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Patterns of Increased Brain Activity Indicative of Pain in a Rat Model of Peripheral Mononeuropathy

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Regional changes in brain neural activity were examined in rats with painful peripheral mononeuropathy (chronic constrictive injury, CCI) by using the fully quantitative ^{14}C-2-deoxyglucose (2-DG) autoradiographic technique to measure local glucose utilization rate. CCI rats used in the experiment exhibited demonstrable thermal hyperalgesia and spontaneous pain behaviors 10 d after sciatic nerve ligation when the 2-DG experiment was carried out. In the absence of overt peripheral stimulation, reliable increases in 2-DG metabolic activity were observed in CCI rats as compared to sham-operated rats within extensive brain regions that have been implicated in supraspinal nociceptive processing. These brain regions included cortical somatosensory areas, cingulate cortex, amygdala, ventral posterolateral thalamic nucleus, posterior thalamic nucleus, hypothalamic arcuate nucleus, central gray matter, deep layers of superior colliculus, pontine reticular nuclei, locus coeruleus, parabrachial nucleus, gigantocellular reticular nucleus, and paragigantocellular nucleus. The increase in 2-DG metabolic activity was bilateral in most brain regions of CCI rats. However, somatosensory regions within the thalamus and the cerebral cortex were activated in CCI rats. High levels of 2-DG metabolic activity were observed within the cortical hind limb area, ventral posterolateral thalamic nucleus, and posterior thalamic nucleus contralateral to the ligated sciatic nerve, and these levels were higher than ipsilateral corresponding regions in CCI rats. In addition, patterns of increased neural activity found in the brain of CCI rats showed some similarities and differences to those found in the brain of rats exposed to acute nociception induced by noxious heat or formalin stimulation. Thus, these CCI-induced *spontaneous* increases in neural activity within *extensive* brain regions of CCI rats previously implicated in sensory–discriminative and affective–motivational dimensions of pain as well as centrifugal modulation of pain are likely to reflect brain neural processing of spontaneous pain. Implications of increased brain neural activity in mechanisms of neuropathic pain are discussed with emphasis on correlations between spatial patterns of altered brain neural activity and pain-related behaviors in CCI rats and clinical symptoms in neuropathic pain patients.

[Key words: neuropathic pain, 2-deoxyglucose, hyperalgesia, spontaneous pain, nerve injury, brainstem, cerebral cortex, thalamus]

Peripheral nerve injury sometimes results in a chronic neuropathic pain syndrome characterized by hyperalgesia, spontaneous pain, radiation of pain, and nociceptive responses to normally innocuous stimulation (allodynia) (Bonica, 1979; Thomas, 1984; Price et al., 1989). Most of these symptoms have been recently observed in a rodent model of painful peripheral mononeuropathy induced by loose ligation of the rat's common sciatic nerve (chronic constrictive injury, CCI) (Bennett and Xie, 1988). For example, one prominent change seen in CCI rats is the appearance of ongoing behaviors such as typical hind paw guarding positions following sciatic nerve ligation, suggesting the presence of persistent spontaneous pain (Bennett and Xie, 1988; Attal et al., 1990; Mao et al., 1992a,b). However, based on these behaviors alone, it is difficult to determine whether CCI rats truly experience spontaneous pain or these ongoing behaviors simply occur as a defense against pains evoked by mechanical or thermal stimuli from the affected area. Some evidence indicating the presence of spontaneous pain in CCI rats derives from recent ^{14}C-2-deoxyglucose (2-DG) autoradiographic studies that show that spinal cord neural activity (inferred from increased local glucose utilization rate) increases substantially in the absence of overt peripheral stimulation in CCI rats 10 d after sciatic nerve ligation (Price et al., 1991; Mao et al., 1992a). Consistent with spontaneous increases in spinal cord neural activity in CCI rats, electrophysiological studies have indicated increases in spontaneous neuronal discharges both in spinothalamic tract neurons (Palecek et al., 1991) and in the ventrobasal thalamic complex (Guilbaud et al., 1990) of CCI rats 2–3 weeks after peripheral nerve injury. In view of the critical roles of supraspinal structures in nociceptive processing, more conclusive evidence for the existence of spontaneous pain in CCI rats should be provided if increased neural activity occurs in brain regions that have been implicated in pain.

Therefore, it would be of great interest to examine the spatial distribution of neural activity throughout the entire brain of CCI rats, examining brain structures implicated in supraspinal nociceptive processing. The advantage of using a neural imaging approach such as the 2-DG technique is that it allows simultaneous examination of multiple CNS areas affected by a stimulus or behavioral condition. As reported previously, increases

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in spinal cord neural activity in CCI rats exhibit specific patterns with respect to the spatial distribution of spontaneously increased neural activity (Price et al., 1991; Mao et al., 1992a). These patterns of increased neural activity can serve as the guidelines for examining spatial patterns of increases in neural activity in specific brain regions of CCI rats. For instance, spinal cord regions showing increased neural activity in these CCI rats include superficial laminae I–II, deep dorsal horn laminae V–VI, and the ventral horn lamina VII (Price et al., 1991; Mao et al., 1992a), regions highly implicated in spinal cord nociceptive processing and representing the main origin of ascending spinothalamic, spinoreticular, and spinomesencephalic tracts. These pathways transmit somatosensory information to many brain structures involved in supraspinal nociceptive processing (Willis, 1985; Price, 1988).

Given the bilateral pattern and the regional specificity (laminae I–IV, V–VI, and VII) of increased spinal cord neural activity in CCI rats (see results in Price et al., 1991; Mao et al., 1992a), several hypotheses may be made with respect to the possible spatial distribution of increases in brain neural activity. First, increases in neural activity should occur bilaterally in broad supraspinal regions of CCI rats in association with ascending nociceptive pathways (including the spinothalamic tract), particularly those in connection with spinoreticular and spinomesencephalic tracts, such as brainstem reticular formation and central gray matter. Second, since at the spinal level maximal increases in neural activity occur in somatotopically appropriate L_1–L_5 spinal laminae I–IV and V–VI (the main origin of the spinothalamic tract) in CCI rats, thalamic nuclei receiving direct input from these spinal cord regions, such as the ventral posterolateral thalamic nucleus and posterior thalamic nucleus, should exhibit somatotopically organized activation. Likewise, cortical somatosensory areas that, in turn, receive input from these thalamic nuclei should be activated as well. Moreover, greater neural activity should be observed on the side contralateral to nerve injury than on the ipsilateral side. Third, since spinothalamic and spinoreticular tracts are likely to participate in both sensory and affective dimensions of pain (Willis, 1985; Price, 1988), regions that may participate in neural processing of affective–motivational states related to pain, such as the amygdala and the cingulate cortex, also would be expected to show increases in neural activity following CCI.

In addition, since similarities and differences in spatial patterns of spinal cord neural activity are observed between acute pain (Coghill et al., 1991) and persistent neuropathic pain in this CCI model (Price et al., 1991; Mao et al., 1992a), accordingly, similarities and differences might be seen between patterns of brain neural activity in CCI rats and in rats exposed to formalin injection, an acute noxious stimulus (Porro et al., 1991b). General similarities also might be expected between patterns of brain neural activity in CCI rats and human subjects during noxious heat stimulation (Talbot et al., 1991) in similar functional activity mapping studies. The presence of similarities and differences between patterns of brain neural activity under acute and chronic pain conditions should provide insights into CNS mechanisms of postinjury neuropathic pain and possibly other chronic pain conditions. These possibilities were investigated in CCI rats 10 d after sciatic nerve injury by utilizing the fully quantitative 2-DG technique, a functional mapping technique that enables simultaneous examinations of both relative intensity of neural activity and its spatial distribution (Kennedy et al., 1975; Sokoloff et al., 1977; Singer and Mehler, 1980; Kozlowski and Marshall, 1983; Di Rocco et al., 1989; Coghill et al., 1991; Price et al., 1991; Mao et al., 1992a).

Materials and Methods

Subjects. Adult male Sprague–Dawley rats (Hilltop) weighing 300–350 gm were used. Animals were individually housed in cages with water and food pellets available ad libitum. The animal room was artificially illuminated from 0700 to 1900 hr. All experimental procedures were approved by our Institutional Animal Care and Use Committee. Lumbar spinal cords of the rats used in the present study have been analyzed in our previous 2-DG studies (Price et al., 1991; Mao et al., 1992a).

Surgical preparation of CCI rats. Rats in both the ligation and the sham operation group (n = 6/group) were anesthetized with 50 mg/kg pentobarbital. For sciatic nerve ligation, the right sciatic nerve was exposed and loosely ligated with four ligatures (4-0 chromic gut) according to the method of Bennett and Xie (1988). For sham operation, the nerve was exposed as above but not ligated. All animals received intramuscular potassium penicillin (30,000 IU/rat) postoperatively.

Behavioral assessments. Thermal hyperalgesia and spontaneous pain behaviors were examined in both ligated and sham-operated rats on days 0 (baseline), 5, 7, and 10 postsurgery. Thermal hyperalgesia to radiant heat was assessed by using a foot withdrawal test. Foot withdrawal latency was defined as the time elapsed from the onset of radiant heat to the hind paw withdrawal. Since the foot withdrawal latency taken from the hind paw directly touching the ground differs from that slightly elevated from the ground, a condition sometimes seen in the nerve-ligated hind paw of CCI rats, we tested CCI rats only during the period when their affected hind paws were in contact with the ground. Foot withdrawal latency difference scores (contralateral side minus ipsilateral side) were used to determine the degree of hyperalgesia as described in detail elsewhere (Mao et al., 1992a,b). Spontaneous pain behaviors were evaluated by observing postsurgical hind paw guarding positions including (1) placement of only the medial portion or the heel of the ligated hind paw onto ground and (2) lifting of the ligated hind paw as described in our previous experiments (Mao et al., 1992a,b).

^{14}C-2-DG administration. The procedures for 2-DG administration were exactly as those described in our previous 2-DG experiments (Price et al., 1991; Mao et al., 1992a). Briefly, on the tenth day after nerve ligation or sham operation, animals were anesthetized with isoflurane (4.5% for induction, 1.5% for maintenance in a 70% N_2O and 30% O_2 mixture). The femoral vein and artery contralateral to the ligated or sham-operated side were catheterized with PE-50 tubing filled with heparinized saline. The surgical field was infiltrated repeatedly with a local anesthetic, bupivicaine, before and after surgery to minimize confounding nociceptive input. Animals were allowed to recover completely from anesthesia for at least 1 hr after catheterization to regain a physiologically stable and awake condition.

Afferent feedback from flexor motor reflexes occurring in CCI rats could conceivably contribute to the increases in 2-DG metabolic activity in CCI rats. In order to examine this possibility, one-half of the animals from each group (ligation and sham operation) were intubated and paralyzed with curare (1.5 mg) under the initial anesthesia induced by isoflurane as described in detail elsewhere (Price et al., 1991; Mao et al., 1992a). The initial anesthesia was discontinued after surgery, and rats were artificially ventilated during the experimental period. End-tidal CO_2 was monitored, and respiratory rate/volume was adjusted to maintain end-tidal CO_2 at 4.5%. Anesthesia was discontinued in both paralyzed and nonparalyzed preparations because anesthesia would substantially alter brain sensory and motor activity. In addition, surgical procedures eliminating sensory awareness (e.g., decerebration) would also substantially alter brain neural activity. CCI produces an ongoing sensory abnormality in the normally behaving (nonparalyzed) preparation. However, based on the absence of differences in spinal cord 2-DG metabolic activity between paralyzed and nonparalyzed rats (Price et al., 1991; Mao et al., 1992a), the introduction of paralysis does not seem to alter the magnitude of abnormal sensory input produced by CCI. Moreover, both paralyzed and nonparalyzed animals did not receive any additional noxious stimulation. Thus, both paralyzed and nonparalyzed animals were very likely to receive similar sensory inputs related to an ongoing sensory abnormality. Furthermore, paralysis did not appear to produce any additional stress in both sham-operated and CCI animals. Neither heart rates (before vs after: 187.3 ± 15.4 vs 185.5 ± 8.9; paired t test, $P > 0.05$) nor blood pressure (systolic/diastolic: 93.7 ± 5.9/71.6 ± 5.4 vs 91.7 ± 7.1/68.8 ± 4.3; paired t test, $P >$

0.05) were changed upon administration of curare. Thus, paralysis does not appear to exacerbate stress in sham-operated animals or pain and stress in CCI animals.

The catheterized paralyzed or nonparalyzed animal was comfortably positioned in a well-ventilated, tube-shaped plastic restrainer (internal diameter 6.5 cm × internal length 25 cm) with fore and hind paws extending through holes at the bottom of the restrainer. The use of a restrainer in nonparalyzed rats was to secure the intravascular cannulas implanted for the blood sampling, a procedure necessary for the fully quantitative 2-DG autoradiography. A restrainer also was used with paralyzed rats in order to apply the same experimental conditions as those applied with nonparalyzed rats. All animals were habituated to restraint 1 hr per day for 5 d prior to 2-DG administration. The placement of a nonparalyzed rat into a restrainer does not immobilize the rat. The rat can still move to a certain degree within the restrainer. In fact, rats usually voluntarily enter a tube-shaped restrainer during the period of habituation before the 2-DG administration. No signs of distress were observed for these rats during restraint as indicated by the observed lack of aggressive behaviors and by the presence of stable blood pressure values before and throughout the experiment. *Neither* noxious *nor* overt innocuous peripheral stimulation was delivered to animals within each group during the following 45 min blood sampling period. At the beginning of a 45 min blood sampling period, ^{14}C-2-DG (50 μCi) was injected into the femoral vein and 13 scheduled blood samples were drawn from the femoral artery to determine plasma glucose and 2-DG radioactive levels for the performance of the fully quantitative ^{14}C-2-DG autoradiography. Blood pressure and heart rates were monitored during the experimental period. End-tidal CO_2 also was monitored in paralyzed, artificially ventilated rats throughout the experiment. On completion of the blood sampling procedure, rats were killed with pentobarbital and perfused with saline and 10% formalin solution. The brain and spinal cords were carefully removed, immediately frozen in cooled 2-methylbutane (−50°C), and then stored at −70°C for later cryostat sectioning and autoradiography. Lumbar spinal cords of these rats have been analyzed previously as described in detail elsewhere (Price et al., 1991; Mao et al., 1992a).

Autoradiography and image processing. Coronal sections of the brain (20 μm per section) were made by using a cryostat at −20°C. Autoradiography was carried out by exposing x-ray film (Kodak SB-5) to brain sections and methyl methacrylate standards (Amersham) for 5 d. Autoradiographic images were digitized and analyzed with a commercially available image processing system (MCID, Imaging Research Inc.). Thirty-six regions of interest (ROIs) selected from the medulla to the cerebral cortex were sampled by manually outlining the territory of each ROI based on the atlas of Paxinos and Watson (1986). The histological verification of these regions was made by using dark-field microscopic observations, and microscopic photos were taken when necessary. Four sections were sampled for each ROI per rat. Whenever applicable, ipsilateral and contralateral portions of an ROI were sampled separately. Optical density was then converted into local glucose utilization rate (LGUR; μmol/100 gm/min) according to the method of Sokoloff et al. (1977).

Statistical data analyses. Mean individual LGUR was first calculated for each ROI for both ligated or sham-operated rats. Mean group LGUR of each ROI was then obtained by averaging individual means of each ROI from each group. Consistent with the lack of differences seen in spinal cord 2-DG metabolic activity between paralyzed and nonparalyzed rats in each group (Price et al., 1991; Mao et al., 1992a), there was also no difference in brain 2-DG metabolic activity (pooled from all 36 brain regions analyzed) between paralyzed and nonparalyzed rats in each group (paralyzed CCI rats vs nonparalyzed CCI rats: 67.2 ± 9.7 vs 62.6 ± 13.2; paralyzed sham rats vs nonparalyzed sham rats: 40.3 ± 4.5 vs 42.5 ± 6.9; Student's t tests, each $P > 0.05$). In addition, LGUR in each sampled brain ROI also was not different between paralyzed and nonparalyzed rats in both ligation and sham operation groups (analysis of variance, ANOVA, $P > 0.05$). All of these indicate a negligible influence from the flexor afferent feedback on brain 2-DG metabolic activity. Thus, data from paralyzed and nonparalyzed rats were pooled to yield group means (the ligation group and the sham-operation group).

Two-way ANOVA was used to examine overall differences in brain LGUR between ligation and sham operation groups as well as differences in each ROI between groups. Univariate tests were used to further analyze differences between ROIs, if applicable, ipsilateral or contralateral to surgical procedures (nerve ligation or sham operation).

Results

Behavioral abnormalities in CCI rats

Behavioral assessment confirmed that CCI rats used in the experiment had demonstrable thermal hyperalgesia, that is, higher foot withdrawal latency difference scores, on days 3, 5, and 10 after nerve ligation as compared to sham-operated rats (ANOVA, $P < 0.01$; see results in Mao et al., 1992a). CCI rats used in this experiment all exhibited foot withdrawal latency difference scores higher than 2 sec. Accordingly, these animals had reliably higher spinal cord 2-DG metabolic activity as compared to sham-operated controls (Price et al., 1991; Mao et al., 1992a). All CCI rats also showed typical postsurgical guarding positions such as placement of only the medial portion or the heel of the ligated hind paw onto ground and frequent lifting of the ligated hind paw.

Global changes in brain 2-DG metabolic activity in CCI rats

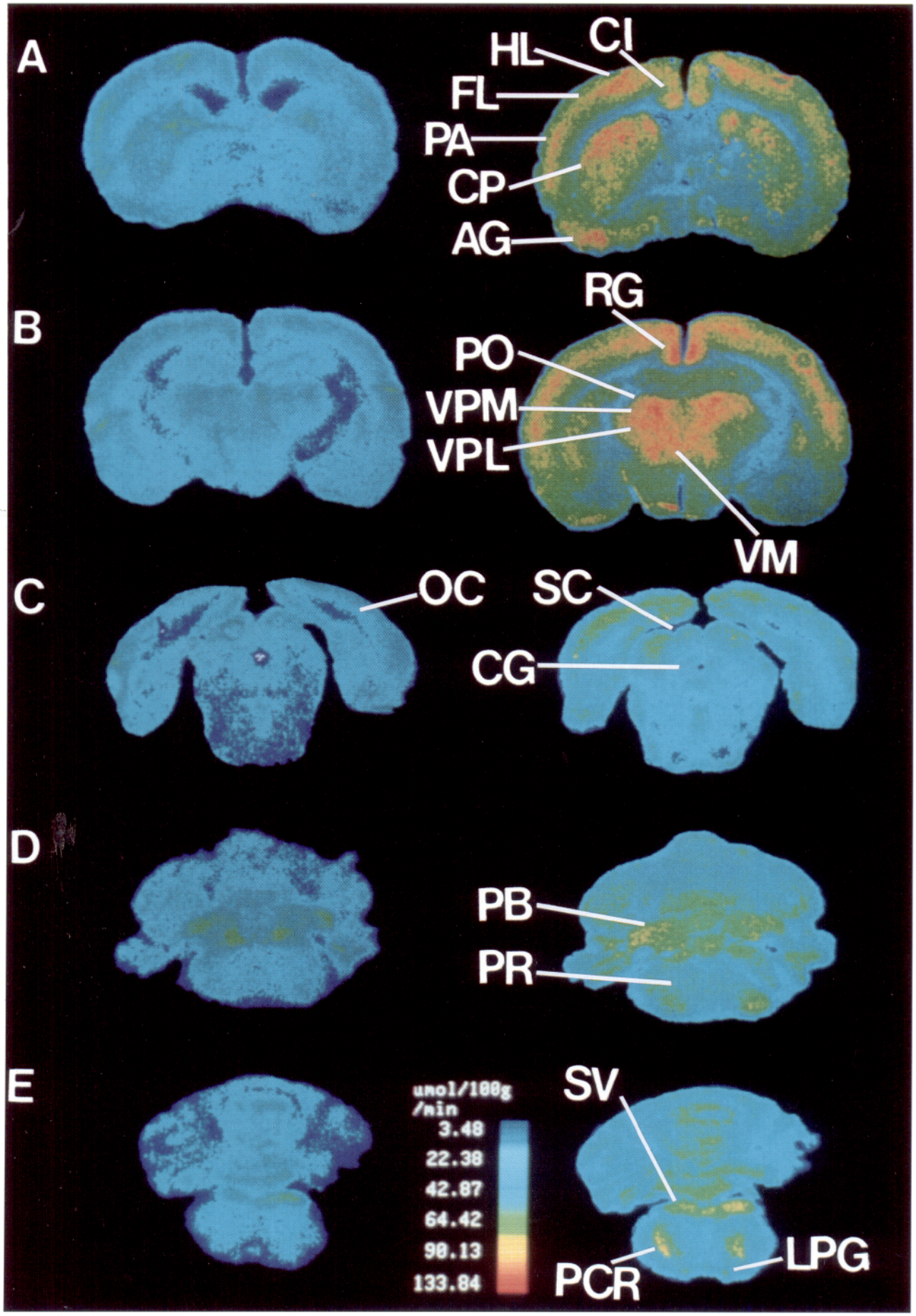
Brain 2-DG metabolism in sham-operated rats ranged from 29 ± 8 (hypothalamic arcuate nucleus) to 57 ± 7 (the posterior thalamic nucleus) μmol/100 gm/min (Fig. 1, left column; Table 1). No significant differences in 2-DG metabolic activity between sampled brain regions ipsilateral and contralateral to surgery were found in sham-operated rats. Thus, sham surgery had a negligible effect on LGUR. In contrast to sham-operated rats, CCI rats examined 10 d after sciatic nerve ligation exhibited strikingly increased brain 2-DG metabolic activity (Fig. 1, right column; Table 1). Overall LGUR pooled from 36 sampled ROIs was reliably higher in CCI rats than in sham-operated rats (ligation, 64.9 ± 13.1, vs sham operation, 41.4 ± 6.2; Student's t test, $P < 0.01$). LGUR in the thalamus and the cerebral cortex was comparatively higher than that in lower brainstem regions of sham-operated rats. Accordingly, reliably higher LGUR also was observed at cortical and thalamic levels in comparison with lower brainstem regions in CCI rats (cerebral cortex + thalamus, 73.8 ± 9.4, vs lower brainstem, 56.1 ± 5.8; Student's t test, $P < 0.05$; Fig. 1, right column; Fig. 2).

Topographic changes in brain 2-DG metabolic activity in CCI rats

Changes in 2-DG metabolic activity occurred within extensive brain regions from the medulla to the cerebral cortex of CCI rats 10 d following sciatic nerve ligation. However, as indicated for specific areas described below, increases in brain neural activity occurred in specific nuclei and were topographically organized.

Medulla. There was an general elevation of LGUR in CCI rats at the medullary level, particularly within both dorsal and ventral portions of the medullary reticular formation (Fig. 1*E*, Table 1). As shown in Figure 1, reliably higher LGUR was observed in CCI rats within a clearly outlined medullary region, the parvocellular reticular nucleus, both ipsilateral and contralateral to the side of nerve ligation as compared to sham-operated rats ($P < 0.01$; Table 1). Reliable increases in LGUR also occurred in bilateral ventral gigantocellular reticular nucleus, lateral paragigantocellular nucleus, and the entire nucleus raphe magnus ($P < 0.01$; Table 1).

LGUR within the vestibular nuclei was not different between CCI rats and sham-operated rats ($P > 0.05$; Table 1), although there seemed to be a slight increase within bilateral vestibular nuclei in CCI rats (Fig. 1*E*). In addition, 2-DG metabolic activity in caudal and interpolar portions of the spinal trigeminal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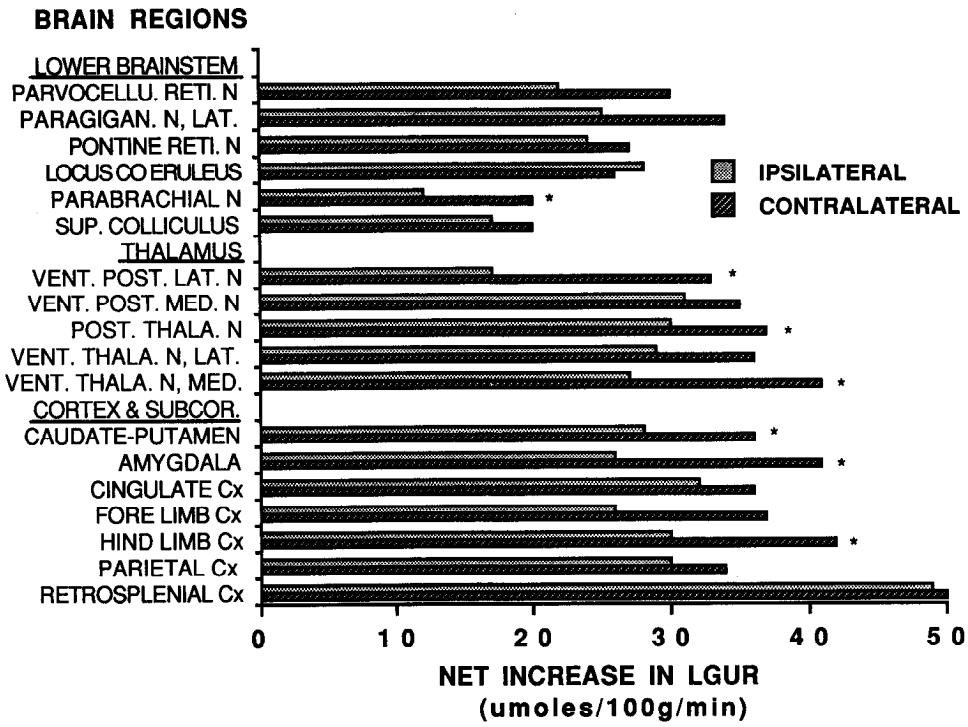

Figure 2. Net increases in 2-DG metabolic activity in sampled bilateral brain regions that were found to have significant elevations in 2-DG activity (see Table 1). Net increases (ligation group minus sham operation group) in LGUR (μmol/100 gm/min) were calculated for each side (ipsilateral or contralateral) of a bilateral ROI exhibiting statistical significance between two groups.

nuclei remained unchanged in CCI rats in comparison with corresponding regions of sham-operated rats ($P > 0.05$; Fig. 1*E*, Table 1).

Pons. LGUR within ventral and caudal pontine reticular nuclei was reliably higher in CCI rats than in sham-operated rats, and similar increases in LGUR were observed in bilateral locus coeruleus of CCI rats (Fig. 1*D*, Table 1). As shown in Figure 1, the activation of the parabrachial nucleus complex, including lateral and medial parabrachial nuclei, was lateralized in CCI rats. Thus, although both ipsilateral and contralateral parabrachial nuclei showed increased LGUR in CCI rats as compared to sham-operated rats, higher LGUR occurred in the contralateral (seen in the left half of a section, the side opposite to the ligated sciatic nerve) parabrachial nuclei as compared to the corresponding ipsilateral (right half of a section) region in CCI rats ($P < 0.05$, paired *t* test; Table 1).

Midbrain. Reliable increases in LGUR were observed within the dorsolateral portion of the central gray matter, the dorsal raphe nucleus, and bilateral reticular tegmental nucleus ($P < 0.05$; Fig. 1*C*, Table 1). Increases in LGUR within the superior colliculus of CCI rats were heterogeneous; that is, reliable increases in LGUR were seen in deep layers of bilateral superior colliculus ($P < 0.05$) but not in superficial layers of this nucleus as compared to sham-operated rats ($P > 0.05$; Fig. 1*C*, Table 1).

Although there appeared to be a slight increase in 2-DG metabolic activity within occipital areas in CCI rats (Fig. 1*C*, Table 1), no significant difference in LGUR was detected within these areas between CCI rats and sham-operated rats ($P > 0.05$; Table 1).

Thalamus and hypothalamus. At the thalamic and hypothalamic level, two apparent changes occurred with regard to increased 2-DG metabolic activity in CCI rats: (1) LGUR was substantially higher within the thalamus and the hypothalamus than that seen in the lower brainstem (Figs. 1*B–E*, 2), and (2) more brain regions were activated as compared to the number of regions showing reliable increases in LGUR within the lower brainstem (Fig. 1*B–E*). However, regions exhibiting increased 2-DG metabolic activity at the thalamic and hypothalamic level were segregated into several distinguishable groups in CCI rats (Fig. 1*B*).

2-DG metabolic activity reliably increased in thalamic somatosensory relay nuclei. These nuclei included ventral posterolateral thalamic nucleus, ventral posteromedial thalamic nucleus, posterior thalamic nucleus, and ventral medial and ventral lateral thalamic nuclei. While increases in LGUR were observed bilaterally within these nuclei as compared to sham-operated rats ($P < 0.01$), higher LGUR occurred on the side contralateral to the injured sciatic nerve than on the ipsilateral side, particularly in ventral posterolateral thalamic nucleus, posterior tha-

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Figure 1. Topographic presentation of coronal sections showing local glucose utilization in the brain of a CCI rat (*right column*) and a sham-operated rat (*left column*). Sciatic nerve ligation produced substantial increases in metabolic activity within cortical hind limb (*HL*) and fore limb (*FL*) area, retrosplenial granular cortex (*RG*), cingulate cortex (*CI*), parietal area (*PA*), caudate-putamen complex (*CP*), amygdala (*AG*), posterior thalamic nucleus (*PO*), ventral posterolateral thalamic nucleus (*VPL*), ventral posteromedial thalamic nucleus (*VPM*), ventral medial thalamic nucleus (*VM*), occipital cortex (*OC*), central gray matter (*CG*), superior colliculus (*SC*), pontine reticular nucleus (*PR*), parabrachial nuclei (*PB*), parvocellular reticular nucleus (*PCR*), spinal vestibular nuclei (*SV*), and lateral paragigantocellular nucleus (*LPG*) as compared to corresponding regions of sham-operated rats. This increase was more pronounced on the contralateral side (left side of an image) of CCI rats than on the ipsilateral side. The anteroposterior coordinates (from bregma) of each coronal section (from *A* to *E*) are −0.80 mm, −3.14 mm, −7.80 mm, −9.80 mm, and −11.96 mm, respectively, based on the Paxinos and Watson atlas (Paxinos and Watson, 1986). The *color bar* represents the calibration of the LGUR (μmol/100 gm/min) for these images.

Table 1. Regional changes in LGUR (μmol/100 gm/min)

Brain regions	Ligation Mean ± SE		Sham Mean ± SE
Medulla			
Spinal trigeminal N, caudal	46 ± 5	(C)	38 ± 3
	47 ± 5	(I)	37 ± 5
Spinal trigeminal N, interpolar	48 ± 6	(C)	38 ± 4
	54 ± 7	(I)	36 ± 5
Medullar reticular formation, dorsal	55 ± 5	(C)	42 ± 6
	57 ± 6	(I)	42 ± 5
Medullar reticular formation, ventral	52 ± 4	(C)	39 ± 3
	50 ± 3	(I)	38 ± 5
Lateral reticular N	47 ± 5	(C)	32 ± 4
	50 ± 6	(I)	38 ± 5
Vestibular N	53 ± 5	(C)	46 ± 4
	60 ± 7	(I)	53 ± 5
Parvocellular reticular N	73 ± 7**	(C)	43 ± 5
	67 ± 3*	(I)	45 ± 6
Lateral paragigantocellular N	62 ± 6**	(C)	28 ± 4
	58 ± 6**	(I)	33 ± 3
Gigantocellular reticular N, ventral	61 ± 8**		31 ± 4
Raphe magnus N	56 ± 4*		34 ± 5
Pons			
Pontine reticular N, caudal	76 ± 9*	(C)	49 ± 6
	69 ± 5**	(I)	45 ± 4
Pontine reticular N, ventral	56 ± 3*		36 ± 6
Locus coeruleus	58 ± 3**	(C)	32 ± 4
	58 ± 11*	(I)	30 ± 3
Parabrachial N, lateral-medial	61 ± 1**†	(C)	41 ± 2
	52 ± 1**	(I)	40 ± 3
Midbrain			
Central gray matter			
Dorsal lateal	58 ± 4*	(C)	42 ± 5
	57 ± 5*	(I)	41 ± 5
Ventral lateral	60 ± 5	(C)	46 ± 6
	60 ± 5	(I)	45 ± 6
Raphe N, dorsal	62 ± 3*		46 ± 7
Reticular tegmental N	55 ± 5*	(C)	36 ± 5
	56 ± 6*	(I)	36 ± 4
Superior colliculus	57 ± 1*	(C)	37 ± 3
Deep layers	55 ± 1*	(I)	38 ± 3
Upper layers	40 ± 2	(C)	38 ± 2
	42 ± 2	(I)	37 ± 2
Thalamus and hypothalamus			
Ventral posterolateral N	82 ± 10*†	(C)	49 ± 6
	66 ± 9	(I)	49 ± 6
Ventral posteromedial N	88 ± 11*	(C)	53 ± 6
	82 ± 10*	(I)	51 ± 6
Posterior thalamic N	94 ± 7*†	(C)	57 ± 7
	82 ± 4*	(I)	52 ± 5
Ventral lateral thalamic N	92 ± 11*	(C)	56 ± 7
	80 ± 12*	(I)	51 ± 5
Ventral medial thalamic N	82 ± 1**†	(C)	41 ± 3
	69 ± 2**	(I)	42 ± 3
Central lateral-central medial N	63 ± 2*		41 ± 3
Dorsomedial hypothalamic N	62 ± 9	(C)	43 ± 6
	62 ± 8	(I)	42 ± 5
Hypothalamic arcuate N	62 ± 9*		29 ± 8
Lateral geniculate N	42 ± 1	(C)	39 ± 2
	42 ± 1	(I)	42 ± 4

Table 1. Continued

Brain regions	Ligation Mean ± SE		Sham Mean ± SE
Cortex and subcortical regions			
Parietal area 1	80 ± 14*	(C)	46 ± 6
	76 ± 13*	(I)	46 ± 7
Parietal area 2	82 ± 14*	(C)	48 ± 6
	79 ± 12*	(I)	48 ± 5
Retrosplenial granular cortex	92 ± 5**	(C)	42 ± 2
	89 ± 4**	(I)	40 ± 1
Hind limb area	82 ± 6**,†	(C)	40 ± 5
	69 ± 6*	(I)	39 ± 5
Fore limb area	77 ± 6**	(C)	40 ± 5
	67 ± 5*	(I)	41 ± 5
Amygdala	81 ± 5**,†	(C)	40 ± 5
	66 ± 5*	(I)	40 ± 6
Cingulate area	82 ± 14*	(C)	46 ± 6
	78 ± 14*	(I)	46 ± 8
Caudate-putamen N	86 ± 5**,†	(C)	50 ± 4
	75 ± 5*	(I)	47 ± 4
Occipital area	45 ± 1	(C)	41 ± 3
	42 ± 1	(I)	39 ± 3

Whenever applicable the metabolic rate calculated from contralateral (C) and ipsilateral (I) brain regions is given separately in the table. *, $P < 0.05$; **, $P < 0.01$ (ANOVA); comparisons made between ligation and sham groups. †, $P < 0.05$ (paired t test), comparisons made between contralateral and ipsilateral side of an ROI in CCI rats.

lamic nucleus, and ventral medial thalamic nucleus ($P < 0.05$, paired t tests; Fig. 1*B*, Table 1). In contrast to increases in LGUR within the thalamic somatosensory relay nuclei, no differences in 2-DG metabolic activity were observed in the lateral geniculate body, a sensory relay nucleus within the visual system, between CCI rats and sham-operated rats ($P > 0.05$; Table 1).

Comparatively smaller but significant increases in 2-DG metabolic activity were observed within the central medial and central lateral nuclei in CCI rats as compared to sham-operated rats ($P < 0.05$; Fig. 1*B*, Table 1). Similar increases in LGUR occurred within the hypothalamus such as the hypothalamic arcuate nucleus ($P < 0.05$; Fig. 1*B*, Table 1).

Cortex and subcortical areas. At the cortical and subcortical level, regions with increased 2-DG metabolic activity in CCI rats were largely divided into two groups: the cortical somatosensory areas and the limbic structures. With respect to the first group, reliable bilateral increases in LGUR were observed within parietal cortex and within cortical hind limb and fore limb somatosensory areas of CCI rats in comparison with corresponding regions of sham-operated rats ($P < 0.01$; Fig. 1*A*). Appropriately, peak activity occurred within the hind limb area (Fig. 1*A*). Consistent with somatotopical activation of thalamic somatosensory projection nuclei in CCI rats, increases in LGUR within the cortical hind limb area were also lateralized; that is, reliably higher LGUR was seen in the contralateral hind limb area as compared to the ipsilateral hind limb area in CCI rats ($P < 0.05$, paired t test; Fig. 1*A*, Table 1). The activation of cortical regions was largely concentrated within the middle and deep layers of the cortex (Fig. 1*A,B*).

Two regions classified as limbic structures, that is, the bilateral amygdala and cingulate cortex, were significantly activated in CCI rats as compared to sham-operated rats ($P < 0.01$; Fig. 1*A*, Table 1). LGUR within the contralateral amygdala was reliably higher than that of the ipsilateral amygdala in CCI rats ($P < 0.05$, paired t test; Fig. 1*A*, Table 1).

Retrosplenial granular cortex, a cortical area seen in the thalamic section (Fig. 1*B*), was highly activated in CCI rats. 2-DG metabolic activity in this cortical area increased bilaterally and homogeneously in CCI rats as compared to sham-operated rats ($P < 0.01$). Moreover, this area exhibited the highest increase in LGUR among 36 sampled brain regions in CCI rats (Fig. 2).

2-DG metabolic activity within the bilateral caudate and putamen complex, a portion of the subcortical basal ganglia, also was increased in CCI rats in comparison with sham-operated rats ($P < 0.01$; Fig. 1*A*, Table 1). Moreover, significantly higher LGUR was seen in the contralateral caudate and putamen complex than in the corresponding ipsilateral region in CCI rats ($P < 0.05$, Table 1).

Characteristics of spatial patterns of increased brain 2-DG metabolic activity in CCI rats

The spatial patterns of strikingly increased 2-DG metabolic activity described above for CCI rats were characterized by several distinct features (see above for detailed statistical analyses). First, LGUR was increased bilaterally in most sampled ROIs of CCI rats as compared to corresponding regions of sham-operated rats (Table 1, Fig. 2). Second, consistent with somatotopic projections of the ascending spinothalamic tract and its corresponding cortical areas, LGUR was reliably higher within appropriate contralateral thalamic and cortical regions, including the ventral posterolateral thalamic nucleus, posterior thalamic nucleus, and cortical hind limb area in CCI rats (Figs. 1, 2). Third, although there occurred a general elevation in background 2-DG metabolic activity in CCI rats, peak increases in metabolic activity (ligated rats minus sham-operated rats) were observed, among 36 sampled ROIs, in the contralateral cortical hind limb area,

amygdala, ventral posterolateral thalamic nucleus, posterior thalamic nucleus, and bilateral retrosplenial granular cortex in five out of six CCI rats (Figs. 1, 2). Finally, LGUR in brain regions known not to be directly involved in somatosensory processing, such as those within the visual pathway (lateral geniculate body, occipital cortex) and the vestibular pathway (medullary vestibular nuclei), remained unchanged in CCI rats.

Discussion

The present findings that striking increases in 2-DG metabolic activity occur in widespread brain regions of CCI rats provide the first functional mapping of supraspinal neural activity resulting from a chronic pain condition. These increases in 2-DG metabolic activity occurred in CCI rats with demonstrable thermal hyperalgesia and spontaneous pain behaviors. The fact that peak increases in metabolic activity occurred in contralateral brain regions previously implicated in supraspinal nociceptive processing suggests a causal relationship between increases in brain neural activity (as measured by 2-DG metabolic activity) and neuropathic pain behaviors in CCI rats. These striking increases in neural activity, which occur in the absence of overt peripheral stimulation, provide further evidence that ongoing pain behaviors reflect persistent spontaneous pain in CCI rats and are not exclusively defensive behaviors directed toward avoiding pain. This overall conclusion is supported by the following discussion, which deals with methodological considerations and anatomical–functional interpretations of brain regions showing increased neural activity.

Methodological considerations

One major limitation of currently applied autoradiographic functional activity mapping techniques, including the ^{14}C-2-DG technique, is their potential inability to distinguish excitatory from inhibitory activity, since both excitatory and inhibitory events involve energy consumption, and therefore increases in local glucose utilization (Ackermann et al., 1984; Nudo and Masterton, 1986). However, studies of combined 2-DG measurement and electrophysiological recording of primate somatosensory cortex strongly suggest that neural excitation is a major contribution to increases in 2-DG metabolic activity (Juliano and Whitsel, 1987), and in fact, inhibitory neural events are shown to decrease local glucose utilization in similar 2-DG studies (Sharp et al., 1988). Consistent with these observations, recent electrophysiological studies have reported increased background neuronal discharges within the lumbar spinal cord (Palecek et al., 1991) and the thalamic ventrobasal complex (Guilbaud et al., 1990) of CCI rats, in which substantial increases in 2-DG metabolic activity are detected in previous (Price et al., 1991; Mao et al., 1992a) and present 2-DG experiments. Thus, increases in 2-DG metabolic activity observed in brain regions of CCI rats are more likely to reflect excitatory than inhibitory activity. An additional limitation of this 2-DG technique relates to its lack of cellular resolution; that is, changes in 2-DG metabolic activity are undistinguishable between neuronal perikarya and nerve fibers (Di Rocco et al., 1989). An alternative functional activity mapping technique utilizing the c-fos–like protein expression provides such cellular resolution (Hunt et al., 1987).

Despite its limitations, the 2-DG technique is a well established autoradiographic mapping technique (Sokoloff et al., 1977). It is different from the c-fos method, which can label only certain types of neurons and therefore may be an incomplete marker of neural activity (Bullitt, 1990). Moreover, the 2-DG technique is based on the well-understood mechanism that the increase in local cerebral glucose utilization is proportional to the increase in neural activity (Sokoloff et al., 1977). This technique provides a unique means to allow simultaneous examination of both relative intensity of neural activity and its spatial distribution within the CNS by measuring LGUR during a relatively long period (30–45 min). Thus, the 2-DG technique is particularly suitable for mapping CNS neural activity during prolonged noxious stimulation (Coghill et al., 1991) or ongoing nociception such as in this CCI model (Price et al., 1991; Mao et al., 1992a). Comparable electrophysiological single-unit examinations of the spatial distribution of neural activity within the entire CNS would be extremely time consuming and thereby impractical. Nevertheless, the use of different methodologies should provide better understanding of CNS nociceptive processing than any one technique alone.

Functional relationships to brain regions implicated in nociceptive processing

Given the chronic course of behavioral and physiological changes subsequent to nerve ligation in this CCI model, it is conceivable that a number of potential changes only indirectly related to chronic neuropathic pain may have occurred and have contributed to regional increases in brain 2-DG metabolic activity in CCI rats. Such changes might occur in brain regions involving regulation of motor activity, food and water intake, cardiovascular activity, and respiratory functions. This may partially explain the bilateral activation of certain brain regions in CCI rats. However, the extensive activation of brain regions observed in the present 2-DG experiment most likely reflects CNS processing of neuropathic pain in CCI rats for several reasons. First, increases in brain neural activity of CCI rats reflect the anatomic and functional continuation of ascending spinal cord somatosensory pathways (e.g., spinoreticular, spinomesencephalic, and spinothalamic tracts), whose spinal cord origins (laminae I–IV, V–VI, VII) exhibit substantially elevated neural activity in these same CCI rats (cf. results in Price et al., 1991; Mao et al., 1992a). Second, brain regions that are very unlikely to be involved in pain, such as those within the visual pathway (lateral geniculate body, occipital cortex) and the vestibular pathway (medullar vestibular nuclei), remained unchanged in CCI rats as compared to sham-operated rats. Third, a large body of evidence indicates that the increase in brain neural activity in CCI rats exhibits regional specificity with respect to known anatomical and electrophysiological involvement of these regions in supraspinal nociceptive processing. Nevertheless, a complete resolution of this interpretational problem awaits the analysis of brain activity by a variety of noxious stimuli with varying nonspecific sequelae. The relationships between observed increases in neural activity and evidence for involvement in nociceptive processing or pain modulation will be discussed for individual brain regions in turn.

Medulla. The activation of lateral paragigantocellular nucleus, gigantocellular reticular nucleus, nucleus raphe magnus, and parvocellular reticular nucleus at the medullary level is consistent with their possible roles in nociception and pain modulation (Basbaum and Fields, 1984; Lovick, 1987; Mayer and Price, 1989; Zhuo and Gebhart, 1991; Kiefel et al., 1992). Extracellular recordings made from the gigantocellular reticular nucleus indicate that neuronal discharges of nociceptive neurons within

this nucleus reliably increase in response to peripheral noxious heat (Sotgiu, 1988) or mechanical (pinch) stimulation (Drower and Hammond, 1988), and the electrical activation of this nucleus with low-intensity stimulation facilitates spinal cord nociceptive transmission as assessed using the tail-flick test (Zhuo and Gebhart, 1990b, 1991). On the other hand, most of these nuclei are also known to be major origins of descending nociceptive inhibitory pathways (Lovick, 1986b, 1987; Zhuo and Gebhart, 1990a,b, 1991; Cho and Basbaum, 1991) and to relay descending pain modulatory pathways from upper brain regions (Lovick, 1986a; Ness and Gebhart, 1986; Mokha et al., 1987; Drower and Hammond, 1988; Kiefel et al., 1992). Since the activation of descending inhibition by noxious or environmental peripheral input has been known to play an important role in endogenous analgesic systems (Mayer and Price, 1989), the activation of these nuclei could relate to both nociceptive processing and descending modulation.

It should be noted that both lateral paragigantocellular nucleus and gigantocellular reticular nucleus are also implicated in the autonomic regulation of cardiovascular functions (Lovick, 1986b, 1987; Van Bockstaele et al., 1989; Aicher and Randich, 1990). Thus, it will be of interest to determine whether the activation of these nuclei is related to abnormal sympathetic responses in CCI rats, reflected for example by differences in skin temperature between ligated and nonligated hind paws in CCI rats (Bennett and Ochoa, 1991).

Pons and midbrain. The observation of increases in 2-DG metabolic activity within the pontine reticular nucleus, locus coeruleus, dorsal raphe nucleus, reticular tegmental nucleus, and central gray matter confirms the previous behavioral and electrophysiological data that these regions participate in central nociceptive processing (Basbaum and Fields, 1984; Willis, 1985; Ness and Gebhart, 1986; Yezierski and Schwartz, 1986; Mayer and Price, 1989; Muller and Klingberg, 1989; Kiefel et al., 1992). Of further significance is that higher metabolic activity is seen in deep layers than in superficial layers of the superior colliculus. This observation is consistent with electrophysiological studies showing that wide-dynamic-range and nociceptive-specific neurons occur in deep but not superficial layers of the superior colliculus (McHaffie et al., 1989). The involvement of this midbrain nucleus in nociceptive processing has also been reported in several recent electrophysiological studies (Larson et al., 1987; McHaffie et al., 1989; Nelson et al., 1989). In view of its possible role in sensory–motor integration (McHaffie et al., 1989), the superior colliculus may have specific roles in abnormal behaviors in CCI rats related to orientation to the location of peripheral nociception.

The activation of the parabrachial nucleus at the pontine level is of particular interest since this pontine nucleus is strategically located within a recently delineated spinopontoamygdaloid pathway (Ma and Peschanski, 1988; Bernard et al., 1989; Hylden et al., 1989; Berkley and Scofield, 1990; Bernard and Besson, 1990). This spinopontoamygdaloid pathway originates from superficial spinal laminae I–II and is relayed within the pontine parabrachial nucleus to the amygdala (Ma and Peschanski, 1988; Bernard et al., 1989; Hylden et al., 1989; Berkley and Scofield, 1990; Bernard and Besson, 1990), a structure that was found to be highly activated in the present experiment. Projections from laminae I–II cells to the parabrachial nucleus were found to be bilateral and some of them were shown to be collaterals of the spinothalamic tract (Hylden et al., 1989). The majority (69%) of neurons within this pathway (recordings made from antidromically identified parabrachial–amygdaloid projection neurons) were classified as nociceptive-specific neurons, and *no* wide-dynamic-range neurons were recorded within this pathway (Bernard and Besson, 1990).

The role of this spinopontoamygdaloid pathway in pain processing is not yet clear. It is unlikely that this pathway participates in neural processing of sensory–discriminative dimensions of pain since little evidence exists indicating the involvement of the amygdala in sensory discrimination of pain. This ascending pathway is, however, likely to be involved in affective–motivational dimensions of pain, particularly in the coordination of nociceptive, emotional, and autonomic responses to noxious stimulation (Bernard et al., 1989; Berkley and Scofield, 1990; Bernard and Besson, 1990), since the amygdala is known to be a forebrain region implicated in stress, arousal, and fearful reactions (Werka and Marek, 1990; Tanaka et al., 1991). On the other hand, both anatomical and electrophysiological studies have indicated an efferent pathway originating from the amygdala, directly or indirectly (via the medial hypothalamus) synapsing within the periaqueductal gray, to the spinal cord dorsal horn (Hopkins and Holstege, 1978; Krettek and Price, 1978; Beitz, 1982; Watson et al., 1983; Zhang et al., 1991). This efferent pathway from the forebrain has been implicated in descending modulation of pain (Basbaum and Fields, 1984; Shaikh et al., 1991; Zhang et al., 1991). It should be noted that structures within both the spinopontoamygdaloid pathway and the amygdaloid efferent pathway were found to be highly activated in our present and previous (Price et al., 1991; Mao et al., 1992a) 2-DG studies. Thus, there appears to be a neural loop that connects the spinal cord dorsal horn (laminae I–II), pontine parabrachial nuclei, amygdala, periaqueductal gray, and back to the spinal cord. This proposed neural loop might be important in efferent modulation of pain. Interestingly, a recent behavioral study has indicated important roles of the parabrachial nucleus in development of neuropathic pain behaviors (autotomy) following sciatic and saphenous nerve sections (Wall et al., 1988). Destruction of cell bodies with ibotenic acid in the contralateral parabrachial nuclei accelerates the onset of autotomy in nerve-sectioned rats (Wall et al., 1988). It will be of interest to investigate whether such an inhibitory influence from the parabrachial nucleus on development of neuropathic pain is mediated by this proposed neural loop.

Thalamus and hypothalamus. The role of thalamic somatosensory relay nuclei and their adjacent regions in brain nociceptive processing has been well documented (Willis, 1985; Chung et al., 1986; Guilbaud et al., 1986; Taguchi et al., 1987; Downie et al., 1988; Lenz et al., 1988; Price, 1988; Dostrovsky and Guilbaud, 1990). Accordingly, the ventral posterolateral thalamic nucleus, posterior thalamic nucleus, ventral lateral and medial thalamic nuclei, and central medial and lateral thalamic nuclei are shown to be highly activated in the present 2-DG experiment. Moreover, results from our 2-DG labeling are also consistent with the data derived from a recent electrophysiological study (Guilbaud et al., 1990). In that study, abnormal neuronal responses within the ventrobasal thalamic complex (regions corresponding to major thalamic relay nuclei in our 2-DG labeling) were observed in the same CCI model used in the present 2-DG experiment, including increased spontaneous neuronal activity, fading of the response with repetitive stimulation, and prolonged afterdischarges (Guilbaud et al., 1990).

Consistent with the involvement of the hypothalamic arcuate nucleus in pain modulation (Mao and Yin, 1987; Hamba, 1988;

Sathaye and Bodnar, 1989; Wang et al., 1990), increases in 2-DG metabolic activity are also seen in this hypothalamic nucleus in CCI rats. Direct spinohypothalamic projections from both superficial spinal laminae I–II and the base of the dorsal horn have been recently reported (Burstein et al., 1987). This direct spinohypothalamic pathway may at least partially account for the activation of hypothalamic nuclei in CCI rats. The hypothalamic arcuate nucleus is the major origin of β-endorphinergic neurons in the brain (Bloom et al., 1978) and β-endorphin is known to play important roles in pain modulation (Loh et al., 1976). Thus, the activation of the hypothalamic arcuate nucleus may have a descending inhibitory influence on nociceptive processing in CCI rats. On the other hand, since the hypothalamic arcuate nucleus has numerous connections with brainstem structures and functional relationships with the pituitary (Sim and Joseph, 1991; Takeshige et al., 1991a) and is therefore involved in regulation of many physiological reactions including stress and emotional reactions (Takeshige et al., 1991a,b), its activation in CCI rats may reflect possible roles of this hypothalamic nucleus in the affective–motivational dimension of pain in CCI rats.

Cortex and subcortical areas. Regions activated at the cortical level such as parietal areas and the hind limb somatosensory area, areas corresponding to the rat's primary somatosensory area (SI) (Mountcastle and Darian-Smith, 1968), are consistent with those thalamic somatosensory relay nuclei showing increased neural activity in CCI rats. Of particular interest is the fact that peak activity occurred within the hind limb area of the somatosensory cortex (Fig. 1). Nociceptive neuronal responses within these cortical areas have been reported in previous animal and human studies (Lamour et al., 1983a; Willis, 1985; Price, 1988; Talbot et al., 1991). It should be emphasized that the activation of cortical areas in CCI rats presents a general laminar organization (Fig. 1). Thus, increased neural activity occurs mainly within middle and deep layers of the cortex, a feature consistent with the heavy concentration of wide-dynamic-range and nociceptive-specific neurons found in these cortical layers in electrophysiological studies (Lamour et al., 1983a,b).

The caudate and putamen complex is part of the subcortical basal ganglia that is known as a central region involved in coordination of motor activity (Mountcastle and Darian-Smith, 1968). Several recent reports, however, have indicated its role in pain modulation (Ohno et al., 1987; Li and Xu, 1990; Mulgaonker and Mascarenhas, 1991; He, 1992). Given the abnormal motor activity resulting from abnormal guarding postures in CCI rats, the activation of the caudate and putamen complex may be related to pain modulation and/or pain-related abnormal motoric activity.

It is surprising that the greatest increase in 2-DG metabolic activity occurs within the retrosplenial granular cortex of CCI rats (Fig. 2). The retrosplenial granular cortex is an association cortex that has numerous reciprocal connections with the thalamus, hypothalamus, limbic structures, and other cortical areas (Thompson and Robertson, 1987; Groen and Wyss, 1990). To our knowledge, the role of this cortical area in nociceptive processing has not yet been investigated. This area is known to play important roles in learning and memory in many species (Thompson and Robertson, 1987; Groen and Wyss, 1990, 1992). Of interest is that intracellular changes of spinal cord neurons similar to those during mammalian learning and memory processes have been reported in CCI rats (Mao et al., 1992e). This striking increase in neural activity within the retrosplenial granular cortex, which is involved in learning and memory, lends further support to the hypothesis that neural mechanisms of postinjury neuropathic pain may be related to CNS neuronal plastic changes similar to those occurring in learning and memory (Mao et al., 1992c–f).

The activation of the cingulate cortex and amygdala may be related to general arousal, anxiety, and stress (Papez, 1937; Vogt et al., 1979; Werka and Marek, 1990; Tanaka et al., 1991), although these areas have also been implicated in nociceptive processing and pain modulation (Hylden et al., 1989; Al Rodhan et al., 1990; Werka and Marek, 1990; Jones et al., 1991; Talbot et al., 1991). However, since rats used in the present experiment had been habituated for restraint before 2-DG administration and did not receive overt peripheral stimulation during the experimental period, and since bilateral elevations of 2-DG metabolic activity within these areas are observed only in CCI rats but not sham-operated rats handled in the same manner, the activation of the cingulate cortex and amygdala may primarily reflect neural processing related to pain, particularly the affective–motivational dimension of chronic neuropathic pain. In fact, this feature of the bilateral activation of the cingulate cortex in CCI rats is consistent with the clinical observation that the affective-motivational dimension of persistent pain in human patients, including neuropathic pain patients, can be partially relieved after bilateral lesions of this area (Foltz and White, 1962; Hurt and Ballantine, 1973). Thus, it will be of interest to determine whether the activation of the cingulate cortex, amygdala, and retrosplenial granular cortex contributes to the immediate unpleasantness stage or the secondary stage (related to long-term implications and suffering) of the affective dimension of pain (Price and Harkins, 1992).

Comparisons of patterns of increased brain activity between acute and persistent pain and implications for mechanisms of neuropathic pain

Patterns of increased brain neural activity following CCI are similar in many respects to those induced by acute noxious heat or formalin stimulation (Porro et al., 1991b; Talbot et al., 1991). First, lower brainstem regions activated in CCI rats overlap primarily with those activated by hind paw formalin injection, both including medullar and pontine reticular nuclei, nucleus raphe magnus, dorsal raphe nucleus, and central gray matter. These regions receive input from spinoreticular and spinomesencephalic pathways, the spinal cord origins of which are highly activated in similar 2-DG studies (Porro et al., 1991a; Price et al., 1991; Mao et al., 1992a). Second, the increase in 2-DG metabolic activity is bilateral in most sampled brainstem regions but with greater increases within the contralateral side following either unilateral sciatic nerve injury or unilateral hind paw formalin stimulation, a feature consistent with bilateral increases in spinal cord 2-DG metabolic activity in both cases (Porro et al., 1991a; Price et al., 1991; Mao et al., 1992a). The bilateral activation of brain structures following unilateral sciatic nerve ligation is consistent with behavioral observations that mechanical hyperalgesia occurs in hind paws of CCI rats both ipsilateral and contralateral to the side of nerve ligation (Attal et al., 1990). Bilateral activation also occurs in the spinal cord of rats exposed to noxious heat stimulation (Coghill et al., 1991), although much higher contralateral spinal cord neural activity is seen in CCI rats (Mao et al., 1992a) than in rats stimulated with noxious heat (Coghill et al., 1991). Third, cortical and subcortical areas with increased neural activity in CCI rats are

generally consistent with those activated by noxious heat stimulation in awake human subjects (Jones et al., 1991; Talbot et al., 1991) including cortical somatosensory regions, the cingulate cortex, and thalamic regions. Thus, the same brain regions involved in CNS nociceptive processing appear to be activated under both acute and chronic pain conditions.

An important difference in spatial patterns of brain neural activity is that brain regions activated in CCI rats appear to be even more extensive than those CNS regions activated during formalin or noxious heat stimulation (Porro et al., 1991b; Talbot et al., 1991). For example, the activation of the brain is bilateral in many thalamic and cortical regions of CCI rats including the ventral posterolateral thalamic nucleus, posterior thalamic nucleus, cortical hind limb area, fore limb area, and parietal areas. The existence of large and bilateral receptive fields of nociceptive neurons within spinal cord lamina VII (Menetrey et al., 1984) may have conceivably contributed to such bilateral activations in CCI rats. On the other hand, the bilateral activation of brain regions (e.g., the cortical fore limb area) in CCI rats may result from the divergence of neural responses at both spinal and supraspinal levels. For instance, propriospinal connections have been shown to extend from caudal spinal cord segments up to the cervical enlargement (Yezierski et al., 1980; English et al., 1985), a region receiving fore limb somatosensory input. Consistent with these anatomical connections, elevations of spontaneous neural activity are observed across the entire sampled spinal cord lumbar segments (L_1–L_5) with little decrease at both rostral (L_1) and caudal (L_5) extremes in CCI rats (Price et al., 1991; Mao et al., 1992a). Thus, it is likely that changes in neural activity within the spinal cord may extend well beyond the primary afferent terminations of the ligated sciatic nerve in CCI rats and may even include the fore limb area. The divergence of spinal cord activity may, thereby, account for much of the widespread activity in thalamic and cortical somatosensory regions.

The activation of brain structures is not, however, restricted to regions receiving input directly from known spinal cord ascending nociceptive pathways in CCI rats. For example, the activation of the ventral posteromedial thalamic nucleus in CCI rats is unexpected, since this thalamic region receives trigeminothalamic but little, if any, spinothalamic tract input (Yen et al., 1991). Since no reliable increases in metabolic activity were observed in the medullary trigeminal nuclei, the increased neural activity within the ventral posteromedial thalamic nucleus (and possibly other brain areas) is likely to be related to the divergence of excitatory activity within the brain itself (Willis, 1985; Price, 1988). The extension of neural activity to brain areas beyond those related to the anatomic territory of the injured sciatic nerve may account for pain radiation and for the occurrence of sensory abnormalities well beyond the territory of original injury in neuropathic pain patients (Bonica, 1979; Price et al., 1989). Thus, it will be of interest to examine whether similar phenomena can be seen, for instance, in the facial area or the fore limb of CCI rats since substantial increases in neural activity are observed in the ventral posteromedial thalamic nucleus and the cortical fore limb area in this CCI model.

In addition to the extensive activation of many brain regions related to processing of the sensory–discriminative dimension of pain, a number of structures within the limbic system (retrosplenial granular cortex, amygdala, cingulate cortex), the motor system (caudate-putamen complex), and the autonomic system (medullary reticular formation, hypothalamic areas) are also highly activated in CCI rats. Such a multiple-system activation is consistent with clinical characteristics of chronic neuropathic pain syndromes that involve disorders associated with sensory, motor, and autonomic systems (Bonica, 1979; Thomas, 1984; Price et al., 1989). Similar pathological changes are seen in CCI rats including thermal hyperalgesia, spontaneous pain behaviors (abnormal guarding positions), and asymmetry of hind paw skin temperature (Bennett and Xie, 1988; Attal et al., 1990). These symptoms are likely to be mediated by activation of multiple brain regions and their anatomic interconnections.

It is important to point out that sciatic nerve injury in CCI rats activates not only those brain regions implicated in afferent processing of sensory–discriminative and affective–motivational dimensions of pain but also those associated with centrifugal modulation of pain. The latter structures may include the medullary reticular formation, nucleus raphe magnus, parabrachial nuclei, dorsal raphe nucleus, central gray matter, and hypothalamic arcuate nucleus. Such a dual-functional activation of both afferent and efferent nociceptive processing systems in CCI rats may reflect CNS mechanisms of adaptation to a persistent pain condition. Thus, this CCI-induced increase in neural activity across *extensive* brain regions implicated in both sensory–discriminative and affective–motivational dimensions of pain as well as centrifugal modulation of pain may represent patterns of supraspinal processing related to chronic pain conditions.

The use of the 2-DG functional activity mapping technique provides a three-dimensional view of the entire picture of brain nociceptive processes under a persistent pain condition. Such an approach is likely to lead to improved understanding of CNS processing related to acute and chronic pain. For instance, although temporal properties of nociceptive neural activity (such as neuronal discharge frequency, temporal summation) may or may not differ greatly between acute and chronic pain (Willis, 1985; Price, 1988), the spatial distribution of CNS nociceptive processing in the latter appears to be distinguished by greater divergent activation of multiple brain regions. Furthermore, the unexpected and as yet unexplained activation of certain brain regions in CCI rats such as the retrosplenial granular cortex and caudate-putamen complex suggests that studies of the role of these brain regions in the neural processing of pain might provide new insights into the neurobiology of neuropathic pain and pain in general. Thus, results derived from this functional (2-DG) activity mapping study may serve as guidelines for future electrophysiological and anatomic studies to determine the physiological nature of neurons activated in neuropathic pain as well as to delineate the neural circuitry involving supraspinal nociceptive processing under chronic pain conditions.

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