhage, blood products diffusing out of the hematoma activate the complement cascade in microglia in the surrounding brain parenchyma. Whether this is a critical component that contributes to thalamic syndrome is currently unclear. Despite the lack of neuron death in the surrounding parenchyma, microglia and astrocytes remained activated for at least 7 days after onset. It is possible that these microglia become activated by and then subsequently protect nearby neurons from toxic compounds (e.g. products of hemoglobin degradation, glutamate, thrombin) diffusing out of the hematoma. However, their prolonged activation may impair normal neuron signaling through the production and release of reactive oxygen species, inflammatory cytokines, and growth factors. Accordingly, future studies will assess histological and physiological changes occurring outside of the hematoma in the days and weeks after thalamic hemorrhage.

Nociceptive symptoms in experimental animals differed following hemorrhage, similar to that observed in

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humans (Boivie et al., 1989; Greenspan et al., 2004; Bowsher, 2005; Kim et al., 2007). This is likely due to minute differences in hemorrhage development and location within the thalamus. In humans, thalamic syndrome has been linked specifically with stroke localization in the nucleus ventralis caudalis (Lenz et al., 1994a,b). Our lesion, located caudally in the ventrolateral thalamus produced increased pain sensitivity to mechanical stimuli and heat. However, we did not observe any altered sensitivity to cold in our study. Altered pain responses to mechanical stimulation and temperature changes appear common in CPSP. In a detailed sensory study, Boivie et al. (1989) reported that out of a total of 27 patients with CPSP (nine with thalamic lesions), all except for two showed altered temperature sensibility and only one patient did not demonstrate an abnormal sensibility to pinprick stimulation. One study reported increased thresholds for both cold- and heat-induced pain and the presence of cold allodynia in 56% of patients (Vestergaard et al., 1995). Conversely, it was reported that while most patients exhibited decreased cold perception, few exhibited cold allodynia (Greenspan et al., 2004). These findings point to the location of thalamic lesions in determining the manifestation of pain in response to differing stimuli. Indeed a recent study correlated cold allodynia with lesions of the dorsal thalamus, and movement induced allodynia with more anteriorly placed lesions (Bowsher, 2005). Of note, we did not observe any increased sensitivity to cooling of the hind limbs in our model despite enhanced nociceptive sensitivity to heat. This may be due to the placement of the lesion (ventrolateral) in our model, as opposed to a dorsally placed lesion which correlates with cold allodynia (Bowsher, 2005). It has also been recently reported that lesions localized to the ventrocaudal thalamus are sufficient to cause deficiencies in cold sensation together with CPSP (Kim et al., 2007). Conversely, heat pain perception was not affected by these selective lesions (Kim et al., 2007). Perception of heat was only impaired with lesion extension posterior to the ventral caudal nucleus (Kim et al., 2007). Thus the sensitivity to heat in our model may be a result of the posterior extension of the hemorrhage, or inflammatory processes surrounding the hematoma. Interestingly La-Buda et al. (2000) demonstrated that rats develop mechanical and thermal hypersensitivity after kainite-induced lesions of the ventral posterolateral thalamus. Similar to our study, their lesions that were confined to the same region of the thalamus, significantly reduced the latency of withdrawal to radiant heat applied to the hind paw. Furthermore, it has been shown that electrolytic lesions of the lateral or medial thalamus increased the sensitivity of rats to mechanical and heat stimuli (Saadé et al., 1999). Thus it is possible that due to differences in lesion development or thalamic architecture, rats show a more pronounced tendency to develop a noxious hypersensitivity to heat.

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Table 1. Nociceptive scores for thalamic hemorrhage

Table of nociceptive scores between animals that received thalamic hemorrhages (experiments 1–6) and animals that received control sham saline injections (Ctrl 1–6). Scores were determined by the same investigator in a blinded manner as described in the Experimental Procedures section. Animals that received thalamic hemorrhages showed various levels of increased nociceptive sensitivity in hind limb and forelimb pinch tests. Furthermore, experimental animals also displayed reduced weight bearing and exploratory activity with the affected limbs. One experimental animal (experiment 2) did not show any apparent differences from that of control animals.
rather than cold following lesions of the posterolateral thalamus. Given previous findings and the present data, it is important to note that similar to human CPSP after thalamic stroke, rats develop the characteristic hallmarks of altered nociceptive responses to mechanical and thermal stimuli.

The time course of CPSP development is also accelerated in our rat model, relative to human CPSP which typically develops months after thalamic stroke (Jensen and Lenz, 1995). Our findings show that hypersensitivity to mechanical and thermal stimuli was 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. Symptoms after hemorrhage were persistent for at least 3 weeks in our testing, and 6 weeks following electrolytic lesions (Saadé et al., 1999). Together, these experiments show that the development of hypersensitivity in rats is greatly accelerated in comparison to humans. It is unclear why this occurs, however the rapid and consistent development of symptoms in rats is beneficial in terms of studying the mechanisms that underlie these processes.

Stroke is the leading cause of disability in the industrialized world, occurring at a rate of approximately 500,000–700,000 cases annually in North America alone. Epidemiological studies have revealed that up to 8% of stroke patients are affected by CPSP (Andersen et al., 1995). Previous methods of treating CPSP including the administration of lidocaine, opiates, tricyclic antidepressants, or antiepileptic drugs, have had minimal success and significant negative side effects, however therapeutics such as amitriptyline, gabapentin, and lamotrigine have shown clinical efficacy (Nicholson, 2004). Despite widespread research into peripheral pain, much less is known about the pathophysiology of central pain syndromes. The accelerated development of nociceptive hypersensitivity after thalamic hemorrhage in rats will make it possible to study the processes that underlie thalamic central pain. This model may also serve as the basis for developing and testing central pain therapeutics in vivo, providing new avenues for the future of pain treatment.

Fig. 7. Results of thermal nociceptive testing in six rats that had received thalamic hemorrhages (mean ± SEM). Significant differences in the latency to withdraw the contralateral (affected) vs. ipsilateral (unaffected) limb in response to elevated temperature were observed at 1 (A), 2 (B), and 3 (C) weeks after hemorrhage. At all time points, the latency to withdraw the contralateral hind limb was two- to threefold lower than the ipsilateral limb (left panel). Additionally, the percentage of total limb retractions (right panel) was heavily biased toward the contralateral hind limb, in comparison to the unaffected ipsilateral limb. * P < 0.05.
REFERENCES


Weng HR, Lenz FA, Vierck C, Dougherty PM (2003) Physiological changes in primate somatosensory thalamus induced by deafferentation are dependent on the spinal funiculi that are sectioned and time following injury. Neuroscience 116:1149–1160.


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