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BASIC SCIENCE

# Sustained Neuronal Hyperexcitability Is Evident in the Thalamus After a Transient Cervical Radicular Injury

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**Study Design.** This study used extracellular electrophysiology to examine neuronal hyperexcitability in the ventroposterolateral nucleus (VPL) of the thalamus in a rat model of painful radiculopathy.

**Objective.** The goal of this study was to quantify evoked neuronal excitability in the VPL at day 14 after a cervical nerve root compression to determine thalamic processing of persistent radicular pain.

**Summary of Background Data.** Nerve root compression often leads to radicular pain. Chronic pain is thought to induce structural and biochemical changes in the brain affecting supraspinal signaling. In particular, the VPL of the thalamus has been implicated in chronic pain states.

**Methods.** Rats underwent a painful transient C7 nerve root compression or sham procedure. Ipsilateral forepaw mechanical allodynia was assessed on days 1, 3, 5, 7, 10, and 14 and evoked thalamic neuronal recordings were collected at day 14 from the contralateral VPL, whereas the injured forepaw was stimulated using a range of non-noxious and noxious mechanical stimuli. Neurons were classified on the basis of their response to stimulation.

**Results.** Behavioral sensitivity was elevated after nerve root compression starting at day 3 and persisted until day 14 ($P < 0.049$). Thalamic recordings at day 14 demonstrated increased neuronal hyperexcitability after injury for all mechanical stimuli ($P < 0.024$). In particular, wide dynamic range neurons demonstrated significantly more firing after injury compared with sham in response to von Frey stimulation ($P < 0.0001$). Firing in low threshold mechanoreceptive neurons was not different between groups.

**Conclusion.** These data demonstrate that persistent radicular pain is associated with sustained neuronal hyperexcitability in the contralateral VPL of the thalamus. These findings suggest that thalamic processing is altered during radiculopathy and these changes in neuronal firing are associated with behavioral sensitivity.

**Key words:** radiculopathy, pain, hyperexcitability, thalamus, neuron, brain, allodynia, neck, VPL, radicular.

**Level of Evidence:** N/A

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Cervical radiculopathy is common, with a high prevalence of chronic pain.[1] The cervical nerve roots are at risk for injury from either neck trauma and/or disc disease.[2–4] Even "transient" nerve root compression is sufficient to induce persistent mechanical and thermal behavioral sensitivity that are associated with axonal damage and degeneration in the root and inflammation at the injury site and in the spinal cord.[5–8] Spinal regulation of the glutamatergic system, together with nociceptive neuropeptides and neuronal hyperexcitability, are also altered in association with sustained sensitivity.[7,9–11] Although many of the spinal responses that initiate and maintain persistent pain have been defined,[12,13] less is known about the neuronal mechanisms in the brain that contribute to persistent radicular pain.

Many studies of pain report modified activity in a variety of brain regions, involving the primary and secondary somatic areas, insula, thalamus, and cingulate cortex.[14–21] Increased blood flow, sodium channel expression, neuronal bursting, background activity, afterdischarge, and altered thalamocortical rhythms are reported in chronic or neuropathic pain states.[22–27] Although neuronal activity in the thalamus has been investigated, the focus is largely on pain from spinal cord injury and/or inflammatory exposures.[28–30] Thalamic responses have been investigated in a model of chronic nerve ligation, with resolving pain.[22] Furthermore, Yamashiro *et al*[31] demonstrated that thalamic neurons in the rat respond similarly to that of the human after multilevel (C5–T1) dorsal root sectioning. Despite the high incidence of radicular pain and the role of the thalamus in pain regulation, very little is known about thalamic responses in radicular pain, especially after a transient neural tissue injury. This study used our rat model of transient nerve root compression applied for 15 minutes that induces pain, axonal degeneration and disrupted axonal transport in the nerve root, and spinal neuronal

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dysfunction[5,6,9,32,33] to define neuronal responses in the ventroposterolateral nucleus (VPL) of the thalamus in the context of behavioral sensitivity. In contrast to sustained nerve root deformation, a transient trauma to the nerve root is considered as the one which is removed soon after its application. Because transient root compression produces behavioral sensitivity that is present at 14 days and persists for 6 weeks,[5,34] we hypothesized that neuronal activity in the thalamic nuclei would also increase. Neurons were classified on the basis of their response to mechanical stimulation[11,35] to investigate activity by neuronal phenotype.

## MATERIALS AND METHODS

### Surgical Procedures

Male Holtzman rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 250 to 350 g were housed under USDA- and AAALAC-compliant conditions with a 12:12-hour light-dark cycle and access to food and water *ad libitum*. All procedures were approved by our Institutional Animal Care and Use Committee and carried out according to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.[36]

Rats underwent a transient compression injury to the right C7 dorsal nerve root (n = 6) or sham surgery (n = 5), in accordance with previously published procedures[6,8,9,32,37,38] (Figure 1). All surgical procedures were performed under inhalation isoflurane anesthesia (4% induction, 2% maintenance). A midline incision was made from the occiput to the T2 spinous process, followed by paraspinal muscle dissection on the right side, a C6–C7 hemilaminectomy, and partial facetectomy. The right C7 root was compressed by a 10*g*-force microvascular clip (WPI, Sarasota, FL) for 15 minutes (Figure 1). A sham control group underwent the same surgical procedures but only root exposure with no compression. After surgery, a 2-layered closure was performed using 3-0 absorbable suture and skin staples. Rats were monitored while recovering in room air.

### Behavioral Assessment

Rats were evaluated by a tester blinded to procedures on postoperative days 1, 3, 5, 7, 10, and 14 by measuring mechanical allodynia in the ipsilateral forepaw (Figure 1).[9,10,32] Baseline measurements were obtained prior to surgery to control for any individual differences in behavior. Rats were acclimated prior to each testing session. Von Frey filaments with increasing strengths (1.4 g, 2 g, 4 g) (Stoelting, Wood Dale, IL) were used to stimulate the plantar surface of the ipsilateral forepaw. Each testing session consisted of 3 rounds of 10 stimulations by each filament, with a 10-minute rest period between rounds. For each filament, the total number of paw withdrawals was counted for each rat and averaged for each group, and was compared over time between groups using separate repeated-measures analysis of variances for each filament. *Post hoc* analysis of variances compared responses at each day. Behavioral data are presented as means ± standard error of mean. All statistical analyses were performed using JMP8 (SAS Institute Inc., Cary, NC).

### Electrophysiological Procedures

Electrophysiological recordings were made in the contralateral VPL of the thalamus on day 14 (Figure 1). Rats were anesthetized with 45 mg/kg of sodium pentobarbital injected intraperitoneally. The anesthesia plane was maintained and monitored using further pentobarbital injections (5–10 mg/kg intraperitoneally) as needed. A midline incision over the skull was made from the surgical incision to the frontal bone and soft tissue dissected to reveal the coronal, sagittal, and lambdoid cranial sutures. A craniotomy over the left hemisphere, beginning at bregma and extending caudally 8 mm and laterally 8 mm, exposed the cortex overlying the thalamus. A midline neck incision exposed the midcervical trachea, which was cannulated for mechanical ventilation with room air at 40 to 50 breaths per minute, with a tidal volume of 2.5 to 3.0 mL (CWE, Inc., Ardmore, PA). The end tidal $CO_2$ concentration was monitored. To minimize respiratory-related movement during recordings a right-sided

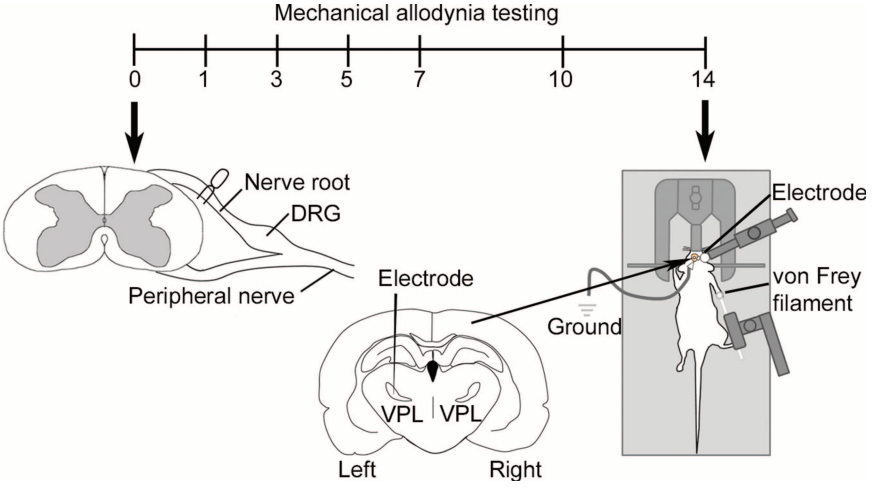


**Figure 1.** Schematic of the experimental protocol showing radicular injury produced by a clip on the dorsal root or sham surgery at day 0. Mechanical allodynia was assessed on the days indicated; on day 14, electrophysiological recordings were made from the contralateral VPL of the thalamus during stimulation protocol of the forepaw. VPL indicates ventroposterolateral nucleus; DRG, dorsal root ganglion.

thoracotomy was performed using the lateral intercostal approach. The rat was placed in a stereotactic frame with ear bars; the head was adjusted so bregma and lambda were in the horizontal plane (Figure 1). Core temperature was maintained at 36ºC to 37ºC using a heating plate and an isolated rectal probe (model TCAT-2DF; Physitemp Instruments Inc., Clifton, NJ). The dura was removed and the brain was bathed in 37ºC mineral oil.

Extracellular voltage potentials were recorded using a glass-insulated tungsten electrode (125-μm shank, 20º taper to <1-μm tip; FHC, Bowdoin, ME). Signals were processed with a 60-Hz noise eliminator (HumBug; Quest Scientific, North Vancouver, Canada) and digitally sampled and stored at 25 kHz (Micro1401; CED, Cambridge, England). Starting at −2.5 mm from bregma and 2.2 mm left lateral, neurons were identified by lowering the electrode 5 to 7 mm below the pial surface at 1-mm intervals in the anterior-posterior and medial-lateral planes based on known coordinates and somatotopy of the rat VPL.[39,40] Mechanosensitive-specific neurons were identified by brushing the right forepaw with a cotton swab. A stimulation protocol was performed applying stimuli to the area of the paw with the maximal response, with at least 60 seconds between each stimulus. On the basis of prior methods recording from spinal neurons,[11,41–43] 5 consecutive 1-second stimulations were applied at 1-second intervals using von Frey filaments (1.4 g, 4 g, 10 g, 26 g), followed by a 10-second noxious pinch using a 60g vascular clip (WPI, Sarasota, FL).

Recordings were spike-sorted using Spike2 software (CED, Cambridge, England). The total numbers of spikes evoked by each stimulus and during the rest period after it were counted and summed. The duration of the each stimulus train was identified; baseline activity was determined by counting the number of spikes in the same time period prior to the first stimulus and was subtracted from the total spike count to evaluate evoked responses.[41,43] Spike counts were log-transformed to account for a positive skew in the spike totals distribution; a normal distribution was verified. Neurons were classified on the basis their response to von Frey stimulation[35]: a wide dynamic range (WDR) neuron demonstrated a graded response to increasing von Frey filament strengths; a low-threshold mechanoreceptive neuron responded to both non-noxious and noxious stimuli; a nociceptive specific neuron only responded to noxious stimuli. To ensure that recordings were from comparable regions in the VPL, the probe locations for each recording were compared using *t* tests between the 2 groups, using the coordinates of the probe's depth (*Z*-direction; distance from pial surface), lateral distance from the midline (*X*-direction), and distance in the anteroposterior plane from bregma (*Y*-direction). Firing responses for all neurons, and for each phenotype separately, were compared between groups for each filament using separate analysis of variances with the *post hoc* Tukey HSD test. Differences in the proportion of neurons by phenotype were compared between injury and sham using the Pearson $\chi^2$ tests. Spike counts are presented as mean ± standard error of mean.

## RESULTS

Overall, transient nerve root compression induced significant mechanical allodynia in response to all filaments ($P = 0.020$ for 1.4 g; $P = 0.0015$ for 2 g; $P = 0.0011$ for 4 g) (Figure 2). Sham responses were unchanged from baseline for all filaments. For testing with the 2-g filament, the injury group had significantly more paw withdrawals than shams on day 5 ($P < 0.005$) and the number of paw withdrawals remained elevated until day 14 ($P < 0.02$) (Figure 2). The number of paw withdrawals in the injury group for the 4-g filament also was significantly higher than sham starting on day 3 ($P < 0.045$), remaining elevated until day 14 ($P < 0.019$) (Figure 2). The number of withdrawals evoked on the day of neuronal recording (day 14) was significantly greater for

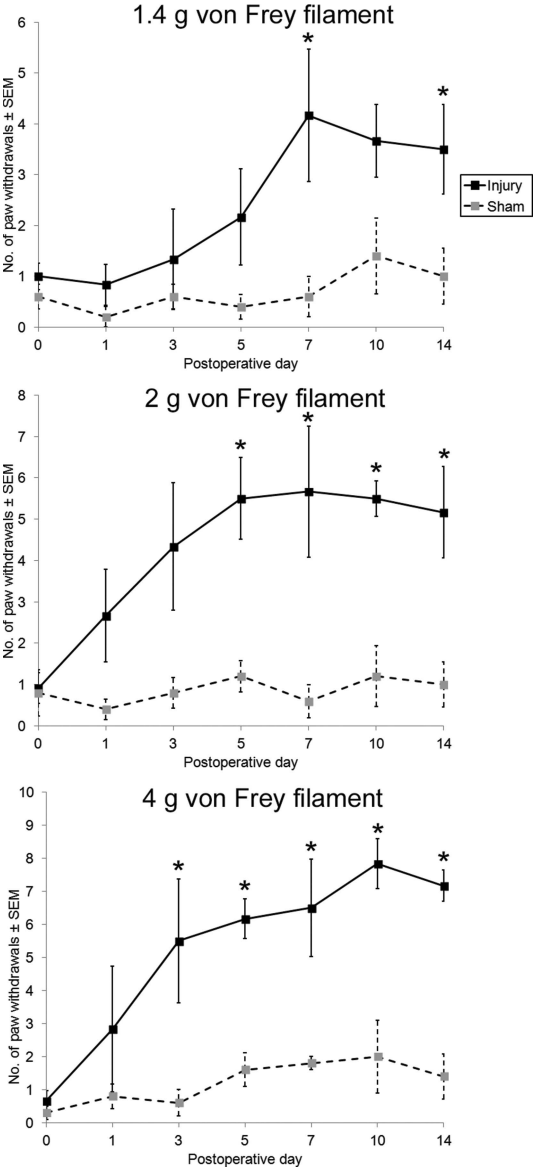


**Figure 2.** Overall, behavioral sensitivity ($P < 0.02$) was detected after injury, with significance (*$P < 0.045$) for testing with each of the 1.4-g, 2-g, and 4-g filaments, indicated. SEM indicates standard error of mean.

injury than sham for all filaments: 1.4 g ($P = 0.049$), 2 g ($P = 0.012$), and 4 g ($P = 0.0001$) (Figure 2).

A total of 57 neurons (38 from injury; 19 from sham) were recorded from all rats, with 5.18 ± 2.32 neurons from each rat. Although 6.33 ± 2.16 neurons were recorded from each rat in the injury group and 3.8 ± 1.8 neurons were recorded from each rat in the sham group, there was no statistical difference between the groups. The electrode positions for making recordings varied little between rats and groups (Figure 3). The depth ($Z$) of the recording electrode was not different between the groups. Similarly, the recording locations in the anteroposterior plane were not different between groups (Figure 3). The left lateral ($X$) position of the probe was significantly different between injury and sham ($P < 0.003$), despite being very similar (Figure 3). Hematoxylin and eosin histological assays confirmed that the probe tracks terminated in the VPL (Figure 3).

Neuronal firing in the VPL significantly increased after a painful injury for all stimuli ($P < 0.0024$) (Figure 4). This was observed for each filament, with greater increases elicited by the stronger filaments (Figure 4). Specifically, stimulating the forepaw with the 1.4-g filament evoked nearly 3 times the number of spikes in the VPL after injury (24.1 ± 4.6 spikes) compared with sham (8.3 ± 2.6 spikes) ($P < 0.001$). Evoked firing by the 4-g filament was similarly significantly greater in the injury group (38.9 ± 8.1 spikes) than the sham group (16.3 ± 4.1 spikes) ($P < 0.034$) (Figure 4). Both the 10-g and 26-g filaments evoked robust increases in the neuronal spikes in the thalamus after injury compared with sham ($P < 0.005$ for 10 g; $P < 0.009$ for 26 g) (Figure 4).

After injury, 58% of the neurons were classified as WDR, 39% as low threshold mechanoreceptive and 3% as nociceptive specific. In contrast, 31% were classified as WDR, 53% as low threshold mechanoreceptive and 16% as nociceptive specific in the sham group. Although there seems to be a shift in the neuronal phenotype toward increased WDR neurons after injury, this was not significant. However, analysis of spike counts by neuronal phenotype showed that the WDR neurons exhibited significant increases in the overall firing response ($P < 0.0001$) and for each von Frey filament after injury compared with sham ($P < 0.014$ for 1.4 g; $P < 0.019$ for 1.4 g; $P < 0.016$ for 10 g; $P < 0.018$ for 26 g). Firing in the other neuron types was not different between groups for any filament.

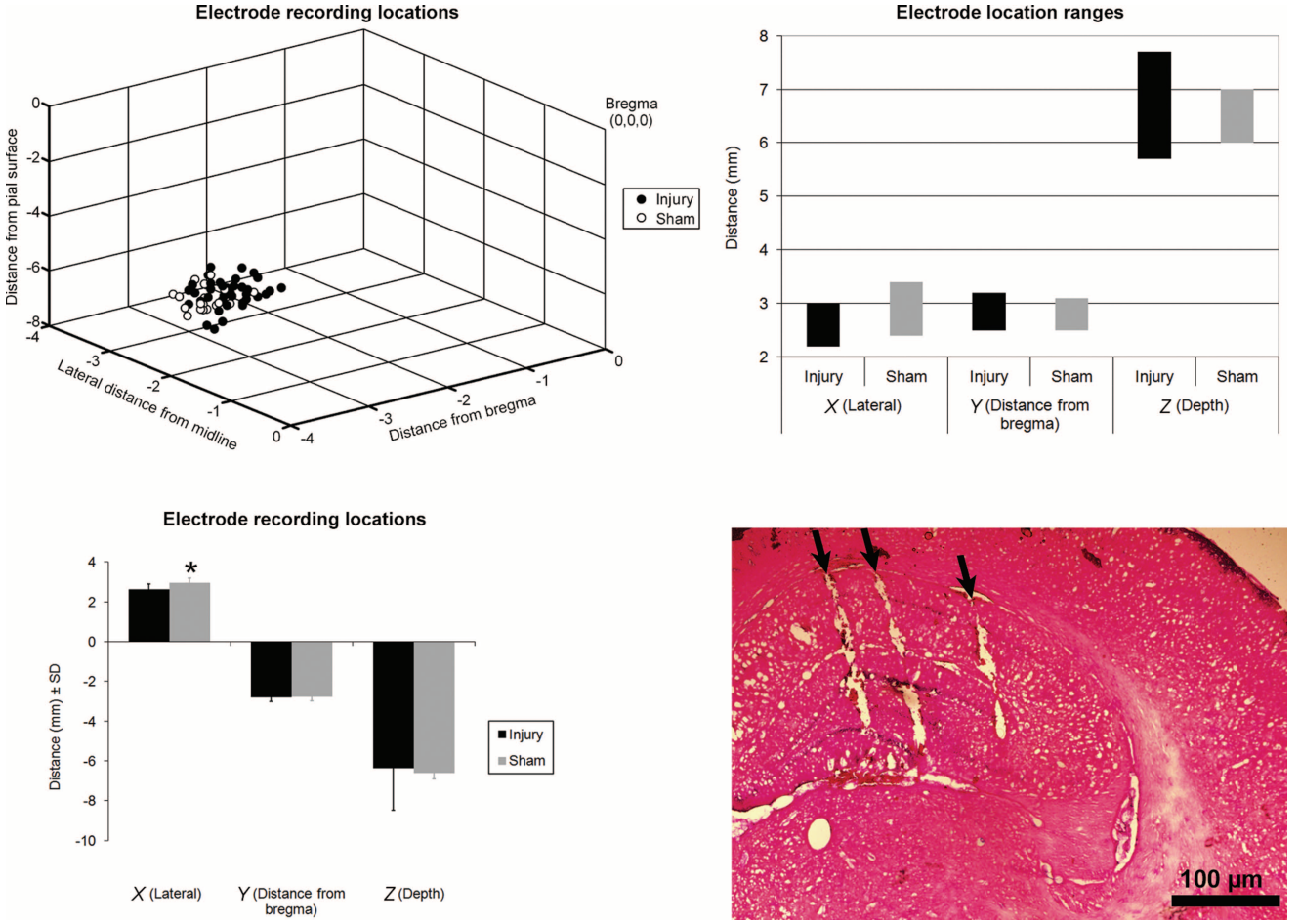


**Figure 3.** Recording locations were within the rat VPL, in relation to distances from the pial surface ($Z$), lateral from midline ($X$), and from bregma ($Y$). A representative coronal section of the VPL uses arrows to indicate residual electrode tracks. VPL indicates ventroposterolateral nucleus.

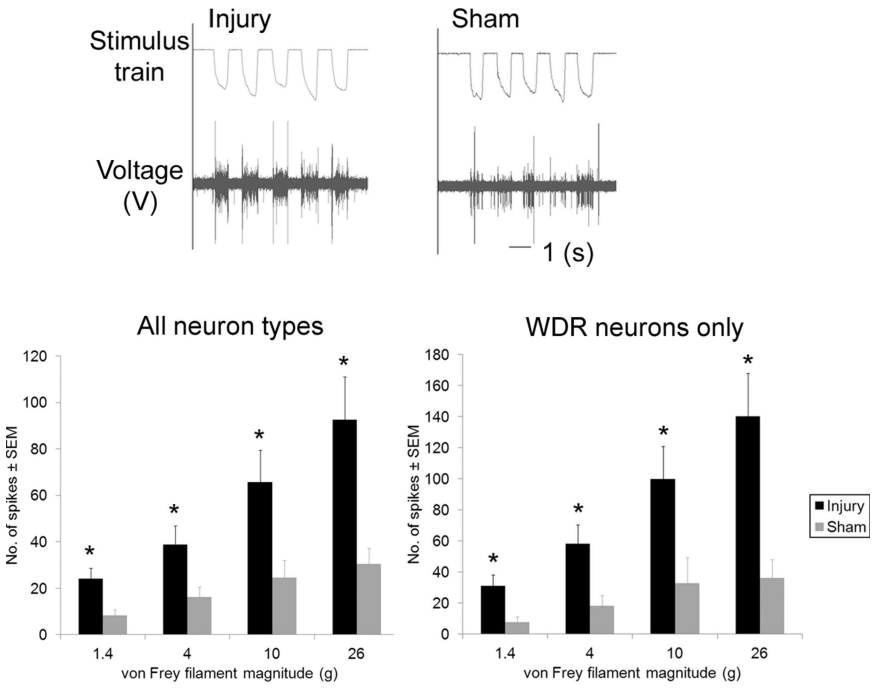


**Figure 4.** Neuronal firing in the VPL increased after injury on day 14. Representative extracellular recording voltages during stimulation show more spikes in response to the paw stimuli after injury. When quantified, firing for all neurons in response to each stimulus was significantly increased after injury over sham ($*P < 0.034$), and for WDR neurons, firing was significantly increased after injury ($*P < 0.019$). VPL indicates ventroposterolateral nucleus; WDR, wide dynamic range; SEM, standard error of mean.

## DISCUSSION

This study is the first to demonstrate that a transient root injury can induce persistent pain associated with increased neuronal firing in the VPL that is detected late after the initial injury (Figures 2, 3), supporting prior reports that radicular injury can produce central sensitization.[44–46] Although the neuronal responses increased in response to all stimuli after injury, the increase was most robust for the noxious stimuli (10-g and 26-g filaments) and is attributable to the responses in WDR neurons (Figure 4). Together with clinical reports of altered thalamocortical activity, structural changes in the brain and altered brain chemistry in patients experiencing pain,[23,47,48] our data provide further evidence that there is a specific and sensitive relationship between neuroplastic changes in the thalamus and persistent pain. Moreover, the fact that such changes are evident at day 14 and seem to persist even after the tissue injury has been removed can explain the occurrence of central sensitization that can be detected in the absence of evidence of neural tissue injury or nerve root compromise.

Transient root compression induced hyperexcitability in WDR neurons in the VPL at day 14 in response to all of the filaments (Figure 4), consistent with the notion that central sensitization develops after painful root trauma.[41,43,49–51] Central sensitization has been hypothesized to develop in the brain from many molecular and biochemical changes.[49,50,52] Previous work with this same injury model has shown that spinal neuronal hyperexcitability is elevated at day 7 after compression, but only in the deeper laminae[7,11] and only for noxious stimuli.[7] Neuronal hyperexcitability in the VPL at day 14 is more robust than spinal cord responses, with elevated evoked responses even for non-noxious stimuli (1.4 g) and increasing in a graded fashion for the noxious 10-g and 26-g filaments (Figure 4). Of note, the process of identifying spikes is indeed subjective and may vary by analyzer; however, to minimize potential confounding effects due to that, all electrophysiology analyses in this study were performed by a single blinded observer. This robust thalamic hyperexcitability may be due to the fact that thalamic processing of pain, together with central sensitization, produces more aberrant firing in the supraspinal processing center of the brain where pain is perceived. Other models of painful chronic nerve constriction have reported afterdischarge firing in the thalamus.[22,53] It should be noted that use of isoflurane as the anesthetic agent for the initial procedures may have had differential effects across the sham and injury groups given the nature of different insults and/or duration of anesthetic exposure associated with the different surgical procedures. However, given prior work with this anesthetic in this model and others,[7,11,33,41,43,54] such an effect is not expected to be a major factor. Nonetheless, additional studies investigating these and other potentially confounding issues are needed. Of note, although transient, the compression insult of 15 minutes at 10 g is sufficient to induce sustained axonal transport disruption, axonal swellings in the nerve root, and persistent spinal neuronal dysfunction,[6,9,33,55] and so is more severe than other such transient injuries.

On the basis of the responses of all neurons and by phenotype, it seems that abnormal firing of WDR neurons is responsible for hyperexcitability in the VPL (Figure 4). Increased WDR activity in the thalamus has been reported in thalamic recordings after spinal hemisection.[54] Together, with similar reports in the spinal cord after painful spinal cord injury and spinal nerve ligation,[56,57] our findings further support the critical role of WDR neurons in nociception and stimulus intensity discrimination.[58–60] Although not significant, we identified a shift in the VPL toward WDR neurons after painful root compression that is consistent with similar

proportions reported after spinal cord injury.[54] Previous work in this model identified a shift toward WDR neurons in the superficial spinal dorsal horn.[11] Together, these findings suggest that WDR neurons are particularly important contributors to radicular pain both locally, in the spinal cord, and in supraspinal locations. On the basis of our findings, and previous reports in the literature, the WDR neurons specifically could be a potential target for clinical treatment of radiculopathy. In fact, deep brain stimulation (DBS) in the periaqueductal gray or the VPL is one such treatment that is gaining attention for neuropathic and chronic pain by modulating neuronal activity.[47,61] Although the specific and complete mechanism(s) of action of DBS remain undefined, DBS is gaining recognition for use in pain treatment, and like spinal cord stimulation, may provide a neuromodulatory effect for persistent pain.

Although we present findings associating increased thalamic activity with pain after transient nerve root compression (Figures 2, 4), further work is needed to understand the temporal mechanism(s) responsible. In particular, assessing neuronal firing at early times after the initial injury would define when and how central sensitization modulates modifications in the brain. On the basis of earlier work on this model, it is possible that glia are also activated at both the early onset of pain development[37] and/or later stages when it is maintained,[7,8,10,32,62] and could have regulated neuronal responses (Figure 4). In addition, further work with brain imaging techniques would also identify potential structural changes after radicular injury. Regardless of potential changes, the VPL recordings show no differences in the quantity or overall location of neurons within the VPL between groups (Figure 3) and are consistent with anatomical studies of the rat brain.[39,40] It is important to note that a noninhalation anesthetic was used; other anesthetics, such as inhalation halothane, may ensure a more stable depth of anesthesia. However, thalamic recordings from rats and primates have been reported from others using sodium pentobarbital for sedation[54,63] and Iwata *et al*[53] found no differences in firing rates between isoflurane and pentobarbital anesthesia. Accordingly, those studies support the notion that our anesthetic approach did not affect neuronal activity outcomes.

## CONCLUSION

A transient nerve root compression that produces persistent pain also induces neuronal hyperexcitability in the VPL that is evident at day 14. Results suggest that altered thalamic activity is an important regulator of pain even after transient neural trauma. These findings have potential clinical implications because they suggest that patients can experience radicular pain and present with sustained alterations in the central nervous system even in the absence of a tissue insult. Our findings that sustained thalamic activity persists together with pain is important for improving both pharmacological and nonpharmacological pain therapies, such as targeting specific neuronal channels and inhibiting neuronal activity using DBS,[47,61] and suggests an alternative supraspinal target for clinical pain intervention in addition to local treatments of the suspected site of pathology or injured tissue.

### ➢ Key Points

- ❑ Transient nerve root compression induces sustained allodynia and increased neuronal firing in the VPL of the thalamus at day 14 after injury.
- ❑ Increases in evoked firing after injury are attributable to the WDR neurons.
- ❑ These findings suggest that radicular pain alters thalamic processing of mechanical stimuli for radicular pain.

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