Pain 84 (2000) 233–245

PAIN

www.elsevier.nl/locate/pain

Bilateral behavioral and regional cerebral blood flow changes during painful peripheral mononeuropathy in the rat

Pamela E. Paulson[a,b,*], Thomas J. Morrow[a,b,c], Kenneth L. Casey[a,b,c]

[a]*Neurology Research Laboratories, V.A. Medical Center, Ann Arbor, MI 48105, USA*
[b]*Department of Neurology, University of Michigan, Ann Arbor, MI 48105, USA*
[c]*Department of Physiology, University of Michigan, Ann Arbor, MI 48105, USA*

Received 11 September 1998; received in revised form 17 June 1999; accepted 13 August 1999

Abstract

A unilateral chronic constriction injury (CCI) of the sciatic nerve produced bilateral effects in both pain related behaviors and in the pattern of forebrain activation. All CCI animals exhibited spontaneous pain-related behaviors as well as bilateral hyperalgesia and allodynia after CCI. Further, we identified changes in baseline (unstimulated) forebrain activation patterns 2 weeks following CCI by measuring regional cerebral blood flow (rCBF). Compared to controls, CCI consistently produced detectable, well-localized and typically bilateral increases in rCBF within multiple forebrain structures in unstimulated animals. For example, the hindlimb region of somatosensory cortex was significantly activated (22%) as well as multiple thalamc nuclei, including the ventral medial (8%), ventral posterior lateral (10%) and the posterior (9%) nuclear groups. In addition, several forebrain regions considered to be part of the limbic system showed pain-induced changes in rCBF, including the anterior dorsal nucleus of the thalamus (23%), cingulate cortex (18%), retrosplenial cortex (30%), habenular complex (53%), interpeduncular nucleus (45%) and the paraventricular nucleus of the hypothalamus (30%). Our results suggest that bilateral somatosensory and limbic forebrain structures participate in the neural mechanisms of prolonged persistent pain produced by a unilateral injury. Published for the International Association for the Study of Pain by Elsevier Science B.V.

Keywords: Chronic pain; Chronic constriction injury; Sciatic nerve; Neuroimaging; Regional cerebral blood flow; Rat

1. Introduction

Complete or partial damage to peripheral sensory nerves by traumatic injury, infection, disease or as the result of surgery can often lead to bizarre and intractable abnormalities in pain perception in humans. These changes include spontaneous pain, radiation of pain to a site distal to the injury, increased responsiveness to noxious stimuli (hyperalgesia), and one or more kinds of stimulus-evoked pain when previously innocuous mechanical or thermal stimuli are applied to the affected area (allodynia) (Tasker et al., 1980; Thomas, 1984; Price et al., 1989; Bonica, 1990). These aberrant sensations can be expressed within minutes, days, weeks or even months following the actual traumatic event (Baker and Winegarner, 1969; Sunderland, 1978; Payne, 1990) long after tissue healing has occurred (Bonica 1990). This suggests pathophysiological mechanisms that include not only the changes in the peripheral nervous system due to the initial injury but also more long lasting changes and reorganization within the CNS structures involved in pain processing. To investigate the mechanisms responsible for human neuropathic pain, however, it is necessary to develop animal models that mimic the symptoms observed in the human condition.

Recently, several animal models of neuropathic pain associated with peripheral neuropathy have been described (Bennett and Xie, 1988; Seltzer et al., 1990; Palecek et al., 1992; Kim and Chung, 1992). Although each of these models differs slightly in the specific method used to produce peripheral nerve damage, all produce post-surgical sensory abnormalities that are similar to those seen in humans. The Bennet and Xie model is one of the best characterized animal models used to study chronic pain in peripheral neuropathy. In this model, loose ligatures are placed around the sciatic nerve, which produces axonal swelling and a partial deafferentation manifested as a significant but incomplete loss of axons in the distal portion of the peripheral nerve (Basbaum et al., 1991). One of the prominent behaviors seen following sciatic nerve ligation is the appearance of hind paw guarding, thought to be an indica-

* Corresponding author. V.A. Medical Center, Neurology Research, (11R) 2215 Fuller Rd., Ann Arbor, MI 48105, USA. Tel.: +1-734-769-7100 ext. 5815; fax: +1-734-761-7693.

0304-3959/00/$20.00 Published for the International Association for the Study of Pain by Elsevier Science B.V.
PII: S0304-3959(99)00216-X

tion of ongoing pain. Support for this idea is derived from reports of increased spinal cord neural activity (Price et al., 1991; Mao et al., 1992), and increased spontaneous neuronal discharge in spinothalamic tract neurons (Palecek et al., 1992) and in the ventrobasal thalamus (Guilbaud et al., 1991) in the absence of overt peripheral stimulation. Several imaging studies have attempted to identify the spatial pattern of glucose metabolism or the expression of immediate early genes in the spinal cord following chronic constriction injury (CCI) induced spontaneous pain (Price et al., 1991; Mao et al., 1992). Because most investigations into the neural mechanisms of CCI-induced pain have focused on the spinal cord the functional role of forebrain structures is less clear.

Recent advances in functional brain imaging techniques now provide a powerful method for simultaneously assessing the activation of multiple brain regions during pain and other sensory experiences. Substantial evidence shows that increases in regional cerebral blood flow (rCBF) are tightly and positively coupled to increases in synaptic glucose metabolism and electrical activity (Sokoloff, 1978; Sokoloff, 1981; Mraovitch et al., 1992; Malonek and Grinvald, 1996). Studies in humans using positron emission tomography (PET) have revealed unique patterns of changes in rCBF that are associated with the perception of pain (Jones et al., 1991; Talbot et al., 1991; Casey et al., 1994; Coghill et al., 1994). Such studies provide insights into the function of the pain network as a whole; information that was previously unattainable from clinical data or individual pharmacological, stimulation, lesion or electrophysiological experiments. The experiments described here characterize the CCI-induced changes in baseline cerebral activation patterns of forebrain structures involved in pain processing up to 15 days following the constriction injury.

Some of the results of these studies were presented at the annual meeting of the Society for Neuroscience (Morrow et al., 1995).

2. Methods

2.1. Animal subjects

Thirty-three male, Sprague–Dawley (Charles River) rats weighing between 225 and 350 g served as subjects for these experiments. All animals were housed individually and maintained on a 12/12 light/dark cycle, with lights on at 06:00 h. Ambient temperature in the animal facility was kept at 22 ± 2°C. Food and water were given ad libitum. Our Institutional Animal Care and Use Committee approved all experimental procedures. We conducted all experiments in accordance with the guidelines of the NIH for the ethical use of laboratory animals (Anonymous, 1996) and of the IASP for use of conscious animals in pain research (Zimmermann, 1983).

2.2. Surgery

Animals were anesthetized with xylazine and ketamine (13 and 87 mg/kg i.m., respectively). A chronic constriction injury (CCI) was produced by ligating the common sciatic nerve on the left side using a method similar to that described by Bennett and Xie (1988). Briefly, the common sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection through the biceps femoris. Proximal to its trifurcation, about 7 mm of nerve was freed of adhering tissue and three ligatures were produced using a braided polyglocolic acid suture (4-0 Dexon Plus) at 1 mm intervals. The ligatures just barely constricted the diameter of the nerve when viewed by 30X magnification. This degree of constriction retarded, but did not arrest, the circulation through the superficial epineural vasculature and produced a small, brief twitch in the muscle around the exposure. Sham-operated controls underwent an identical dissection on the left hindlimb, but the sciatic nerve was not ligated. All surgical procedures used sterile techniques as per ULAM regulations.

2.3. Groups

2.3.1. Un-operated controls ($N = 11$)

For baseline measurement of rCBF in the absence of intentional somatosensory stimulation, each rat was allowed to remain undisturbed prior to, during and for 2–5 min following tracer injection (see below).

2.3.2. Sham-operated control group ($N = 4$)

For examining changes in rCBF in the absence of intentional somatosensory stimulation, 2 weeks following a sham CCI procedure each rat was allowed to remain undisturbed prior to, during and for 2–5 min following tracer injection.

2.3.3. Chronic pain group ($N = 18$)

For examining changes in rCBF in the absence of intentional somatosensory stimulation 2 weeks following CCI, we injected each animal with an intravenous bolus of radiotracer. Each rat was then allowed to remain undisturbed prior to, during and for 2–5 min following tracer injection.

2.4. Quantification of behavior

All animals were given 1–2 weeks to acclimate to the colony room before undergoing any surgical procedure. Over this period the rats were handled extensively and acclimated to gentle restraint in a soft towel and habituated to the various behavioral testing procedures. We quantified the following three behaviors, in the order presented: (1) resting paw posture and response to (2) mechanical and (3) thermal stimulation. These three aspects of neuropathic pain were quantified on five separate occasions: 2 days prior to surgery and days 1–2, 5–6, 8–9 and 12–15 post-operative. The rationale for the choice of this testing sequence was that the least stressful test was done first to minimize the influence of one

test on the result of the next. The sequence of behavioral tests was unchanged throughout the test period for all animals.

2.4.1. Resting paw posture

The resting paw posture of each hind paw of each animal was observed without intervention by the experimenter using the method from Attal et al. (1990). Each animal was placed in clear rectangular Plexiglas cage (20 cm wide × 60 cm long × 20 cm high) and allowed to habituate for 15 min. Different positions of the operated or un-operated hind paw were rated for the next 48 min period, using a time sampling technique. Paw position was recorded for 2 min (120 s) every 16 min according to the following scale: 0 = paw pressed normally on floor; 1 = paw rests lightly on floor, toes ventroflexed; 2 = only internal edge of paw pressed to floor; 3 = only heel pressed to floor, hind paw in inverted position; 4 = whole paw is elevated; 5 = animal licks paw. Each hindpaw was rated three times per test day. The average ratings over successive 16 min periods therefore provided an index of pain intensity for each hind paw of each rat for a 48 min period. A weighted pain score for each hind paw of each animal was calculated by multiplying the average amount of time the rat spent in each category according to the following formula: ($t1 + 2\ t2 + 3\ t3 + 4\ t4 + 5\ t5$)/120 s; where $t1$, $t2$, $t3$, $t4$ and $t5$ are the duration (in s) spent in categories 1, 2, 3, 4 and 5, respectively. Therefore, the possible scores range from 0–5; representing a continuum in pain scores of the paw from normal to abnormal.

2.4.2. Mechanical stimulation

The response to mechanical stimuli was quantified by measuring the number of foot withdrawals to application of a von Frey hair. We used the standard Semmes–Weinstein set of von Frey hairs that are used in neurological and psychophysical testing in humans. Five levels of force ranging from 0.217 to 7.37 gm were applied. Each rat was placed in a side-by-side wire hanging cage with a mesh bottom and allowed to habituate for 10 min. A von Frey hair was applied to the plantar surface of the hindfoot until just bent. This procedure was repeated five times during each trial, at a frequency of about one stimulus/s. Each trial was repeated three times at approximately 5 min intervals on each hind paw. On a given test day, this procedure was repeated for all von Frey hairs, in ascending order, starting with the weakest. The occurrence of foot withdrawal in each of these three trials were expressed as a percent total response: (# of foot withdrawals/5 × 100 = % total response).

2.4.3. Thermal stimulation

The response to thermal stimulation was quantified by measuring the number of foot withdrawals after application of a thermal probe. The thermal probe consisted of a feedback-controlled Peltier device (LT-3, Thermal Devices, Inc.) set to one of the following temperatures: 40, 43, 46, 49 or 52°C. Rats were placed in a soft towel restraint so that the hind paws were free and given 10 min to habituate. The tip of the thermal probe was applied to the plantar surface of either the operated or un-operated hind paw of the rat, with a maximum stimulus time set at 45 s to prevent tissue damage. The hind paws were tested alternately with 5 min intervals between consecutive tests. For a given test session, this procedure was repeated for all stimulus temperatures, presented in random order. Animals were tested three times at each temperature and the occurrence of foot withdrawals in the three trials are expressed as a percent total response: (# of foot withdrawals/3 × 100 = % total response).

2.5. Measurement of regional cerebral blood flow (rCBF)

Approximately 2 weeks following CCI surgery, the day of rCBF measurement, we placed each rat in the towel restraint and inserted a flexible 24 gauge intravenous catheter into the tail vein. A rubber capped injection port was attached to the catheter and flushed with approximately 0.25ml of saline solution. Each animal was then permitted to rest quietly in the restraint for approximately 30–40 min to recover from the stress induced by tail vein catheterization.

For imaging regional cerebral blood flow, we used the method of Morrow and colleagues (1998). Briefly, 8-10mCi of technetium ($^{[99m]}$Tc) exametazime in a 0.51 ml total volume was injected through the tail vein catheter as a bolus over 10–15 s. Two to 5 min following tracer injection, the rat was overdosed with chloral hydrate (300 mg/kg, iv), removed from the restraint and decapitated. The brain was removed from the skull and prepared for sectioning by rapid freezing with powdered dry ice. Standard twenty-micron coronal frozen sections were cut at − 18°C using a Hacker-Bright™ cryostat. Three to four consecutive sections taken at fixed intervals were mounted on glass slides and rapidly desiccated on a hotplate at 60°C. Slides were then arranged in a standard X-ray cassette and an autoradiogram was generated by direct apposition of the tissue to the emulsion side of Kodak BioMax™ MR-1 Imaging film (Kodak Inc., Rochester, NY) for a 1.5–3.0 h exposure.

Densitometric analysis of autoradiograms was performed using a microcomputer-assisted video imaging densitometer (MCID system, Imaging Research Inc., St. Catherines, Ontario, Canada). Anatomic location of selected regions of interest (ROIs) was determined using an approach designed to ensure the accuracy and consistency of structural identification (Morrow et al., 1998). Briefly, for each brain section, we overlaid a matching transparent stereotaxic template, adapted from the stereotaxic atlas Paxinos and Watson (1986), on the digitized brain images displayed on the video monitor and aligned the images using prominent landmarks. The template served as a guide to sample

Table 1
Anatomical abbreviations

AD	Anterior dorsal nucleus (thalamus)	PAG	Periaqueductal gray (midbrain)
BLA	Basal lateral amygdyla	PAR	Parietal cortex
CC	Cingulate cortex	PO	Posterior group (thalamus)
CPU	Caudate-putamen	PVN	Paraventricular nucleus (hypothalamus)
HBC	Habenular complex	RS	Retrosplenial cortex
HIP	Hippocampus	VL	Ventral lateral nucleus (thalamus)
HL	Hindlimb area of somatosensory cortex	VM	Ventral medial nucleus (thalamus)
IPN	Interpeduncular nucleus	VPL	Ventral posterior lateral nucleus (thalamus)
MT	Medial thalamus	VPM	Ventral posterior medial nucleus (thalamus)

the ROIs present at a given anterior-posterior level. An activation index (AI) from individual ROIs was calculated as a percent of average total activity of the entire brain.

For the purpose of this study, we limited the regions of interest (ROIs) to 18 structures. Table 1 lists all structures sampled in this study and provides a key for all anatomical abbreviations. We sampled structures known to participate in pain processing and others that are not generally included in pain pathways. Some ROIs were previously identified in human PET as showing increased blood flow during acute pain due to noxious thermal stimuli (Talbot et al., 1991; Casey et al., 1994). Due to our specific interest in the forebrain, we restricted sampling primarily to cortical and thalamic structures and the brainstem PAG. The spinal cord was not examined.

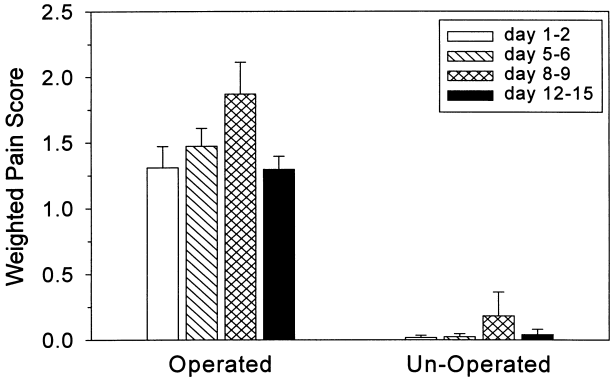

Fig. 1. The bar graph shows the mean weighted pain score for each hind paw of the CCI animals at multiple time points after surgery. The weighted pain scores were calculated by multiplying the amount of time the rat spent in each category (see Section 2). All animals were monitored for spontaneous pain behaviors before surgery and received scores of zero. Notice that the ligated hind paw exhibited a significant increase in spontaneous pain behaviors at all times tested following surgery whereas the un-operated hind paw was not significantly different from zero (no pain behavior) on any test day.

2.6. Data analysis

2.6.1. Behavioral

Parametric statistics, including repeated measures analyses of variance (ANOVA) and Fisher's least significant difference tests for follow-up pairwise comparisons were used to analyze group means for the various behavioral tests. P values < 0.05 were considered significant.

2.6.1.1. Within group comparisons. Each animal was tested repeatedly before surgery and at multiple time points following CCI. A one-way repeated measure ANOVA was used to compare the pre-surgery to post-surgery values for each group.

2.6.1.2. Between group comparisons. A repeated measures ANOVA was used to determine group differences in the time course of the development of CNP as measured with each behavioral test. Post-hoc two way ANOVAs were performed after significant differences were found, comparing each group to every other group.

2.6.2. rCBF analysis

Densitometric analysis of autoradiograms was performed using a microcomputer-assisted video imaging densitometer (MCID™ system, Imaging Research Inc., St. Catherines, Ontario, Canada). Each brain section on film was digitized to produce a 256 level grayscale image. Anatomic location of selected regions of interest (ROIs) was determined by overlaying transparent stereotaxic templates (adapted from the Paxinos and Watson 1986) on digitized brain images displayed on the video monitor. As described previously (Morrow et al., 1998), an index of activation (AI) was calculated for each ROI. Briefly, the MCID system converted sampled film optical densities to apparent tissue radioactivity concentrations (nCi/mg) by comparison with the optical densities of [^{14}C] standards also imaged on each film. The average total brain activity was then estimated by sampling all pixels in each brain section and averaging the activity across all sections for a given animal. AI values were then calculated for each sampled ROI were calculated as a percent difference from the average total activity of the entire brain using the following formula

$$AI = \frac{(\text{Sampled ROI activity} - \text{Total Brain Activity})}{\text{Total Brain Activity}} \times 100\%$$

We then computed an average AI for each ROI. Finally, the within subject mean AIs for each sampled region were averaged across all subjects in an experimental group to compute within group means for each ROI. Distinctly bilateral structures were examined for side to side differences in rCBF using a paired t-test ($P \geq 0.05$). For each ROI, we tested for significant differences in AI between control and CCI groups using a one-way ANOVA. All statistical

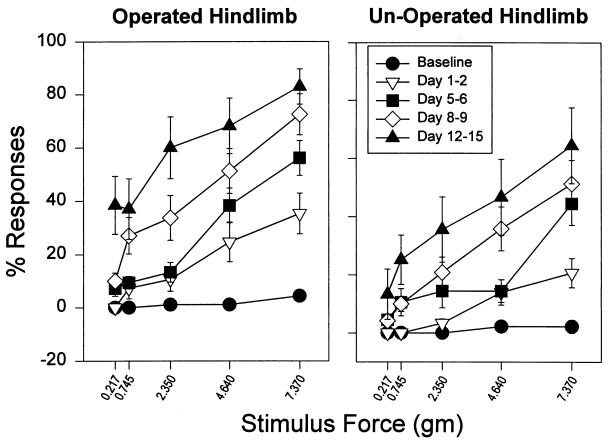

Fig. 2. The average number of total responses made by CCI animals to application of increasing strengths of von Frey hairs when tested before and after surgery. The percent total response was calculated by dividing the number of foot withdrawals elicited by each von Frey hair by the maximum number of responses possible in that trial (see Section 2). Data obtained from stimulation of the ligated and un-operated hindlimbs are shown in the left panel and right panel, respectively. CCI produced mechanical allodynia in both hind paws, although this enhanced behavioral response was more pronounced in the ligated hind paw. Note that allodynia was enhanced over the two week post-op period in which the animals were tested.

analyses were performed using the software package, SPSS for Windows (SPSS Inc., Chicago, Illinois).

3. Results

Animals that underwent the sham operation did not show any changes in spontaneous pain-related behaviors or in their response to thermal or mechanical stimuli relative to preoperative values. Therefore, the data from this group was pooled with un-operated controls for all further comparisons.

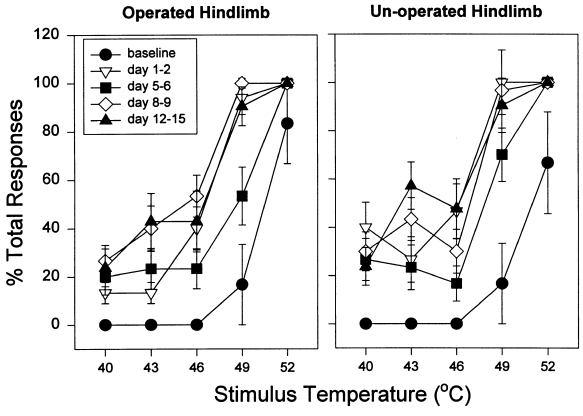

Fig. 3. The average number of total responses made by CCI animals to increasing stimulus temperatures when tested before and after surgery. The percent total response was calculated by dividing the number of foot withdrawals elicited at each temperature by the maximum number of responses possible in that trial (see Section 2). Data obtained from stimulation of the ligated and un-operated hindlimbs are shown in the left panel and right panel, respectively. CCI produced thermal hyperalgesia in both hind paws. Further, thermal allodynia was present at the three lower stimulus temperatures and this was enhanced over the two-week post-op period in which the animals were tested.

3.1. Behavioral effects of chronic constriction injury

3.1.1. Spontaneous pain behaviors

The results of the test for spontaneous pain-related behaviors are shown in Fig. 1. Before CCI, all animals rested with both of their hind paws pressed normally to the floor, reflected by a weighted pain score of 0. Within 1–2 days after CCI, the ligated hind paw showed a significant number of occurrences of abnormal paw posture, characterized primarily by pain behaviors 1 and/or 2: paw rests lightly on floor, toes ventroflexed and/or only internal edge of paw pressed to floor. However, a significant percentage (60%) of the CCI animals also exhibited pain behaviors 4 and 5 on the ligated hind paw: whole paw is elevated and animal licks paw. The intensity and duration of spontaneous pain-related behaviors progressively increased until their peak at days 8–9 post-op. Further, CCI animals continued to show abnormal paw postures of the ligated hind paw for up to 12–15 days after surgery, at which time they were prepared for the measurement of regional cerebral blood flow. In contrast, the un-operated hind paw did not exhibit any significant changes in paw posture at any time after surgery.

3.1.2. Mechanical allodynia

Fig. 2 shows a progressive development of mechanical allodynia over time following CCI in both the operated and un-operated hind paw. The left panel shows the mean percent total response of the ligated hind paw to each of the five stimulus forces before surgery (baseline) and at 1–2, 5–6, 8–9 and 12–15 days after CCI. The right panel shows the mean percent total response of the un-operated hind paw, displayed in the same format. Notice that the ligated hind paw showed a significantly enhanced response to application of the strongest intensity von Frey hair (7.37 gm force) within the first 2 days after CCI. Further, the stimulus intensity necessary to evoke a withdrawal response progressively decreased over time after CCI, such that by 12–15 days after surgery, application of even the weakest von Frey hair (0.217 gm force) produced a significantly greater number of withdrawal responses compared to baseline. Similarly, the un-operated hind paw showed a progressive development of mechanical allodynia over time after CCI. Note, however, that the allodynia in the un-operated hind paw was not evident until 5–6 days following CCI, and then only in response to the stimulation by the strongest von Frey hair. Further, significant group differences in response to von Frey hair stimulation were not seen at any time after CCI in response to the 0.217 gm force in the un-operated hind paw. The apparent differences in the expression of allodynia between the ligated and the un-operated hind paw were confirmed by a one-way ANOVA comparing

Table 2
Regional differences in cerebral blood flow (rCBF) expressed as the mean percent difference from mean whole brain activity, the activation index (AI)[a]

Region of Interest	Control ($n = 10$)	CCI ($n = 14$)
AD	16.29 ± 2.50	**24.87 ± 2.89**
BLA	−1.85 ± 1.19	1.46 ± 3.35
CC	8.98 ± 3.10	**19.33 ± 3.10**
CPU	11.52 ± 3.23	8.21 ± 4.86
HBC	41.51 ± 5.41	**51.43 ± 4.92**
HIP	−3.68 ± 3.61	−5.15 ± 2.07
HL	12.36 ± 1.36	**22.14 ± 2.72**
IPN	29.89 ± 7.20	**41.49 ± 4.18**
MT	6.83 ± 3.80	8.76 ± 2.26
PAG	−4.76 ± 3.39	−6.84 ± 3.39
PAR	15.81 ± 3.09	16.08 ± 2.29
PO	4.23 ± 2.06	**8.23 ± 3.72**
PVN	9.30 ± 6.48	**31.65 ± 5.96**
RS	21.12 ± 1.97	**30.23 ± 3.60**
VL	4.83 ± 1.84	8.24 ± 3.83
VM	3.66 ± 2.17	**9.07 ± 3.62**
VPL	4.28 ± 1.74	**12.48 ± 3.67***
VPM	14.51 ± 2.56	18.25 ± 3.63

[a] Values calculated as group means (see Section 2) ± SEM for all regions of interest (ROIs). The only side-to-side difference in either group was a greater increase in contralateral versus ipsilateral VPL in CCI animals. Therefore, data are presented as the averaged the index of activation (AI) from both sides for each ROI in all animals. Bold values, significant difference between CCI and control animals ($P \leq 0.05$, ANOVA); *significant side to side difference ($P \leq 0.05$, t-test).

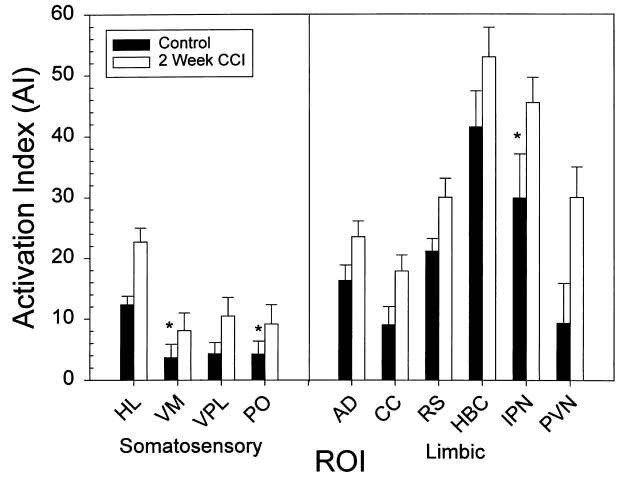

Fig. 4. The bar graph compares the average bilateral level of activation in several ROIs for unstimulated control and unstimulated two-week CCI groups. Data are shown only for ROIs showing a significant difference in activation between these two groups. These differences were significant using a two-tailed t-test with the exception of the three ROIs with an asterisk, which indicates significance only when using a one-tailed t-test. The relative levels of activation are expressed as AI, the mean percent difference from total brain activity (see Section 2). CCI produced an increase in AI in four somatosensory structures and six limbic regions at a time when these unstimulated CCI animals were showing prominent spontaneous pain-related behaviors as well as pronounced allodynia and hyperalgesia in both hind paws.

the response of the two hindlimbs. This comparison revealed side-to-side differences in response to application of von Frey hair at each stimulus intensity except the intermediate monofilament that was used (2.35 gm force).

3.1.3. Thermal hyperalgesia

The line graphs in Fig. 3 show the CCI-induced changes in response to thermal stimulation in the operated and un-operated hind paw. The left panel shows the mean percent total response of the ligated hind paw to each of the five stimulus temperatures before surgery (baseline) and at 1–2, 5–6, 8–9 and 12–15 days after CCI. The right panel shows the mean percent total response of the un-operated hind paw, displayed in the same format. Before surgery, a significant number of hind paw withdrawal responses were observed only in response to the 52°C stimulus. In contrast to the side-to-side differences that we observed in mechanical allodynia, CCI produced a similar change in withdrawal response to thermal stimulation in both hind paws. At all time points after surgery, the 49 and 52°C stimuli elicited an enhanced withdrawal response (hyperalgesia) when compared to baseline. Further, thermal allodynia was observed in response to the three lower stimulus temperatures only at days 8–9 and 12–15 following CCI. No other group differences were significant.

3.2. Effects of chronic constriction injury on regional cerebral blood flow

Control subjects showed no significant side to side differences in rCBF (data not shown). CCI rats showed lateralized differences in rCBF only in the VPL with significantly greater activation on the side contralateral to the ligated hindlimb compared to the ipsilateral side (paired t-test, $P \geq 0.05$). However, this side-to-side difference was superimposed on a bilateral increase in rCBF in VPL as compared to control animals. Because lateralized differences were essentially absent in ligated and unligated groups, we averaged the index of activation (AI) from both sides for each ROI in all animals before computing group means. All subsequent analysis and presentation were performed with this pooled data.

The average bilateral AI values for all ROIs sampled in unstimulated control and CCI groups are shown in Table 2. There was considerable variation in blood flow among ROIs even in the control subjects. Two weeks following surgery unstimulated CCI rats showed significant bilateral increases in rCBF in ten of the eighteen structures sampled when compared to controls. These findings are presented in Fig. 4. We found significant group differences in forebrain activation in four somatosensory structures, the hindlimb region of somatosensory cortex (22%), the ventral medial (9%), the ventral posterior lateral (12%) and the posterior (8%) nuclear groups of the thalamus. Further, CCI resulted in significant increases in rCBF within several forebrain structures of the limbic system. In addition to cingulate cortex

(19%), blood flow increased in the anterior dorsal nucleus of the thalamus (25%), retrosplenial cortex (30%), habenular complex (51%), and the interpeduncular nucleus (41%). CCI animals also showed significantly increased activation in the paraventricular nucleus of the hypothalamus (32%), a structure implicated in the modulation of acute pain. No other group differences were significant.

4. Discussion

We measured regional cerebral blood flow (rCBF) to identify changes in forebrain activation patterns following a chronic constriction injury (CCI) in the rat. This is the first study to use rCBF in an animal model to identify the forebrain neural correlates of chronic neuropathic pain. The data presented here indicate that a unilateral partial deafferentation due to CCI of the sciatic nerve produces bilateral effects in both pain-related behaviors and the pattern of forebrain activation.

4.1. Behavioral effects of chronic constriction injury

We found that CCI in one hind limb produced behavioral signs of spontaneous chronic pain that developed over time and that were most evident in the hind paw in which the sciatic nerve was damaged. The behaviors indicative of spontaneous chronic pain have been well characterized (Bennett and Xie, 1988; Attal et al., 1990; Kupers et al., 1992). Briefly, the CCI rats displayed prominent guarding of the affected hind paw, in a manner similar to that described by previous reports. However, none of our CCI animals developed autonomy; and all animals continued to groom and eat normally throughout the course of this experiment.

In contrast to the unilateral effects of CCI on spontaneous pain behaviors, a different picture emerged when we studied stimulus-evoked pain behaviors. CCI produced signs of hyperalgesia and allodynia in both hind paws. The total number of responses to both innocuous and noxious thermal and mechanical stimuli was increased in both the operated and un-operated hind paw (Figs. 2 and 3). Since we found no behavioral abnormalities indicative of pain in rats prepared with only a sham operation; the bilateral abnormalities we see in our CCI rats must be due to the unilateral sciatic nerve ligation.

Bilateral hyperalgesia and allodynia have generally not been reported previously using this model, with the exception of one report by Attal and colleagues (1990). Methodological variables could account for this discrepancy. For example, we used a different suture material than that used in any previous reports and the use of different types of suture materials has been shown to produce different outcomes (Kajander et al., 1996). Secondly, several laboratories report thermal and mechanical stimulus-evoked responses as a difference score, subtracting the number of responses made by the ligated hindpaw from that of the un-operated hind paw. As stated by Bennett and colleagues 'the difference scores are unambiguous only if the ligation has no contralateral effect' (Bennett and Xie, 1988). In their report, comparisons of the responses obtained pre- and post-operatively from the contralateral side in the same animal were not significantly different. However, in our hands CCI did produce significantly enhanced stimulus-evoked responses in the un-operated hindpaw. In many of the previous reports, experimenters have not asked explicitly whether contralateral measures were the same as untreated controls. For a thorough discussion of evidence of contralateral effects following peripheral nerve lesions, we refer the reader to a recent publication by Koltzenburg and colleagues (1999).

4.2. Forebrain activation pattern during spontaneous chronic pain

Rats with CCI showed changes in rCBF within multiple forebrain structures. Specifically, we found significant increases in the hindlimb, cingulate and retrosplenial cortical areas as well as in several thalamic nuclei (ventral medial, ventral posterior lateral, posterior and anterior-dorsal groups). In addition, the habenular complex, interpeduncular nucleus and the paraventricular nucleus of the hypothalamus showed significant increases in rCBF after CCI. Based on the extensive pattern of increased brain activity in CCI rats reported by Mao and colleagues using the 2-deoxyglucose technique, we expected to see increased rCBF in other forebrain structures as well. However, the $^{[99m]}$Tc -exametazime blood flow method we used in this study has better spatial and temporal resolution than the conventional 2-deoxyglucose method and this may very well account for the discrepancies. For a more thorough comparison of these two techniques, see Morrow et al. (1998).

Because spontaneous pain-related behaviors were displayed primarily in the hindlimb ipsilateral to the constriction injury, we expected to see differences in activation primarily on the contralateral side in those structures known to be part of the ascending spinothalamic system. However, we consistently found bilateral changes in forebrain activation in these unstimulated CCI animals. Bilateral changes in the CNS following a unilateral injury or stimulus is not unexpected (see review by Koltzenburg, 1999). Human PET studies (Casey et al., 1996) have described bilateral changes in the rCBF of forebrain structures during painful tonic deep cold stimulation of one arm. We recently reported that formalin injection into one hindpaw of the rat produces bilateral increases in rCBF in multiple forebrain structures (Morrow et al., 1998). In addition, laboratories using 2-deoxyglucose imaging report bilateral activation in the brain (Mao et al., 1993) and spinal cord (Coghill et al., 1991; Mao et al., 1992) of rats exhibiting chronic pain following a unilateral chronic constriction injury of the sciatic nerve.

4.2.1. Changes in rCBF in somatosensory structures after CCI

We found evidence of increased blood flow in numerous forebrain somatosensory structures associated with the ascending spinothalamic tract. In particular, there was a significant increase in the blood flow to the hindlimb area of the somatosensory cortex. In addition, a number of thalamic structures showed a trend toward increased rCBF in CCI rats when compared to control values, although the increases were significant only in the ventral medial, ventroposterior lateral and posterior nuclear groups (Table 2).

Previous experiments in animals have shown that reorganization can occur at the cortical level following peripheral injury (Pons et al., 1988, 1991; Guilbaud et al., 1991, 1992; Recanzone et al., 1992; Mao et al., 1993; Florence and Kaas, 1995; Chen et al., 1998). Guilbaud et al. (1991) described abnormal spontaneous 'paroxysmal' discharges in S1 neurons that occurred without stimulation and which lasted up to 5 minutes in CCI animals. In addition expansive changes in the receptive field properties of the cortical neurons were noted.

Other studies have shown that the thalamus undergoes functional modifications in pathophysiological chronic pain conditions. For example, patients with chronic pain following spinal injury show increased thalamic receptive field size, and increased neuronal excitability and firing patterns (Tasker et al., 1983; Lenz et al., 1987; Tasker, 1990; Lenz, 1991). Microelectrode recordings in animals with chronic pain (e.g. chronic arthritis, nerve injury) also demonstrate increased responsiveness of thalamic neurons to various stimuli (Rampin and Morain 1987; Albe-Fessard and Rampin, 1991; Guilbaud et al., 1991, 1992). Similarly, Mao and colleagues (1993) found significantly increased metabolic activity in thalamic nuclei of CCI rats using 2-deoxyglucose. Increased thalamic activity could relate to the changes in the activity of somatosensory neurons, dorsal root ganglia and spinal dorsal horn after different injuries such as nerve crush, nerve transection, neuroma and dorsal rhizotomy (Wall and Egger, 1972; Basbaum and Wall, 1976; Dostrovsky et al., 1976; Millar and Basbaum, 1976; Devor and Wall, 1981; Wall et al., 1983; Burchel, 1984; Lisney and Devor, 1987; Calford and Tweedale, 1988).

However, not all laboratories report increased thalamic activity in association with chronic pain. Decreased thalamic activation has been reported in patients with chronic neuropathic or cancer pain using positron emission tomography (Di Piero et al., 1991; Iadarola et al., 1995). Iadarola et al. (1995) suggests that the decrease in thalamic blood flow may reflect excessive inhibition of thalamic activity in an over-compensatory response to the excessive excitatory inputs and that less energy is required to inhibit large groups of neurons than that necessary to excite them.

A disadvantage of blood flow (including human PET) and other metabolic imaging techniques, is the inability to determine the functional valence of brain activation. Although there is substantial evidence that the activity of local neurons is a major factor regulating the specific distribution of blood flow throughout the brain (Sokoloff, 1981; Lou et al., 1987; Iadecola et al., 1996; Malonek and Grinvald, 1996), current methods cannot distinguish between excitatory and inhibitory synaptic activity. Therefore, while abundant clinical and animal data implicate the thalamus as a primary candidate site for pathophysiological changes in subjects with chronic neuropathic pain, the exact mechanism of change is still unclear.

4.2.2. Changes in rCBF in limbic structures after CCI

Several forebrain regions considered part of the limbic system showed pain-induced changes in rCBF, including the anterior dorsal nucleus of the thalamus, cingulate and retrosplenial cortex, the habenular complex, interpeduncular nucleus and the paraventricular nucleus of the hypothalamus. The involvement of limbic structures in nociceptive processing and of structures associated with pain modulation is expected because the limbic system has long been believed to play an important role in neural mechanisms related to the affective-motivational dimension of pain (Melzack and Casey, 1967; Hylden et al., 1986; Geisler et al., 1994; Chapman, 1996; Willis and Westlund, 1997). Recent studies using positron emission tomography also report significant increases in regional blood flow in limbic forebrain structures during acute or chronic pain perception in humans (Talbot et al., 1991; Jones et al., 1991; Casey et al., 1994, 1996; Coghill et al., 1994; Hsieh et al., 1995, 1996; Vogt et al., 1996). Anatomical studies have shown that limbic structures receive spino-reticulo-thalamic input via connections from the thalamic medial-intralaminar nuclei and thalamic reticular nucleus (Kaitz and Robertson, 1981; Robertson and Kaitz, 1981). In addition, there is evidence for extensive reciprocal interconnections between many of these cortical and thalamic regions (Kaitz and Robertson, 1981; Robertson and Kaitz, 1981; Thompson and Robertson, 1987; Cavada and Goldman-Rakic, 1989; Lozsàdi, 1994), thus providing a neural substrate for complex interactions. The activation of limbic structures may be related to either pain perception or the modulation of nociceptive inputs.

4.2.3. Role of the habenulo-interpenduncular complex in pain processing

Extracellular electrical recordings of single units in the anaesthetized rat demonstrate that about two thirds of the neurons in the lateral habenula respond to peripheral noxious stimuli (Benabid and Jeaugey, 1989). The firing pattern of the lateral habenula cells is either excitatory (75%) or inhibitory (24%), is related to the intensity of the stimulus; and their receptive field is large and bilateral. Most of these cells do not respond to non-noxious stimuli.

Electrical stimulation of, or microinjection of, morphine into the habenular complex demonstrate habenular stimulation-induced analgesia in the formalin or tail flick test (Cohen and Melzack, 1986; Cohen and Melzack, 1985;

Cohen and Melzack, 1993; Mahieux and Benabid, 1987). Lesions of the habenular nuclei increase the pain sensitivity, enhance the analgesic activity of morphine and slightly activate behavior (Meszaros et al., 1985). Naloxone significantly decreases habenular stimulation-induced analgesia (Mahieux and Benabid, 1987). These studies suggest that the habenula is a central target at the upper brainstem level for nociceptive inputs.

Lesion of the fasciculus retroflexus, a pathway connecting habenular nuclei with interpeduncular nucleus also enhances pain sensitivity. However, lesion of this pathway does not change the efficacy of morphine analgesia, but does significantly increase the activity of animals (Meszaros et al., 1985). An increase in the activation of the interpeduncular nucleus may be partly responsible for the hyperalgesia seen in the formalin test. Hentall and Budhrani (1990) showed that electrical stimulation in IPN of rat excites 'on cells' and inhibits 'off cells' in the nucleus raphe magnus. The effect of such stimulation should be hyperalgesic based on descriptions of the response properties of neurons in the raphe spinal system (Barbaro et al., 1989; Heinricher et al., 1989; Heinricher and Tortorici, 1994). Lesions of the interpeduncular nucleus influence pain sensitivity to a small degree, do not affect morphine analgesia, but dramatically increase the behavioral activity of animals (Meszaros et al., 1985). The IPN-lesioned-induced behavioral activation does not resemble the aimless excitation of amphetamine-treated or raphe-lesioned rats, and no signs of increased emotionality or irritability were reported (Meszaros et al., 1985). These experiments suggest the habenulo-interpenduncular complex may be involved in the regulation of behavioral activity and the sensitivity to aversive stimuli.

4.2.4. Paraventricular nucleus of the hypothalamus

Previous research indicates that the paraventricular nucleus (PVN) plays a complex role in the production of stress induced analgesia during phasic but not tonic pain (Bodnar et al., 1986; Truesdell and Bodnar, 1987; Pacak et al., 1995; Fuchs and Melzack, 1996). Activation of the PVN in CCI rats exhibiting spontaneous pain may involve a response to either pain or stress. Recent studies have looked at brain activation induced by pain or immobilization stress using the expression of the proto-oncogene, c-fos, as a marker. An increased number of neurons expressing Fos-like immunoreactivity was observed in discrete brain regions, such as the lateral septum, midline nuclei of the thalamus, paraventricular hypothalamic nucleus, brain stem catecholaminergic, and serotonergic neurons, in response to pain or immobilization stress. Distribution patterns of Fos-like immunoreactive neurons were similar in animals subjected to either pain or immobilization (Senba et al., 1993; Smith and Day, 1994).

The paraventricular nucleus (PVN) of the hypothalamus contains vasopressin (VP) parvocellular neurons that project to extrahypothalamic structures involved in pain inhibition and VP has been shown to play an important part in a variety of CNS functions (see Doris, 1984 for review). Substantial evidence exists implicating VP in learning and memory processes, cardiovascular regulation through central pathways or regulation of body temperature during fever. Other areas of central regulation where VP may be important include circadian rhythmicity, control of water intake, control of permeability of brain capillaries to water, central regulation of ACTH release and nociception. Pain thresholds are increased following central administration of arginine vasopressin (AVP). Bodnar and colleagues (1986) examined whether AVP analgesia as measured by the tail-flick test was altered in animals with lesions placed in the PVN. Lesions placed in the PVN eliminated AVP analgesia on the tail-flick test indicating that the PVN is a critical structure for the integrity of AVP analgesia.

Activation of the PVN in our CCI animals may also be an immune response to pain, or the stress of pain. Matsumoto et al. (1997) report that cytokine-induced neutrophil chemoattractant (CINC), a member of IL-8 family in rat and a counter part of human growth-related oncogene product, is synthesized in the PVN and released in peripheral blood in response to formalin injection into the foot pad. This cytokine mediated nociceptive response from CNS to peripheral tissue, would be a novel type of cross-talk between nervous and immune system.

In contrast to the CCI-induced increases in blood flow that we found in the limbic structures discussed above, a number of limbic sites did not show a significant change. Bernard and colleagues (1989, 1990, 1996) have demonstrated a spino(trigemino)pontoamygdaloid pathway that may be involved with the affective-motivational dimension of pain. Conversely, anatomical and electrophysiological studies indicate the existence of an efferent pathway originating in the amygdala, synapsing within the periaquaductal gray and continuing on to the spinal cord dorsal horn that modulates pain (Hopkins and Holstege 1978; Krettek and Price 1978; Beitz 1982; Watson et al., 1983; Zhang et al., 1991; Bernard et al., 1996; Manning 1998). In addition, the basal ganglia receives nociceptive information through several sources including the amygdala, cingulate cortex, prefrontal cortex and the habenula (Ma and Han, 1991; Cenci et al., 1992; Ma et al., 1992; Chudler and Dong, 1995).

In the study presented here, CCI did not significantly increase blood flow to the caudate-putamen, amygdala or the periaquaductal gray, limbic structures previously reported to be affected by peripheral nerve injury (Mao et al., 1993). This discrepancy may be due to the methodological variables previously discussed, or perhaps to differences in sampling techniques. Alternatively, the lack of change in rCBF in these particular limbic structures may indicate differences in a conditioned response to noxious stimuli (Bernard et al., 1989; Berkley and Scofield 1990; Bernard and Besson 1990; for review see Bernard et al., 1996) in our experimental animals compared to those in the Mao study.

A final consideration in the interpretation of these results is whether the increases that we see in regional cerebral blood flow are due to: (1) ongoing pain generated from the periphery, or (2) changes within the CNS. Although we cannot answer this question based on our experimental results alone, previous research suggests that chronic pain is, at least in part, the result of long lasting changes and reorganization within the CNS structures involved in pain processing. For example, marked changes in receptive field size and the physiological activity of single neurons in forebrain structures following peripheral or central lesions have been demonstrated in numerous animal studies (Brandenberg and Mann, 1989; Allard et al., 1991; Calford and Tweedale, 1988; Garraghty and Kaas, 1991; Guilbaud et al., 1991, 1992; Turnbull and Rasmusson, 1991; Rasmusson et al., 1993). In addition to this experimental evidence, there is extensive clinical data indicating receptive field plasticity in the spinal cord, thalamus and somatosensory cortex of humans following deafferentation (Lenz et al., 1987; Tasker et al., 1987; Lenz, 1991; Halligan et al., 1993; Marshall and Halligan, 1993; Aglioti et al., 1994; Schady et al., 1994; Pockett, 1995; Bernard et al., 1996). These findings provide strong evidence that central reorganization occurs following peripheral nerve injury. In addition, they support the contention that the development of chronic neuropathic pain is paralleled by long term modifications in the physiological response properties of neurons within specific forebrain regions.

5. Conclusion

A comparison of the changes in forebrain rCBF produced by acute versus chronic pain may provide insight into the pathophysiology of chronic pain. There are some similarities between forebrain rCBF elicited by an acute formalin injection (Morrow et al., 1998) and the patterns of rCBF changes we report here. For example, the HL, HBC and the IPN are activated in both formalin-injected animals and in CCI rats showing signs of spontaneous chronic pain. In contrast, the robust activation found in the PAG 20 min after injection of formalin, was not found in our chronic pain animals. Conversely, significant increases in activation were found in the cingulate cortex of CCI rats, but not in formalin injected animals. These findings suggest that perhaps a critical difference between acute and chronic pain is in the pattern of forebrain activation, not in the activation of any one specific structure.

Acknowledgements

This work was supported by the Dept. of Veterans Affairs, the National Institutes of Health (PO1-HD33986), and the Veterans Education and Research Association of Michigan. We thank Dr Milton Gross and the staff of the Nuclear Medicine Laboratory at the Ann Arbor VAMC for preparing the radiotracer kits used in these experiments. Our thanks also go to Dr Satoshi Minoshima in the University of Michigan Department of Nuclear medicine for his advice regarding data analysis and to Mr Revius Williams, Director of Ann Arbor VAMC Animal Research Facility for his assistance with animal preparation. We also express our gratitude to Mediphysics Inc., Arlington Heights, IL (a division of Amersham Inc.) for their generous donation of the Ceretec™ radiotracer kits that were used in this study.

References

Aglioti S, Cortese F, Franchini C. Rapid sensory remapping in the adult human brain as inferred from phantom breast perception. Neuroreport 1994;5:473–476.

Albe-Fessard D, Rampin O. Neurophysiological studies in rats deafferented by dorsal root sections. In: Nashold BS, Ovelmen-Levitt J, editors. Deafferentation pain syndromes: pathophysiology and treatment, New York: Raven Press, 1991. pp. 121–139.

Allard T, Clark SA, Jenkins WM, Merzenich MM. Reorganization of somatosensory area 3b representations in adult owl monkeys after digital syndactyly. J Neurophysiol 1991;66:1048–1058.

Anonymous. NIH Guide for care and use of laboratory animals, Washington D.C.: National Academy Press Inc, 1996.

Attal N, Jazat F, Guilbaud G. Further evidence for pain-related behaviors in a model of unilateral peripheral mononeuropathy. Pain 1990;41:235–251.

Baker AG, Winegarner FG. Causalgia: a review of twenty-eight treated cases. Amer J Surg 1969;117:690–694.

Barbaro NM, Heinricher MM, Fields HL. Putative nociceptive modulatory neurons in the rostral ventromedial medulla of the rat display highly correlated firing patterns. Somatosens Res 1989;6:413–425.

Basbaum AI, Wall PD. Chronic changes in the response of cells in adult cat dorsal horn following partial differentiation: the appearance of responding cells in a previously non-responding region. Brain Res 1976;116:181–204.

Basbaum AI, Gautron M, Jazat F, Mayes M, Guilbaud G. The spectrum of fiber loss in a model of neuropathic pain in the rat: An electron microscopic study. Pain 1991;47:359–367.

Beitz AJ. The organization of afferent projections to the midbrain periaqueductal gray of the rat. Neuroscience 1982;7:133–159.

Benabid AL, Jeaugey L. Cells of the rat lateral habenula respond to high-threshold somatosensory inputs. Neurosci Lett 1989;96(3):289–294.

Bennett GJ, Xie YK. A peripheral mononeuropathy in the rat that produces disorders of pain sensation like those seen in man. Pain 1988;33:87–107.

Berkley KJ, Scofield SL. Relays from the spinal cord and solitary nucleus through the parabrachial nucleus to the forebrain of the cat. Brain Res 1990;529:333–338.

Bernard JF, Besson JM. The spino(trigemino)pontoamygdaloid pathway: electrophysiological evidence for an involvement in pain processes. J Neurophys 1990;63:473–490.

Bernard JF, Peschanski M, Besson JM. A possible spino (trigemino)-pontoamygdaloid pathway for pain. Neurosci Lett 1989;100:83–88.

Bernard JF, Bester H, Besson JM. Involvement of the spino-parabrachio - amygdaloid and-hypothalamic pathways in the autonomic and affective emotional aspects of pain. (Review, 80 Refs.). Progress in Brain Res 1996;107:243–255.

Bodnar RJ, Truesdell LS, Haldar J, Aral IA, Kordower JH, Nilaver G. Elimination of vasopressin analgesia following lesions placed in the rat hypothalamic paraventricular nucleus. Peptides 1986;7(1):111–117.

Bonica JJ. Causalgia and other reflex sympathetic dystrophies. The Management of Pain, Philadelphia: Lea and Febiger, 1990. pp. 220–243.

Brandenberg GA, Mann MD. Sensory nerve crush and regeneration and the receptive fields and response properties of neurons in the primary somatosensory cerebral cortex of cats. Exp Neurol 1989;103:256–266.

Burchel KJ. Effects of electrical and mechanical stimulation on two foci of spontaneous activity which develop in primary afferent neurons after peripheral axotomy. Pain 1984;18:249–265.

Calford BC, Tweedale R. Immediate and chronic changes in responses of somatosensory cortex in adult flying-fox after digit amputation. Nature Lond 1988;332:446–448.

Casey KL, Minoshima S, Berger KL, Koeppe RA, Morrow TJ, Frey KA. Positron emission tomographic analysis of cerebral structures activated specifically by repetitive noxious heat stimuli. J Neurophysiol 1994;71:802–807.

Casey KL, Minoshima S, Morrow TJ, Koeppe RA. Comparison of human cerebral activation patterns during cutaneous warmth, heat pain, and deep cold pain. J Neurophysiol 1996;76:571–581.

Cavada C, Goldman-Rakic PS. Posterior parietal cortex in rhesus monkey: I. Parcellation of areas based on distinctive limbic and sensory corticocortical connections. J Comp Neurol 1989;287:393–421.

Cenci MA, Kalen P, Mandel RJ, Bjorklund A. Regional differences in the regulation of dopamine and noradrenaline release in the medial prefrontal cortex, nucleus accumbens and caudate-putamen: a micro-dialysis study in the rat. Brain Res 1992;581(2):217–228.

Chapman CR. Limbic processes and the affective dimension of pain. Prog Brain Res 1996;110:63–81.

Chen R, Corwell B, Yaseen Z, Hallett M, Cohen LG. Mechanisms of cortical reorganization in lower-limb amputees. J Neurosci 1998;18(9):3443–3450.

Chudler EH, Dong WK. The role of the basal ganglia in nociception and pain. Pain 1995;60(1):3–38.

Coghill RC, Price DD, Hayes RL, Mayer DJ. Spatial distribution of nociceptive processing in the rat spinal cord. J Neurophysiol 1991;65:133–140.

Coghill RC, Talbot JD, Evans AC, Meyer E, Gjedde A, Bushnell MC, Duncan GH. Distributed processing of pain and vibration by the human brain. J Neurosci 1994;14:4095–4108.

Cohen SR, Melzack R. Morphine injected into the habenula and dorsal posteromedial thalamus produces analgesia in the formalin test. Brain Res 1985;359:131–139.

Cohen SR, Melzack R. Habenular stimulation produces analgesia in the formalin test. Neurosci Lett 1986;70:165–169.

Cohen SR, Melzack R. The habenula and pain: repeated electrical stimulation produces prolonged analgesia but lesions have no effect on formalin pain or morphine analgesia. Behav Brain Res 1993;54:171–178.

Devor M, Wall PD. The effect of peripheral nerve injury on receptive fields of cells in the cat spinal cord. J Comp Neurol 1981;199:277–291.

Di Piero V, Jones AKP, Iannotti F, Powell M, Perani D, Lenzi GL, Frackowiak RSJ. Chronic pain: a PET study of the central effects of percutaneous high cervical cordotomy. Pain 1991;46:9–12.

Doris PA. Vasopressin and central integrative processes. Neuroendocrinology 1984;38(1):75–85.

Dostrovsky JO, Millar J, Wall PD. The immediate shift of afferent drive of dorsal column nucleus cells following deafferentation: a comparison of acute and chronic deafferentation in gracile nucleus and spinal cord. Exp Neurol 1976;52:480–495.

Florence SL, Kaas JH. Large-scale reorganization at multiple levels of the somatosensory pathway follows therapeutic amputation of the hand in monkeys. J Neurosci 1995;15(12):8083–8095.

Fuchs PN, Melzack R. Restraint reduces formalin-test pain but the effect is not influenced by lesions of the hypothalamic paraventricular nucleus. Exp Neurol 1996;139:299–305.

Garraghty PE, Kaas JH. Functional reorganization in adult monkey thalamus after peripheral nerve injury. Neuroreport 1991;2:747–750.

Geisler JGJ, Katter JT, Dado RJ. Direct spinal pathways to the limbic system for nociceptive information. TINS 1994;17:244–250.

Guilbaud G, Benoist JM, Jazat F, Gauton M. Neuronal responsiveness in the ventrobasal thalamic complex of rats with an experimental peripheral mononeuropathy. J Physiology 1991;64:1537–1554.

Guilbaud G, Benoist JM, Levante A, Gautron M, Willer JC. Primary somatosensory cortex in rats with pain-related behaviors due to a peripheral mononeuropathy after moderate ligation of one sciatic nerve: Neuronal responsivity to somatic stimulation. Exp Brain Res 1992;92:227–245.

Halligan PW, Marshall JC, Wade DT, Davey J, Morrison D. Thumb in cheek? Sensory reorganization and perceptual plasticity after limb amputation. Neuroreport 1993;4:233–236.

Heinricher MM, Tortorici V. Interference with GABA transmission in the rostral ventromedial medulla: Disinhibition of off-cells as a central mechanism in nociceptive modulation. Neuroscience 1994;63:533–546.

Heinricher MM, Barbaro NM, Fields HL. Putative nociceptive modulating neurons in the rostral ventromedial medulla of the rat: firing of on- and off-cells is related to nociceptive responsiveness. Somatosens Res 1989;6:427–439.

Hentall ID, Budhrani VM. The interpeduncular nucleus excites the on-cells and inhibits the off-cells of the nucleus raphe magnus. Brain Res 1990;522:322–324.

Hopkins DA, Holstege G. Amygdaloid projections to the mesencephalon, pons and medullar oblongata in the cat. Exp Brain Res 1978;32:529–547.

Hsieh JC, Belfrage M, Stone-Elander S, Hansson P, Ingvar M. Central representation of chronic ongoing neuropathic pain studied by positron emission tomography. Pain 1995;63:225–236.

Hsieh JC, Ståhle-Bäckdahl M, Hägermark Ö, Stone-Elander S, Rosenquist G, Ingvar M. Traumatic nociceptive pain activates the hypothalamus and the periaqueductal gray: A positron emission tomography study. Pain 1996;64:303–314.

Hylden JL, Hayashi H, Dubner R, Bennett GJ. Physiology and morphology of the lamina I spinomesencephalic projection. J Comp Neurol 1986;247:505–515.

Iadarola MJ, Max MB, Berman KF, Byas-Smith MG, Coghill RC, Gracely RH, Bennett GJ. Unilateral decrease in thalamic activity observed with positron emission tomography in patients with chronic neuropathic pain. Pain 1995;63:55–64.

Iadecola C, Li J, Xu S, Yang G. Neural mechanisms of blood flow regulation during synaptic activity in cerebellar cortex. J Neurophysiol 1996;75:940–950.

Jones AKP, Brown WD, Friston KJ, Qi LY, Frackowiak RSJ. Cortical and subcortical localization of response to pain in man using positron emission tomography. Proc R Soc Lond (Biol) 1991;244:39–44.

Kaitz SS, Robertson RT. Thalamic connections with limbic cortex. II. Corticothalamic projections. J Comp Neurol 1981;195:527–545.

Kajander KC, Pollock CH, Berg H. Evaluation of hindpaw position in rats during chronic constriction injury (CCI) produced with different suture materials. Somatosens Mot Res 1996;13:95–101.

Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992;50:355–363.

Koltzenburg M, Wall PD, McMahon SB. Does the right side know what the left is doing? Trends Neurosci 1999;22:122–127.

Krettek JE, Price JL. Amygdaloid projections to the subcortical structures within the basal forebrain and brainstem in the rat and cat. J Comp Neurol 1978;178:225–254.

Kupers RC, Nuytten D, De Castro-Costa M, Gybels JM. A time course analysis of the changes in spontaneous and evoked behaviour in a rat model of neuropathic pain. Pain 1992;50:101–111.

Lenz FA. The thalamus and central pain syndromes: human and animal studies. In: Casey KL, editor. Pain and central nervous system disease: the central pain syndromes, New York: Raven Press, 1991. pp. 171–182.

Lenz FA, Tasker RR, Drostrovsky JO. Abnormal single unit activity recorded in the somatosensory thalamus of a quadriplegic patient with central pain. Pain 1987;31:225–236.

Lisney SJW, Devor M. Afterdischarge and interactions among fibers in damaged peripheral nerve in the rat. Brain Res 1987;415:122–136.

Lou HC, Edvinsson L, MacKenzie ET. The concept of coupling blood flow to brain fucntion: revision required? Ann Neurol 1987;22:289–297.

Lozsàdi DA. Organization of cortical afferents to the rostral limbic sector of the rat thalamic reticular nucleus. J Comp Neurol 1994;341:520–533.

Ma QP, Han JS. Neurochemical studies on the mesolimbic circuitry of antinociception. Brain Res 1991;566(1-2):95–102.

Ma QP, Shi YS, Han JS. Further studies on interactions between periaqueductal gray, nucleus accumbens and habenula in antinociception. Brain Res 1992;583(1-2):292–295.

Mahieux G, Benabid AL. Naloxone-reversible analgesia induced by electrical stimulation of the habenula in the rat. Brain Res 1987;406(1-2):118–129.

Malonek D, Grinvald A. Interactions between electrical activity and cortical microcirculation revealed by imaging spectroscopy: implications for functional brain mapping. Science 1996;272:551–554.

Manning BH. A lateralized deficit in morphine antinociception after unilateral inactivation of the central amygdala. J Neurosci 1998;18(22):9453–9470.

Mao J, Price DD, Coghill RC, Mayer DJ, Hayes RL. Spatial patterns of spinal cord [14C]-2-deoxyglucose metabolic activity in a rat model of painful peripheral mononeuropathy (published erratum appears in Pain 1992 Dec; 51(3): 389). Pain 1992;50:89–100.

Mao J, Mayer DJ, Price DD. Patterns of increased brain activity indicative of pain in a rat model of peripheral mononeuropathy. J Neurosci 1993;13:2689–2702.

Marshall JC, Halligan PW. Pain pathways and plasticity (letter). Nature 1993;361:410.

Matsumoto K, Koike K, Miyake A, Watanabe K, Konishi K, Kiyama H. Noxious stimulation enhances release of cytokine-induced neutrophil chemoattractant from hypothalamic neurosecretory cells. Neuroscience Res 1997;27(2):181–184.

Melzack R, Casey KL. Sensory motivational and central control determinants of pain: a new conceptual model. In: Kenshalo D, editor. International Symposium on the Skin Senses, 1967.

Meszaros J, Gajewska S, Tarchalska-Krynska B. Habenulo-interpeduncular lesions: the effects on pain sensitivity, morphine analgesia and open-field behavior in rats. Polish J Pharmacol Pharmacy 1985;37(4):469–477.

Millar J, Basbaum AI. Restructuring of the somatotopic map and appearance of abnormal activity in the gracile nucleus after partial deafferentation. Exp Neurol 1976;50:658–672.

Morrow TJ, Paulson PE, Casey KL. Changes in regional cerebral blood flow associated with painful peripheral mononeuropathy in the rat. Soc Neurosci Abst 1995;21(2):1165.

Morrow TJ, Paulson PE, Danneman PJ, Casey KL. Regional changes in forebrain activation during the early and late phase of formalin nociception: analysis using cerebral blood flow in the rat. Pain 1998;75:355–365.

Mraovitch S, Calando Y, Pinard E, Pearce WJ, Seylaz J. Differential cerebrovascular and metabolic responses in specific neural systems elicited from the centromedian-parafascicular complex. Neuroscience 1992;49:451–466.

Pacak K, Palkovits M, Kvetnansky R, Yadid G, Kopin I, Goldstein DS. Effects of various stressors on in vivo norepinephrine release in the hypothalamic paraventricular nucleus and on the pituitary- adrenocortical axis. Ann NY Acad Sci 1995;:115–130.

Palecek J, Palecková V, Dougherty PM, Carlton SM, Willis WD. Responses of spinothalamic tract cells to mechanical and thermal stimulation of skin in rats with experimental peripheral neuropathy. J Neurophysiol 1992;67:1562–1573.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates, New York: Academic Press, 1986.

Payne R. Reflex sympathetic dystrophy syndrome: diagnosis and treatment. In: Fields HL, editor. Pain syndromes in neurology, London: Butterworth, 1990. pp. 107–129.

Pons TP, Garraghty PE, Mishkin M. Lesion-induced plasticity in the second somatosensory cortex of adult macaques. Proc Natl Acad Sci 1988;85:5279–5281.

Pons TP, Garraghty PE, Ommaya AK, Kaas JH, Taub E, Mishkin M. Massive cortical reorganization after sensory deafferentation in adult macaques. Science 1991;252:1857–1860.

Price DD, Bennet GJ, Rafii A. Psychophysical observations on patients with neuropathic pain relieved by a sympathetic block. Pain 1989;36:273–288.

Price DD, Mao J, Coghill RC, D'Avella D, Cicciarello R, Fiori MG, Mayer DJ, Hayes RL. Regional changes in spinal cord glucose metabolism in a rat model of painful neuropathy. Brain Res 1991;564:314–318.

Rampin O, Morain P. Cortical involvement in dorsal horn cell hyperactivity and abnormal behavior in rats with dorsal root section. Somatosens Motor Res 1987;4:237–251.

Recanzone GH, Merzenich MM, Dinse HR. Expansion of the cortical representation of a specific skin field in primary somatosensory cortex by intracortical microstimulation. Cerebral Cortex 1992;2:181–196.

Robertson RT, Kaitz SS. Thalamic connections with limbic cortex I. Thalamocortical projections. J Comp Neurol 1981;195:501–525.

Schady W, Braune S, Watson S, Torebjork HE, Schmidt R. Responsiveness of the somatosensory system after nerve injury and amputation in the human hand. Ann Neurol 1994;36:68–75.

Seltzer Z, Dubner R, Shir Y. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain 1990;43:205–218.

Senba E, Matsunaga K, Tohyama M, Noguchi K. Institution Department of Anatomy and Neurobiology. Wakayama Medical College, Japan. Stress-induced c-fos expression in the rat brain: activation mechanism of sympathetic pathway. Brain Res Bulletin 1993;31(3-4):329–344.

Smith DW, Day TAc-fosexpressioninhypoth. halamic neurosecretory and brainstem catecholamine cells following noxious somatic stimuli. Neuroscience 1994;58(4):765–775.

Sokoloff L. Local cerebral energy metabolism: its relationships to local functional activity and blood flow. Ciba Found Symp 1978;171–197.

Sokoloff L. Relationship among local functional activity, energy metabolism, and blood flow in the central nervous system. Fed Proc 1981;40:2311–2316.

Sunderland S. Nerves and nerve injuries, New York: Churchill Livingstone, 1978.

Talbot JD, Marrett S, Evans AC, Meyer E, Bushnell MC, Duncan GH. Multiple representations of pain in human cerebral cortex. Science 1991;251:1355–1358.

Tasker RR. Pain resulting from central nervous system pathology (central pain). In: Bonica JJ, editor. The management of pain, Philadelphia, PA: Lea and Febiger, 1990. pp. 264–283.

Tasker RR, Organ LW, Hawrylyshyn P. Deafferentation and causalgia. Res Publ Assoc Res Nerve Ment Dis 1980;58:305–329.

Tasker RR, Tsuda R, Hawrylyshyn P. Clinical neurophysiological investigation of deafferentation pain. In: Bonica JJ, Lindblom U, Iggo A, editors. Advances in pain research and therapy, 3. Raven Press: New York, 1983. pp. 713–738.

Tasker RR, Gorecki J, Lenz FA, Hirayama T, Dostrovsky JO. Thalamic microelectrode recording and microstimulation in central and deafferentation pain. App Neurophysiol 1987;50:414–417.

Thomas PK. Clinical features and differential diagnosis of peripheral neuropathy. In: Dyck PJ, Thomas PK, Lambert EH, Bunge R, editors. Peripheral neuropathy, Philadelphia: Saunders, 1984. pp. 1169–1190.

Thompson SM, Robertson RT. Organization of the subcortical pathways for sensory projections to the limbic cortex I. Subcortical projections to the medial limbic cortex in the rat. J Comp Neurol 1987;265:159–174.

Truesdell LS, Bodnar RJ. Reduction in cold-water swim analgesia following hypothalamic paraventricular nucleus lesions. Physiol Behav 1987;39:727–731.

Turnbull BG, Rasmusson DD. Chronic effects of total or partial digit denervation on raccoon somatosensory cortex. Somatosens Mot Res 1991;8:201–213.

Vogt BA, Derbyshire S, Jones AKP. Pain processing in four regions of human cingulate cortex localized with co-registered PET and MR imaging. Eur J Neurosci 1996;8:1461–1473.

Wall PD, Egger MD. Formation of new connections in adult rat after partial deafferentation. Nature Lond 1972;232:542–555.

Wall JY, Felleman DJ, Kaas JH. Recovery of normal topography in the somatosensory cortex of monkey after nerve crush and regeneration. Science Wash DC 1983;221:771–773.

Watson RE, Troiano R, Poulakos J, Weiner S, Block CH, Siegel A. A 14C-2-deoxyglucose analysis of the functional neural pathways of the limbic forebrain in the rat. I. The amygdala. Brain Res Reviews 1983;5:1–44.

Willis WD, Westlund KN. Neuroanatomy of the pain system and of the pathways that modulate pain. J Clin Neurophysiol 1997;14:2–31.

Zhang DX, Owens CM, Willis WD. Two forms of inhibition of spinothlamic tract neurons produced by stimulation of the periaqueductal gray and the cerebral cortex. J Neurophysiol 1991;65:1567–1579.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–110.