Peripheral nerve injuries, resulting from either permanent deafferentation or conditions of nerve inflammation, usually lead to abnormal processing of innocuous and noxious stimuli, causing sensations such as phantom perceptions, hyperalgesia, and allodynia. During the last decade, several animal models for peripheral neuropathy have been developed that use chronic nerve constriction injury: (1) partial tight ligation of the sciatic nerve, (2) loose ligation of the sciatic nerve, and (3) tight ligation of spinal nerves. In these models, the behavior of the animals closely mimics the symptoms observed in patients.

In animals rendered neuropathic by injuring the sciatic nerve, single-unit recordings show that somatosensory neurons in the lateral thalamus have activity patterns distinct from those in normal animals: a higher frequency of spontaneous activity, lower thresholds for noxious activation, and after discharges outlasting the stimulus. The rates of these changes in central representation were essentially zero before ligation, maximal within minutes after ligation, and decreased to a steady sustained rate of change within 1 to 2 hours. The incidence of functional connectivity, as measured by cross-correlations, remained unchanged. However, the strength of functional connectivity increased after ligation. The results show immediate reorganization of lateral thalamic networks with peripheral nerve damage. When the population response is considered as the underlying code, this reorganization does not reflect the behavioral manifestations of hyperalgesia and allodynia, even though some of the individual neuronal responses do reflect properties consistent with the hyperalgesia and allodynia reported within the same time frame after nerve injury in the rat.

Immediate Reorganization of the Rat Somatosensory Thalamus After Partial Ligation of Sciatic Nerve

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Abstract: Nerve injury can result in neuropathic pain, which persists after the injury and may occur after healing is completed. The long-term central reorganization associated with neuropathic pain has been previously studied in animal models. The immediate effects of nerve injury on central representation, however, are poorly understood. We examined the population response properties of closely neighboring neurons located in the hindlimb representation area of the somatosensory thalamus. Changes in the neuronal population properties were characterized before, during, and after (up to 6 hours) partial ligation of the sciatic nerve in the rat. Changes in these properties were observed within minutes after nerve injury. There were changes in neuronal class and receptive field size, emergence of new receptive fields, receptive fields observed before ligation disappeared temporarily after ligation, and changes in number of spikes evoked by the same stimulus. The rates of these changes in central representation were essentially zero before ligation, maximal within minutes after ligation, and decreased to a steady sustained rate of change within 1 to 2 hours. The incidence of functional connectivity, as measured by cross-correlations, remained unchanged. However, the strength of functional connectivity increased after ligation. The results show immediate reorganization of lateral thalamic networks with peripheral nerve damage. When the population response is considered as the underlying code, this reorganization does not reflect the behavioral manifestations of hyperalgesia and allodynia, even though some of the individual neuronal responses do reflect properties consistent with the hyperalgesia and allodynia reported within the same time frame after nerve injury in the rat.

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Groups of thalamic neurons were studied by using our new multielectrode technique. The method allows monitoring of the properties of individual neurons, closely located in a small neighborhood (within a volume of about 100 × 100 × 100 µm³ tissue), and enables the study of the interrelationships among the neighboring neurons in functional connectivity, i.e., spike timing synchronizations as measured by cross-correlation analysis. We hypothesized that after nerve ligation there would be (1) changes in receptive field (RF) size or loss of responsiveness, (2) changes in the response patterns such that neurons would have to be assigned to a different neuronal class (e.g., from non-nociceptive to nociceptive), and (3) changes in spike timing relationships between neighboring neurons.

Materials and Methods

Eight anesthetized adult Sprague-Dawley rats (250 to 300 g) were used in the physiologic study, and another 6 rats were used to examine behavioral measures of pain after nerve ligation. Housing, handling, and experimental procedures were approved by the local Committee for the Humane Use of Animals. Animals were anesthetized with halothane (induction: 4% halothane in a mixture of 70% O₂ and 30% NO₂; maintenance: 0.5% to 1.2% halothane and 70% O₂ and 30% NO₂ during the recording session in which halothane concentration was varied as needed to prevent withdrawal reflexes due to noxious stimulation).

The sciatic nerve was prepared before performing the physiologic recordings. The left sciatic nerve was exposed at high thigh level; a 7-0 nylon suture was passed 1 mm distal to the site where the posterior biceps semitendinosus nerve branches off to capture 30% to 50% of the dorsal part of the sciatic nerve. The wound was closed, and the threads were externalized. After collecting a set of control stimulus response recordings, a partial tight ligation was performed. At the end of the experiments, the sciatic nerve was removed and examined microscopically to ascertain that a given ligation was successful.

The rats were placed in a stereotactic frame, and a craniotomy was performed to access the lateral thalamus contralateral to the lesioned sciatic nerve. Recordings were performed in the hindpaw representation area of the ventral posterolateral nucleus (VPL) and adjacent areas by using single or 4-tip tungsten electrodes (FHC Inc, Bowdoinham, ME). In each animal, boundaries of VPL were established by single tungsten electrode recordings before the multielectrode recordings. Subsequent recordings used the 4-tip electrodes, targeting neuronal groups in or around VPL. Only 1 recording site per animal was investigated. Digitized multiunit signals were recorded on-line with commercial software (DataWave Technologies, Longmont, CO). The collected data were clustered off-line to individual neuronal activity, and peristimulus time-histograms of the responses of individual neurons were created, and the spike timing coordination and locations in space relative to the recording tips were calculated.

Stimuli consisted of innocuous brush and/or tap, pressure, and noxious pinch applied bilaterally to 3 to 4 sites on the contralateral and ipsilateral body (extremities and tail) every 15 to 20 minutes. After stable recording conditions were achieved, 3 to 4 repetitions of the stimulation sequence were performed before the ligation. Five to 7 minutes after ligation, the first series of stimuli was applied by stimulation every 10 to 15 minutes (within the first hour after ligation), followed by stimulation every 20 to 40 minutes. In later phases of the experiments, stimuli were applied every hour, altogether for up to 6 hours.

A given unit was classified as responsive to a specific stimulus only if its mean firing rate changed significantly (t test, P < .05) and by at least 30%. Neurons were classified as low threshold (LT) when the increase in activity was not different between innocuous and noxious stimulation, as wide dynamic range (WDR) when noxious stimulation elicited a higher neuronal activity as compared with innocuous stimuli, and as nociceptive specific (NS) when only noxious stimulus intensities led to increased neuronal activity.

Search strategy was to find neurons with RFs on the contralateral ventral hindpaw by using innocuous and, more sparingly, noxious stimuli. Once this was accomplished, other parts of the animal’s body, i.e., the tail and lower trunk, were stimulated to determine inputs from these regions. The area of the RFs of these multiunit recordings was only systematically mapped with brush and touch stimuli. In contrast, noxious stimuli were not used to map the borders of these RFs. Even if no responses to stimulation of other body parts were found, these were stimulated in the same manner as the RF on the hindpaw during the experiment: brush/tap/touch, innocuous pressure, noxious pinch. Thus, an increase in RF size is defined as either an increase in area due to innocuous stimulation or, in the case of noxious stimulation, “new” responses from other parts of the body.

Spike timing synchronization between pairs of neighboring neurons was determined under different stimulus conditions before and after the ligation. These cross-correlations were calculated with a window width of 200 ms, a bin size of 1 ms, and smoothed with a Gaussian function. Statistically significant cross-correlation peaks or valleys were defined as 3 or more bins exceeding 2 standard deviations (SD) from the mean spike count and lasting for at least 4 bins within the first 30 ms. Cross-correlation strength was calculated by measuring the area of the peak or valley 2 SD above the mean count, multiplied by bin size and divided by the total number of spikes. The latter expresses the cross-correlation strength in extra spikes observed above the mean number of spikes (see Apkarian et al and Abeles).

In some animals, at the end of the experiment the recording site was marked with a small electrolytic
Peripheral Injury and Thalamic Reorganization

(15 µA for 10 seconds), and the deeply anesthetized animal was perfused with 4% paraformaldehyde. The brain was cut into 50-µm coronal sections and counterstained with cresyl violet to localize the recording site.

Three rats with partial nerve ligation and 3 sham-operated control animals underwent the formalin test 2 days after the surgical procedures. Formalin (50 µL, 5%) was injected into the paw contralateral to the operated side, and the behavior of the rats was videotaped and rated on the formalin test scales for the number of protective behaviors, number of paw jerks, and number of paw licks during 5-minute periods for 60 minutes after the injection. The sum of these was considered as an overall measure of pain behavior. The ligated rats showed higher overall pain behavior in the early phase (first 15 minutes after formalin injection: mean ± SD of overall pain rating, 369 ± 121 in ligated rats and 168 ± 94 in control rats) and a similar overall pain behavior in the late phase as compared with the control rats. However, the later phase in the ligated animals was also abnormal, because the number of licks was dramatically reduced, being similar to the early phase of the control rats (number of licks in the first 15 minutes: 41 ± 13.3 in ligated rats and 59 ± 32.3 in control rats; in contrast, in the next 45 minutes the number of licks: 82 ± 38 in ligated rats and 433 ± 42 in control rats). These results show that the partial sciatic nerve ligation procedure we have adapted from Seltzer et al\(^2\) causes abnormal pain behavior as they reported previously.

Results

Only neurons with stable response properties before ligation were considered. Altogether 32 lateral thalamic neurons were characterized in five 4-tip electrode recordings (n = 28 neurons; mean ± SD, 5.6 ± 2.1 neurons per recording site in a tissue volume of about 100 × 100 × 100 m\(^3\)) and 3 single electrode recording sessions (n = 4 neurons). Before using 4-tip electrodes the target region in the lateral thalamus was mapped with single electrodes. The boundaries of VPL were determined during this initial mapping. Criteria to assign recording sites to VPL were (1) the well-known sequence of RFs from proximal to distal body sites and from medial to lateral sequence of head, upper limb, lower limb, and tail; (2) small RFs; and (3) brisk responses to hair. The 4-tip electrodes were placed in relation to these boundaries. Their locations were determined on the basis of the responses obtained while lowering the electrodes to the target. In 3 animals the 4-tip recording electrode sites were recovered and ascertainment to be located in hindlimb VPL. According to these criteria, 18 neurons were located inside VPL, and 14 were located in adjacent regions. According to the search paradigm, most RFs of the neurons were located on the contralateral foot and tail.

Figure 1. RF size and properties of 5 neighboring VPL neurons before and after partial sciatic nerve ligation. The colored areas indicate the location of the RF, the color indicates the responses to tap, pinch only, and tapping and pinch. Symbols during ligation: plus, change in ongoing activity during partial nerve injury; Ø, no change in ongoing activity during ligation. The first test was performed 5 minutes after ligation; the following tests were performed 3 times each 20 minutes, then every 40 minutes, and then every 60 minutes, respectively.
data of the 32 neurons were pooled, because no differences between cells located inside VPL versus outside VPL were found before and after ligation with respect to changes in RF size and neuronal class (eg, from LT to WDR) due to ligation. After ligation 19 of 32 neurons (59%) exhibited changes in RF size and 9 of 32 exhibited changes in neuronal class (28%, ie, their classification as LT, WDR, NS). All 9 neurons that showed changes in neuronal class also underwent changes in RF size.

Fig 1 is an example of changes in response properties of 5 neighboring neurons after ligation. Three neurons lost their RFs after ligation (units 1, 2, and 3), although they maintained ongoing activity. One of these neurons (unit 1) lost its tail RF after ligation, a body part not innervated by the ligated sciatic nerve. One neuron had a temporary loss of RF (unit 5). Another neuron showed fluctuations in responsiveness, with changes in neuronal class, and temporary expansion of RF (unit 4).

Fig 2 shows the response of 5 neighboring neurons to noxious pinch and to the ligation. Four neurons were NS type (1 was nonresponsive), and their RFs covered the contralateral (to the recording site) hindpaw and the tail (Fig 2, left panel). At least 3 of the neurons were activated by the ligation (Fig 2, middle panel). Six hours after ligation 4 of the neurons had increased RF sizes, ie, now responding to noxious pinch of the ipsilateral hindpaw (one of these did not respond to pinching of the contralateral hindpaw any more), and all had increased responses to noxious stimulation after ligation (Fig 2, right panel).
Before ligation 11 of 32 neurons were LT, 7 were WDR, 8 were NS, and 6 did not respond to any stimulus. After ligation at the end of the experiment, 9 neurons were LT, 3 were WDR, 10 were NS, and 10 did not respond to any stimulus. Although the overall number of neurons assigned to the different classes were similar before and after ligation, many neurons had to be assigned to a different neuronal class at the end of the experiment, as is depicted in Fig 3. One neuron that was unresponsive before and after ligation is included because it did exhibit a vigorous response to the ligation.

Before ligation 11 of 32 neurons were LT, 7 were WDR, 8 were NS, and 6 did not respond to any stimulus. After ligation 4 to 6 times within the first hour after ligation. In this early phase after ligation, 13, 6, 9, and 8 changes in RF size were observed from test to test, and 15, 7, 10, and 7 changes were observed with respect to neuronal class (n = 28 neurons). Thus, immediately after nerve injury, there seems to occur a large disturbance in the balance of inputs to the thalamus.

Fig 4 shows the rate of fluctuations in RF sizes and in neuronal classes after ligation. If a neuron showed at least one change in RF size or neuronal class from 1 hour of testing to the next one, it was counted as 1 fluctuation. These data were then pooled for the example given in Fig 4A (8 neighboring neurons of a single recording site) and in Fig 4B for 5 recording sites (28 neurons; 4 neurons were excluded from this analysis because their time course was not properly documented). In Fig 4A, the units were tested during a period of 1 hour before and 6 hours after ligation. Before ligation the RF sizes and neuronal classes were stable. This changed dramatically after ligation. Most of the 8 neurons changed response properties within the first hour after ligation. Fig 4B shows the number of neurons changing response properties during 3 hours after ligation for all 28 neurons studied. The mean rate of change per hour per neuron in RF size or neuronal class in the first 3 hours after ligation (averaged across all tests applied in this time) was 2.21 ± 0.57 or 2.22 ± 0.53 (±SEM), respectively.

None of the recording sites showed complete cessation of responsiveness to lower body stimulation after the ligation, although most recording sites had sciatic nerve inputs. The sciatic nerve inputs could be determined from RF properties and ligation effects. Neurons that increased their firing rates during the partial nerve constriction, called ligation responsive, were assumed to have prominent sciatic nerve inputs. The majority of the studied neurons had RFs on the contralateral hindlimb (26 of 32) and responded to the ligation (24 of 32). Of the 32 neurons, 20 responded to the ligation and changed their stimulus-response properties after ligation, 4 responded but had no changes in response properties after ligation, and 8 did not respond to ligation but exhibited changes in response properties after ligation. Thus, changes in response properties after ligation occurred independently of whether neurons were directly affected by the ligation.

Fig 5 shows 2 examples (units 2 and 3 in Fig 1) of cross-correlations between pairs of neurons, recorded from the same cluster located inside VPL, before and after ligation. In Fig 5A, the cross-correlations between 2 nociceptive neurons (1 WDR, 1 NS) are shown. Although both neurons lost their RFs completely after ligation, their cross-correlations did not change. In Fig 5B, a second example is shown in which both neurons were LT before ligation. After ligation,
one was unresponsive and the other temporarily unre-
sponsive, but their cross-correlations were strength-
ened after ligation.
A small fraction of pairs of neurons exhibited repro-
ducible significant cross-correlations. For example, in an
8-unit cluster 24 of 28 possible pairs of cross-correlations
were observed, although only 6 pairs occurred consis-
tently. For 3 experiments, the overall ratio of repro-
ducibly occurring pairs to all possible pairs was 12:55. All
12 pair cross-correlations were preserved after the liga-
tion. However, the connection strengths before versus
after ligation for all stimulus conditions was significantly
higher after ligation (0.02 ± 0.03, mean ± SD) than
before (0.04 ± 0.08; t test, P = .007). Fig 6 shows
strength-distance relationship between all pairs and
stimulus before (Fig 6A) and after ligation (Fig 6B). The
figure shows the increased cross-correlation strength
after ligation and illustrates a lack of distance depen-
dence. We also did not observe stimulus-dependent
changes in cross-correlations, because basically all corre-
lations were positive. Moreover, we rarely observed
inhibitory cross-correlations (in a time window of ± 10
ms; 0.2% incidence).

**Discussion**

The present results show that changes in the response
properties of somatosensory neurons in the lateral thal-
amus can be observed within a few minutes after a par-
tial peripheral nerve injury, and that most changes occur
within the first hour. These changes include changes in
RF size, changes in neuronal class, temporary loss of RFs,
coupled with a strengthening of functional connectivity.
Such fluctuations in responsiveness have not been

Figure 4. (A) Number of neurons with changes in responsiveness after ligation for an 8-neuron cluster located in VPL. Filled
squares, dashed lines: changes in neuronal class; filled circles, solid line: changes in RF size. (B) Number of neurons with changes
after ligation for 28 neurons located in and around VPL (symbols and lines as in A).
Peripheral Injury and Thalamic Reorganization

reported before. Thus, all 3 hypotheses, ie, expected changes in RF size and neuronal class and changes in cross-correlations, were confirmed. Because the short time scale in which we observed these changes is not compatible with anatomic reorganization, we conclude that unmasking of previously ineffective connections must be the underlying mechanism for the reorganization observed in the present study. The observed short-term reorganizations observed in the present study are in agreement with deafferentation experiments performed by others at the level of the spinal cord, nucleus gracilis, thalamus, and cortex.15-19

In our experiments, 25% of the thalamic neurons lost their RF after partial nerve ligation, 25% increased their RF size (eg, from hindpaw to hindpaw plus tail), and about 50% did not change their RF size. However, we did not observe recording sites in which all neurons became silent after ligation. This is in contrast to complete interruption of the afferent input. In an early investigation of the hindpaw representation in the gracile nucleus, all neurons became unresponsive to hindpaw stimulation after cold block of the spinal cord at the L4 level.15 It is very likely that the observed differences in the proportion of neurons changing (or losing) response properties are due to the differences between partial and complete deafferentation, because partial nerve ligation disrupts only part of the input, whereas complete denervation deprives an input domain completely.

Pettit and Schwark16 performed a study with an experimental paradigm that is more comparable with the one used in the present study, ie, partial deafferentation of the innervation domain of the sciatic nerve. They investigated the reorganization of RFs on the cat hindpaw in gracile nucleus neurons by using lidocaine injections into the original RF. In agreement with the present study, they made 3 major observations: (1) after blocking the input from the original RF, new, often bigger RFs appeared in neighboring skin areas of the foot minutes after blocking; (2) new RFs could change response classes (eg, from rapidly adapting to slowly adapting), and (3) RF sizes can fluctuate over time (they recorded up to 6 hours after block). Thus, at the dorsal column nuclei level comparable changes as in the somatosensory thalamus can occur.

Nicolelis et al17 also used local anesthetics to deprive neurons in the thalamic ventral posteromedial nucleus (VPM) of rats from their original whisker inputs by small lidocaine injections. In agreement with the present study, they found that peripheral sensory deprivation changes spatial aspects of the responses of VPM neurons within minutes after block induction.

Given the data discussed previously, it is clear that in the somatosensory system reorganization at the level of the gracile nucleus and the thalamus sets on immediately, and that this reorganization may involve changes in RF size and/or neuronal class, ie, changes in the magnitude of inputs from a particular sensory channel (eg, LT) and/or changes in the quality of inputs

Figure 5. Cross-correlations between 2 different pairs of neurons (units 2 × 3, A; 1 × 5, B in Fig 1) within the same cluster. The times (in hours) when the cross-correlations were calculated are shown in the upper left of each panel (negative numbers are before, positive numbers after partial sciatic nerve ligation). The strength of the correlations are illustrated by the size of the peak relative to baseline firings. The y-axis is not labeled because it varies between graphs to better illustrate the peak correlation in each case.

Figure 6. Connection strength between pairs of neurons with significant cross-correlations before (left panel) and after ligation (right panel), pooled for all stimulus conditions. The connection strength after ligation was significantly higher than before ligation.
from different sensory channels (eg, non-nociceptive and nociceptive). These changes in RF size and neuronal class fluctuated at a mean rate of about 2 changes per hour. The short period of time of the onset of these changes excludes morphologic changes. Thus, we conclude that the nerve injury unmasks pre-existing, non-dominant inputs from a variety of sensory channels. We suppose that in intact animals there exists a stable input equilibrium of dominant and latent inputs from different sources, which is immediately disrupted by partial deafferentation. The observed fluctuations in RF size and neuronal class indicate that partial deprivation of dominant inputs induces competition between available inputs. Even thalamic inputs from outside the sciatic innervation domain, like the tail, are affected (Fig 1, units 1 and 3), showing that peripheral nerve injury can have an impact on relatively distant input domains. It should be emphasized that the majority of the changes occurred immediately after the nerve ligation and thereafter stabilized to a lower rate of change. This pattern of change cannot be explained as because of sensitization as a result of repeated stimulation, especially because the stimulus repetition interval was longer than 10 minutes and there was no observable evidence of skin damage with the noxious stimuli.

In this respect, it is interesting that a “response” to ligation tended to be followed by changes in responsiveness, because this was the case in about two thirds of the neurons. In case of an increase in spike rate during ligation, one would expect that this is the result of the barrage of ectopic action potentials fired during injury by those axons, which provide the dominant input to the thalamic neurons. Thus, one would expect that “responding” neurons would lose their original RF and be unresponsive or gain a new RF on a different body site. This, indeed, was observed in some of the neurons. But there were also units that responded to ligation but had no changes in responsiveness or vice versa. Thus, there must be a considerable divergence of afferent inputs originating from the injured axons.

We hypothesized that peripheral nerve injury would not only lead to changes in RF size and neuronal class but also to changes in the cross-correlations between closely located neurons. Overall, we do observe increased functional connectivity strengths after ligation. This change in connectivity is consistent with the unmasking of pre-existing, normally ineffective synaptic connections because it implies expansion of shared information between neighboring neurons (see Wall20). We interpret these changes as reflecting increased synchronization of the common afferent input to the local population of neurons.9 In the present study in the rat, basically all cross-correlations were excitatory, and thus we did not observe changes in cross-correlations with respect to the kind of stimulus applied (brush, pressure, pinch) and as a function of distance between pairs of neurons. This is in contrast to our findings in the squirrel monkey in which most cross-correlations were inhibitory during noxious stimuli and showed an opponent relationship between distance and functional connectivity strength.21 One explanation could be that the rat ventrobasal complex is virtually void of inhibitory interneurons.22

The presented short-term changes are in disagreement with reported long-term physiologic changes and are also not related to the short- or long-term behavioral changes, persistent allodynia and hyperalgesia, observed with this model.1,5 Although our results do show immediate fluctuations in response properties, they do not show any indication of a net increase in nociceptive responses in the deafferented portion of the lateral thalamus. This in fact was the starting hypothesis, in that we expected to see increased responsiveness to noxious inputs and long after discharges as the basis for an explanation for initiation of the changes leading to neuropathic pain. This dissociation between lateral thalamic network properties and the animal’s behavior may be a reflection of the region as not being necessary for the ligation-induced behavior in this early phase, which is consistent with recent human brain imaging studies.23,24 On the other hand, data reported by Kolhekar et al25 suggest that N-methyl-D-aspartate receptors in the thalamic hindpaw representation area are involved in the development of hyperalgesia in neuropathic pain (they tested 3 to 24 hours after induction). Also, the study by Guilbaud et al2 in which rats with neuropathic pain were studied long after the injury (2 weeks after injury in rats with chronic constriction injury), as well as physiologic studies in humans with chronic pain,3,26-28 indicate abnormal discharge patterns in the lateral thalamus, with evidence for an increase in the net number of nociceptive cells, even though electrical stimulation in this region in patients with chronic pain can reproduce their pain. Therefore, the role of the lateral thalamus in the onset and long-term maintenance of neuropathic pain remains unsettled.

References


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