# Central Pain Following a Collagenase-Induced Hematoma in the Basal Ganglia and Thalamus Can Be Reversed with Gabapentin

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***Summary***
The objective of this study was to evaluate pain sensitization in rats following the induction of an intracerebral haemorrhage located in the basal ganglia and/or thalamus using the Rosenberg model (intracerebral injection of collagenase). Thirty male Sprague-Dawley rats weighing between 175-300 g were used. In a first experiment, 3 groups of 6 animals were used to evaluate pain threshold using the Hargreaves test (thermal sensitivity). Following 3 days of behavioural testing (baseline values), animals in each group were injected intracerebrally either with 0.5, 1 or 2 µL of a collagenase solution (0.5 U/2 µL Type VII collagenase) which induced a hematoma in the right caudoputamen nucleus and/or thalamus. They were then tested for the next 9 consecutive days. No pain-related behavioural changes were observed following injections with 0.5 and 1 µL of collagenase. However with 2 µL, reaction times were significantly faster on days 3, 4, 5, 6 ($p < 0.0001$) and 7 ($p < 0.006$) in the right and left hind paws compared to baseline values. The lesion was localized only in the caudoputamen nucleus for animals receiving 0.5 and 1 µL of collagenase whereas lesions extended in the ipsilateral thalamic nuclei (lateral-dorsal and lateral-posterior nuclei) for animals receiving 2 µL of collagenase. In a second experiment, gabapentin reversed mechanical allodynia, evaluated with von Frey filaments, and hyperalgesia, evaluated with Hargreaves test, in rats (n=6) following a collagenase-induced (3 µL) hematoma. In conclusion, these preliminary results suggest that central pain was induced in rats with a collagenase-induced intracerebral haemorrhage localized in the caudoputamen nuclei most probably associated with lesions to the thalamus, and concurrent allodynia and hyperalgesia were reduced with gabapentin treatment.

***Introduction***
Intracerebral haemorrhage, mainly caused by trauma and stroke in humans, is one of the principal causes of incapacitation and death in the population of industrialized countries (*Fearside & Simpson, 1997; Graham & Gennarelli, 1997*). Cerebrovascular accidents often cause an intracerebral hemorrhage leading to severe and debilitating neurological symptoms and they are the third highest cause of mortality in the United States, following cardiovascular disease and cancer (*Fearside & Simpson, 1997*).

Traumatic cerebral haemorrhages are also seen in veterinary medicine. Nuclear magnetic resonance has shown that dogs may suffer from cerebrovascular accidents leading to brain haemorrhage (*Platt & Garosi, 2003*). Primary and secondary hypertension in dogs predisposing to intracranial haemorrhage (*Dukes, 1992*) is known to occur with hypothyroidism and hyperlipoproteinemia (*Thomas, 1996*). Diseases such as sepsis, coagulopathy, neoplasia and

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heartworms are also associated with cerebral infarction in dogs (*Platt & Garosi, 2003*). Therefore, different pathologies in veterinary medicine of companion animals cause intracerebral haemorrhaging; however, little is known about the development and perception of pain associated with these cerebral insults. Mainly cats and monkeys have been used in research to understand the role of central nervous system structures in the production of central pain (*Craig, 1998*). A recent paper has shown that central pain occurs in rats following lesions in the thalamus (*Wasserman & Koeberle, 2009*).

Central pain has been well documented in humans. Causes include stroke, multiple sclerosis, Parkinson's disease, spinal cord injury and brain trauma. The main cause has been identified as central post-stroke pain (*Andersen et al., 1995*). Central pain in humans also occurs following haemorrhaging in the basal ganglia (*Kim, 2003*). Most animal models of central pain are based on brain stem and spinal cord injuries (*Pagni*, 1989) obtained by dropping a weight (*Anderson 1982; Greenberg et al., 1978*), compression (*Tarlov, 1972*), crushing (*Rivlin & Tator, 1978*), photochemically induced injury (*Hao et al., 1992; Watson et al., 1986*), excitatory neurotoxin methods (*Gorman et al., 2001; Wilcox, 1988*) and spinal hemisection (*Bennett et al., 2000; Christensen et al., 1996 & 1997*). In rats, central pain has been shown to occur following lesions in the thalamus (*Wasserman & Koeberle, 2009*).

The objective of the present studies was to evaluate if central pain occurs following the induction of an intracerebral haemorrhage using the Rosenberg model (intracerebral injection of collagenase) where the lesion in mainly in the caudoputamen nuclei. Two behavioural tests were used to evaluate neuropathic pain, the Hargreaves test and von Frey filaments to evaluate thermal hyperalgesia and mechanical allodynia respectively. To confirm that the changes that occurred with brain trauma were related to pain, and not motor skills, gabapentin, a commonly used central analgesic for the treatment of neuropathic pain was administered.

***Materials and Methods***

Thirty male specific pathogen free Sprague-Dawley rats (Crl:CD (SD) IGS BR stock; Charles River, St-Constant, Qc, Canada) weighing between 175-300 g were used in this study. Following arrival, they were housed in a standard environment (fresh filtered air: 15 changes/h, temperature: 21 ± 3°C, humidity: 40-60 % and light-dark cycle: 12h:12 h). They were pair-housed in polycarbonate cages (Ancare, Bellmore, NY, USA) on hardwood bedding (Beta chip, North-Eastern Products Co., Warrenburg, NY, USA) and acclimated to their environment for 7 days prior to the initiation of the study. Rats received tap water and rodent chow (Charles River Rodent Chow 5075, St-Constant, Qc, Canada) *ad libitum*. The University of Montreal's Faculty of Veterinary Medicine Institutional Animal Care and Use Committee approved the experimental protocol prior to animal use in accordance with the guidelines of the Canadian Council on Animal Care (*CCAC, 1993*).

*Experimental design*

For the first experiment, eighteen intact rats were trained for 5 days on the Hargreave's test only. Behavioural testing was performed for 3 days to obtain baseline values of thermal sensitivity. Rats were then injected intracerebrally with 0.5 µL (n = 6 rats; Group 1), 1 µL (n = 6 rats; Group 2) or 2 µL (n = 6 rats; Group 3) of a collagenase solution (0.5 U/2 µL) to produce a hematoma centered in the caudoputamen nuclei (basal ganglia) in the right cerebral hemisphere. Following the surgery, thermal sensitivity of both left and right hind paws was determined daily using the Hargreaves test for 9 consecutive days. Thermal sensitivity to a progressive increase in intensity of heat from a light source (Plantar test Hargreaves method, IITC Life Sciences, Series 8, Model 390, Woodland Hills, CA) was used. The beam of light was applied on the left and right hind paws and animals voluntarily retracted their hind paw from the light source upon reaching the threshold of intolerable pain sensation. The maximum duration over which the hind paw was exposed was set at 20 sec. Reaction times

were taken on three occasions, with a 20 min delay between trials, and the daily reported value for each animal was the mean of 3 trials for both the right and left hind paw.

In a second experiment, a group of animals (n = 12) underwent training for 5 days on the Hargreaves test and von Frey filaments. Following training, baseline values were recorded for 3 days. Hargreaves test was performed in a similar fashion to the first experiment of this study. Von Frey filaments were used to evaluate mechanical sensitivity according to a previously published method (*Vachon et al., 2004*). Briefly, paw withdrawal thresholds were evaluated on the plantar surface of the right and left hind paw using von Frey monofilaments (0.4 g – 30 g; Stoelting, Wood Dale, IL, USA). Monofilaments were applied for 2 s in the central region of the plantar surface. The threshold was taken as the lowest force that caused at least 3 withdrawals out of 5 consecutive stimuli. Values reported are the threshold force in grams corresponding to the hind paw withdrawal. Following the collection of baseline data, rats were injected intracerebrally with 3 µL of a collagenase solution (0.5 U/2 µL) to produce a hematoma centered in basal ganglia of the right cerebral hemisphere. Both tests were to be performed once on the 2nd day following surgery to establish that neuropathy was present and then twice daily (once without and once with treatment) on subsequent days for the treatment group (n = 6) and saline gavage for the control group (n = 6). Treated animals received 100 mg/kg gabapentin (*Nakazato-Imasato et al., 2009*) by gavage (100 mg/mL; 1% methylcellulose solution) 30 min prior to behavioural testing. A rest period of 1 h was respected between behavioural evaluations. The person doing the evaluations was blinded for both experiments.

Prior to and following the intracerebral collagenase injection, animals were evaluated daily, prior to experimentation, with selected motor behavioural tests that have previously been described (*Lema et al., 2004 & 2005*). Rats were acclimated to the apparatus of both tests for 20 min prior to each daily testing. This exam consisted of general activity, evaluated as the immediate exploration of the immediate environment when the animal was placed on a novel hard surface and locomotion (linear locomotion or unilateral rotation), observed at the same time. Paresis was evaluated by holding the rat by the tail and lowering it toward a hard surface (visual positioning test). A normal response consists in a full extension and reaching for the hard surface. Tail rigidity was evaluated by simply elevating it at midlength and observed for flexibility or rigidity.

*Surgical method*

The surgical procedures have been previously described (*Lema et al., 2004 & 2005*). Following the induction and maintenance of anaesthesia with isoflurane (Aerrane, Baxter, Mississauga, ON, Canada), rats were placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA). A sagittal skin incision was made and a burr hole (1.5 mm diameter) was drilled in the bone at the following stereotaxic coordinates: antero-posterior 0.0 mm and lateral 3.0 mm, in reference to bregma, so that the injection site would be in the right caudoputamen nuclei (*Paxinos & Watson, 1998*). To induce the hematoma, a collagenase solution composed of 2.5 U collagenase Type VII (Sigma-Aldrich, Oakville, ON, Canada) in 10 µL saline (Abbott Laboratories, St-Laurent, QC, Canada) was injected over a period of 10 min via the burr hole in the right caudoputamen nucleus, 6 mm below the dura mater with a 5 µL Hamilton syringe. The needle was removed 5 min following the end of the injection and the skin was sutured. During the surgery body temperature was monitored with a rectal thermometer (Thermalert TH-8, Physitemp, Clifton, NJ, USA) and it was maintained within normal limits (35.5 - 37.5°C) by keeping animals on an electric heating pad. Following surgery, no analgesics were administered and animals were maintained under a heating lamp until recovery. In a previous study (*Ferland et al., 2007*), buprenorphine was shown to improve motor performance, reduce hematoma size and increase the number of necrotic neurons in the marginal zone of the lesion. It was therefore decided not to admin-

ister analgesics post-operatively since this could change the outcome of the study. We also wanted to reproduce the Rosenberg model in which no analgesic is administered either pre- or post-operatively.

*Histological methods*

Following completion of the behavioural testing animals were anesthetized to a surgical plane using pentobarbital (100 mg/kg, IP) (MTC Pharmaceuticals, Cambridge, Ontario, Canada). They were then perfused through the heart, first with a physiological dextrose-sucrose solution (100 mL/rat; solution composition (1L): 8 g NaCl, 4 g dextrose, 8 g sucrose, 0.23 g calcium chloride) followed by a 10% buffered formalin solution (100 mL/rat). All chemicals used for perfusion solutions were ordered from Sigma (Sigma Inc., St-Louis, MO, USA). For microscopic evaluation, brains were fixed in formalin for 48 h and embedded in paraffin. Five µm horizontal sections were taken and stained with haematoxylin, eosin, phloxin and saffron stain. All lesions were confirmed by a certified veterinary pathologist (Dr Pierre Hélie DVM, DACVP, Department of Veterinary Pathology, University of Montreal)

*Statistical analyses*

An analysis of variance using a linear model with repeated measures and *post hoc* Tukey tests were used to assess whether the changes in the Hargreaves and von Frey tests were significantly different from pre-operative values. For the analysis of variance time as the inter subject factor and treatment as the intra subject factor were used. Statistical analyses were carried out with SAS (Version 9.1, Cary, N.C.). The level of significance was set at 0.05.

***Results***

Daily motor behaviours observed following the induction of cerebral lesions were decreased exploration of the immediate environment, unilateral circling, hemiparesis of the right anterior right limb and tail rigidity which were more pronounced in animals that received 2 and 3 µL of the collagenase solution. These are typical motor changes observed following the induction of hematoma in the caudoputamen nucleus (*Lema et al., 2004 & 2005*).

In the first experiment, in groups 1 and 2 (n = 6/group) receiving respectively 0.5 and 1 µL of the collagenase solution intracerebrally, no change was observed in the Hargreaves test when comparing pre and post-surgery reaction times (results not shown). For animals receiving 2 µL of collagenase (n = 6), reaction times for both right and left hind paws were significantly decreased on days 3, 4, 5, 6 ($p < 0.001$) and 7 ($p < 0.01$) compared to baseline values (Figure 1). These rats returned to baseline reaction times for both right and left hind paws on days 8 and 9 (p = n.s.).

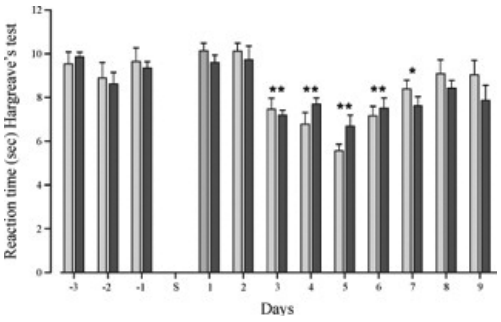


**Figure 1.** Histogram representation of the mean (± SE) thermal sensitivity (Hargreave's test) results in male Sprague-Dawley rats (right hind paw/light gray, left hind paw/dark) prior to (Days -3, -2, -1) and following (Days 1 - 9) the stereotaxic injection of 2 µL of a collagenase solution (0.5 U/2 µL) in the right caudoputamen nucleus. (S surgery). ** $p < 0.001$, * $p < 0.01$

Photomicrographs of histological sections at 48 h post collagenase-induce lesions are shown in Figure 2. In all groups, the caudoputamen nucleus presents a rarefaction of the neuropil, forming a cavity which may extend to the external capsule. The cavity is lined by few reactive astrocytes. The lesion was mainly localized in the caudoputamen nucleus for animals receiving 0.5 and 1 µL of collagenase. Lesions of the internal capsule were observed in all groups. For group 3 animals receiving 2µL of

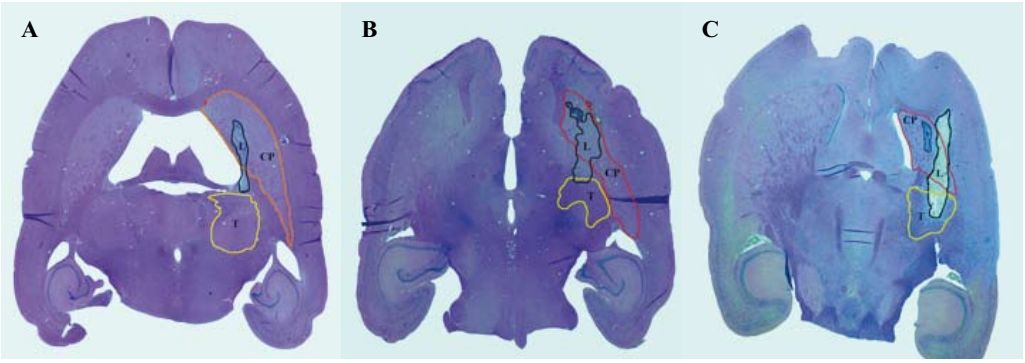


**Figure 2.** Extent of the intracerebral hemorrhage in male Sprague-Dawley rats following stereotaxic injections of 0.5 µL (**A** ; Group 1), 1 µL (**B** ; Group 2) and 2 µL (**C** ; Group 3) of a collagenase solution (0.5U /2 µL) in the caudoputamen nucleus**. L** lesioned area **CP** caudoputamen nucleus **T** thalamic nuclei

collagenase intracerebrally, lesions extend to the ipsilateral thalamic nuclei. The lateral-dorsal and lateral-posterior nuclei of these animals presented small foci of spongiosa lined by numerous microglial cells and rare gemistocytes as well as necrotic neurons.

The effect of gabapentin on mechanical allodynia (von Frey filaments) and thermal hyperalgesia (Hargreaves test) for the second experiment are presented in Figure 3A and B respectively. On the second and third day following the surgery, pain thresholds were decreased in both von Frey and Hargreaves tests ($p<0.05$-$0.001$). However following the administration of gabapentin (100 mg/kg PO) on the 3rd day, pain thresholds were not different from baseline values for both tests. On the fourth day, prior to gabapentin treatment, animals did not show allodynia or hyperalgesia which suggests a residual effect of treatment. Since no difference from baseline was noted on the 4th day, animals were not treated

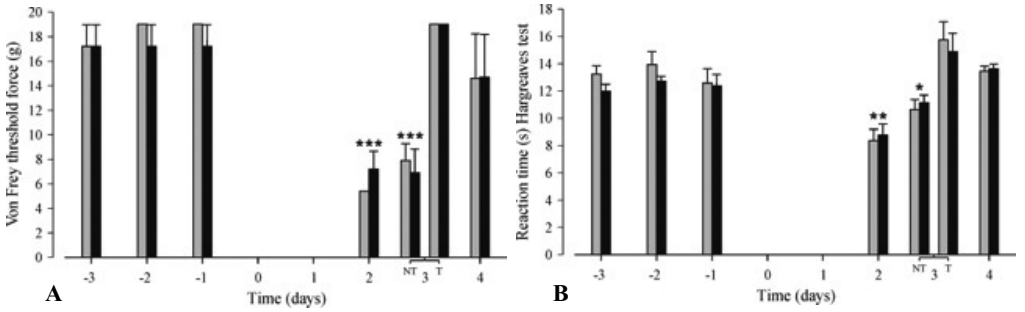


**Figure 3.** Effects of gabapentin (100 mg/kg) on von Frey (**A**) and Hargreaves (**B**) tests in male Sprague-Dawley rats (right hind paw/light gray, left hind paw/dark) following a stereotaxic injection of 3 µL of a collagenase solution (0.5U /2 µL) in the caudoputamen nucleus. No significant changes from baseline occurred in control animals receiving saline by gavage (Data not shown). Saline **NT** and gabapentin **T** treatment groups *** $p < 0.001$, ** $p < 0.01$, * $p< 0.05$

with gabapentin on the 4th post surgical day. For the control group (results not shown), mechanical allodynia and thermal hyperalgesia persisted (Allodynia mean daily values (± SE) Right/Left hind paw : 5.1 ± 0.8/ 6.2 ± 0.7; 6.2 ± 0.6/ 5.8 ± 1.1; 5.5 ± 0.9/5.0 ± 0.6 s and hyperalgesia mean daily values (± SE) Right/Left hind paw : 8.1 ± 0.7/ 9.2 ± 0.5; 9.2 ± 0.7/8.8 ± 0.6; 7.8 ± 1.1/ 8.2 ± 0.8 s) following the collagenase injection. For these animals, the lesion was more extensive in the caudoputamen nucleus and it was also present in the lateral-dorsal and lateral-posterior nuclei of the thalamus (lesion not shown).

As previously shown in other studies (*Lema et al., 2004 & 2005),* clear spaces, interpreted as edema, extended in the corpus callosum and into the contralateral basal ganglia and thalamus for the groups that received 2 and 3 µL of the collagenase solution. This may explain the bilateral effects on central pain induction.

***Discussion***

Animal models have played an important role in elucidating the cascade of cellular and biochemical events occurring after traumatic brain injury. Previous studies have reported traumatic brain injury with models such as injections of autologous blood, balloon distension, inertial acceleration, force impact and cold (*Finnie & Blumberg, 2002*). Rosenberg *et al.* (*1990*) have established the collagenase-induced intracerebral hematoma model, which is highly reproducible and shows many characteristics of the intracerebral haemorrhagic process in mammals. Only one recent paper has shown that collagenase-induced thalamic lesions can cause central pain in rats (*Wasserman & Koeberle, 2009*). Since we had experience with the Rosenberg model, and that the brain lesion caused by the collagenase injection in the basal ganglia may also affect the thalamus (main central relay of sensory perception), we decided to evaluate pain perception in this model.

Results show that central pain, when evaluated with the Hargreaves and von Frey filaments tests, was observed following the production of an intracerebral hematoma when the collagenase injection is in the basal ganglia. However central pain sensitization was noted only in animals where the lesion extended in the posterior thalamus, and not when lesions occurred in the caudoputamen nucleus only. When using this traumatic brain injury model, previous studies (*Lema et al., 2004 & 2005*) have shown that animals had poor motor coordination and a deficit in motor initiation. Since the basal ganglia plays a role in the initiation of movement and postural control, animals would be expected to have increased response times when evaluated on reaction time tests. However, results show a decreased reaction time to a thermal painful stimulus suggesting the establishment of central pain. In Huntington's disease, where there is a neurodegenerative process of the basal ganglia, reflexes are usually decreased although muscle rigidity is present (*Côté, 1981*). We therefore do not attribute the results to a change in reflex excitability at the level of the spinal cord. Also, in our experiment, normal responses were seen with von Frey and Hargreaves tests following gabapentin treatment in animals with the induced hematoma and these findings are highly suggestive of a central analgesic effect. We chose gabapentin as a central analgesic since it has been identified as part of the first line medications for the treatment of neuropathic pain by the International Association for the Study of Pain (*Dworkin et al., 2007*). However in a recent review, gabapentin was suggested as a second-line treatment for central post-stroke pain in humans *(Kumar et al., 2009*).

Lesions in the basal ganglia or thalamus will cause motor and sensory problems in the contralateral side of the lesion. In our study, animals showed pain-related behaviours in both hind paws. In the model established by Wasserman and Koeberle (2009) the total amount of collagenase injected was only 0.025 U and pain sensation was altered only on the contralateral side. We injected 0.125, 0.25 and 0.5 U in the first experiment and 0.75 U in the second experiment. The lesions were therefore more extensive than those seen in the experiments by Wasserman and Koeberle (*2009*). Animals that received 0.5

and 0.75 U had lesions that were not only localized on the ipsilateral side but oedema was observed all along the corpus callosum and in the contralateral basal ganglia and thalamus. This could very well explain why pain-related behaviours were observed in both hind paws and not only unilaterally as demonstrated in the study by Wasserman and Koeberle (*2009*). One study has shown that unilatereal thalamic lesions can in some instances cause a bilateral pain syndrome in humans (*Kim, 1998*).

To better characterize this model, behavioural testing should be performed for a longer time (weeks or months) and should evaluate different pain modalities other than mechanical allodynia with von Frey filaments and thermal hyperalgesia with the Hargreaves test. Compared to our previous results where the reaction time on the Hargreaves test decreased to 5 s in neuropathic animals with sciatic nerve ligation model, the animals with the caudoputamen-thalamus lesions had a longer reaction time (7-9 s) following the 2µL collagenase solution injection which suggests that the pain is not as severe in these animals as seen with sciatic nerve constriction models used in neuropathic research (*Guénette et al., 2007; Beaudry et al., 2010*). However with the 3µL collagenase injection, pain threshold was similar to the animals with sciatic nerve injury. Our results also show that animals appeared more affected on mechanical allodynia than thermal hyperalgesia. Interestingly it is also common in humans to have a very severe pain, mainly a mechanical allodynia, following central lesions such as a stroke (*Kumar et al., 2009*).

Lesions at any level of the neuraxis can cause central pain when pain pathways and associated structures are implicated. Lesions in the dorsal horn of the spinal cord, ascending spinothalamic pathways, brain stem, thalamus, subcortical white matter and cerebral cortex have all been reported to cause central pain (*Bowsher, 1996; Bowsher et al., 1998; Cassinari & Pagni, 1969; Garcin, 1968; Leijon 19889; Tasker, 2001*). Lesions that produce central pain have best been studied in central post-stroke pain and it was found that 60% of lesions involve the thalamus (*Bowsher, 1996*). Haemorrhages in the basal ganglia produce sensory symptoms such as dysesthesia, mainly sensory deficits (*Bowsher et al., 1998; Kim, 2003; Siddal et al., 2003*). Pain associated with lesions of lenticulo-capsular nuclei is associated with lesions in the posterior limb of the internal capsule (*Kim, 2003*) and thalamus (*Groothuis et al., 1977*). Loss of lateral thalamic substrate results in the disinhibition or release of the medial thalamus, which is thought to be important for the emotional aspect of pain (*Craig, 1998*). In the present study, lesions in the posterior thalamus were also seen in rats with the highest volume of intracerebrally injected collagenase (2 and 3 µL). Interestingly, pain was noted only on the 3$^{rd}$ day following the lesion following the collagenase injection. Post-stroke pain usually develops some time after the brain insult in 10-35 % of human patients and the time of onset of pain may be relatively rapid (within 1 month) (*Andersen et al. 1995; Hansen , 2004; Widar et al., 2002*). With spinal cord injuries onset time may be as long as 2 years (*Siddal et al., 2003*). In most cases central pain tends to persist and it is constantly present, with no pain-free intervals. However, in some cases, pain free intervals occur a few hours a day only.

In conclusion, a transient central pain was induced in rats with an intracerebral haemorrhage localized in the caudoputamen and thalamic nuclei following the stereotaxic injection of collagenase. The contralateral central pain is most probably associated with lesions of the lateral posterior thalamus mainly affecting spinothalamocortical tracts neurons implicated in pain perception. The ipsilateral central pain could be associated with the oedema observed in the contralateral non-lesioned basal ganglia and thalamus. Bilateral central pain occurred only when massive lesions were induced. Finally, findings support the alleviating effect of gabapentin on both allodynia and hyperalgesia in this model suggesting that it may be a treatment for central pain originating from thalamic lesions.

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