Altered Receptive Fields and Sensory Modalities of Rat VPL Thalamic Neurons During Spinal Strychnine-Induced Allodynia

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Sherman, Stephen E., Lei Luo, and Jonathan O. Dostrovsky. Altered receptive fields and sensory modalities of rat VPL thalamic neurons during spinal strychnine-induced allodynia. J. Neurophysiol. 78: 2296–2308, 1997. Allodynia is an unpleasant sequela of neural injury or neuropathy that is characterized by the inappropriate perception of light tactile stimuli as pain. This condition may be modeled experimentally in animals by the intrathecal (i.t.) administration of strychnine, a glycine receptor antagonist. Thus after i.t. strychnine, otherwise innocuous tactile stimuli evoke behavioral and autonomic responses that normally are elicited only by noxious stimuli. The current study was undertaken to determine how i.t. strychnine alters the spinal processing of somatosensory input by examining the responses of neurons in the ventroposterolateral thalamic nucleus. Extracellular, single-unit recordings were conducted in the lateral thalamus of 19 urethan-anaesthetized, male, Wistar rats (342 ± 44 g; mean ± SD). Receptive fields and responses to noxious and innocuous cutaneous stimuli were determined for 19 units (1 per animal) before and immediately after i.t. strychnine (40 μg). Eighteen of the animals developed allodynia as evidenced by the ability of otherwise innocuous brush or air jet stimuli to evoke cardiovascular and/or motor reflexes. All (3) of the nociceptive-specific units became responsive to brush stimulation after i.t. strychnine, and one became sensitive to brushing over an expanded receptive field. Expansion of the receptive field, as determined by brush stimulation, also was exhibited by all of the low-threshold mechanoreceptive units (14) and wide dynamic range units (2) after i.t. strychnine. The use of air jet stimuli at fixed cutaneous sites also provided evidence of receptive field expansion, because significant unit responses to air jet developed at 13 cutaneous sites (on 7 animals) where an identical stimulus was ineffective in evoking a unit response before i.t. strychnine. However, the magnitude of the unit response to cutaneous air jet stimulation was not changed at sites that already had been sensitive to this stimulus before i.t. strychnine. The onset of allodynia corresponded with the onset of the altered unit responses (i.e., lowered threshold/receptive field expansion) for the majority of animals (9), but the altered unit response either terminated concurrently with symptoms of allodynia (6) or, more frequently, outlasted the symptoms of allodynia (10) as the effects of strychnine declined. The present results demonstrate that the direct, receptor-mediated actions of strychnine on the spinal processing of sensory information are reflected by changes in the receptive fields and response properties of nociceptive and nonnociceptive thalamic neurons. These changes are consistent with the involvement of thalamocortical mechanisms in the expression of strychnine-induced allodynia and, moreover, suggest that i.t. strychnine also produces changes in innocuous tactile sensation.

INTRODUCTION

Before reaching the thalamus and cortex, both low-threshold and nociceptive afferent sensory information are subject to processing and modulation within the spinal cord or the dorsal column nuclei. γ-Aminobutyric acid (GABA) and glycine are important inhibitory neurotransmitters in the spinal dorsal horn. Recent studies examining synaptic structure and immunohistochemical staining for GABA, glycine, components of their receptors, and associated proteins have provided convincing evidence that GABAergic neurons synapse presynaptically with myelinated, but not unmyelinated, primary afferents, whereas cells staining for glycine, or costaining for both glycine and GABA, synapse with postsynaptic dendrites as well as with presynaptic terminals of myelinated primary afferents (Todd 1990, 1996; Todd et al. 1996). These anatomic observations are consistent with functional electrophysiological data implicating GABAergic interneurons in presynaptic inhibition of primary afferents (Davidson and Southwick 1971; Levy 1977; Wall 1995) and demonstrating the involvement of both glycine and GABA in the postsynaptic inhibition of large-fiber primary afferent input (Bagust et al. 1981; Game and Lodge 1975).

The vital role of glycine in modulating afferent sensory input is highlighted by behavioral pharmacological studies demonstrating that glycine receptor antagonism by strychnine results in profound sensory disturbances. After intrathecal (i.t.) strychnine, lightly touching or stroking the hair evokes vigorous scratching and biting of the stimulation site, vocalization, attempts to escape the stimulus, and aggressive behavior (Beyer et al. 1985, 1988; Onaka et al. 1996; Sosnowski and Yaksh 1989; Yaksh 1989). All of these behaviors usually are elicited only by high-intensity and potentially tissue-damaging (nociceptive) stimuli, not light touch. Thus the temporary removal of spinal glycinergic inhibition with i.t. strychnine results in a disturbance of sensation in which light tactile stimuli appear to be perceived as aversive—a condition termed allodynia. In anesthetized rats, low-intensity tactile stimuli evoke an abrupt motor withdrawal response, tachycardia, and hypertension after i.t. strychnine (Sherman and Loomis 1994–1996; Yaksh 1989). In view of the allodynia observed in conscious animals, these cardiovascular and motor reflexes have been interpreted as nociceptive and, because of the innocuous nature of the stimuli, as evidence of allodynia (Sherman and Loomis 1994; Yaksh 1989). A possible explanation of these observations is that low-threshold mechanoreceptive (LTM) afferent inputs potentially can activate spinal nociceptive neurons to produce nociceptive responses, but that these inputs usually are subject to glycinergic inhibition and are thus subliminal or relatively weak under normal circumstances.

The mechanisms underlying strychnine-dependent allodynia remain to be elucidated. It has been suggested that removal of spinal glycinergic inhibition (by antagonism with strychnine) results in enhanced responses of spinal wide dynamic range (WDR) neurons to low-threshold cutaneous stimuli (Peng et al. 1996; Sorkin and Puig 1996; Yaksh...
Indeed, strychnine does potentiate the responses of some spinal WDR neurons to low-threshold inputs, but responses of nociceptive specific (NS) and LTM neurons also are modified (Lin et al. 1994; Sorkin and Puig 1996; Wilcox et al. 1996). Although it is possible that these three types of spinal neurons might contribute to the gross effects of strychnine on behavior, interpretation of these data in the context of allodynia is limited because none of these studies has attempted to link electrophysiological changes in the spinal cord with gross symptoms of allodynia, and the involvement of supraspinal sites (necessary for the production of complex behavioral responses to tactile stimuli) has not been established.

Strychnine leads to the inappropriate interpretation of a low-threshold input as noxious and causes behavioral changes that likely involve cortex. However, the precise manner by which this abnormal sensory information is processed at supraspinal sites remains to be elucidated. It generally is assumed that the sensory-discriminative and affective-motivational aspects of pain are processed by different regions of thalamus and cortex. The medial thalamus contains many NS neurons and has been implicated repeatedly in mediating the affective-motivational aspects of pain (Ammons et al. 1985; Bushnell and Duncan 1989; Dostrovsky and Guilbaud 1990; Groenewegen and Berendse 1994), whereas the lateral thalamus contains LTM, WDR, and NS neurons and is believed to be important in the sensory discriminative aspects of pain in addition to tactile sensation (Guilbaud et al. 1980, 1994). We recently have reported that when strychnine-induced allodynia is present (as evidenced by touch-evoked cardiovascular and motor reflexes), NS neurons in rat medial thalamus become responsive to low-threshold cutaneous stimuli and thus might contribute to the expression of allodynia (Sherman et al. 1997). It is not known if sites in lateral thalamus are involved in mediating allodynia after i.t. strychnine, but spinal NS and WDR neurons, with projections to VPL thalamus, exhibited altered responses to cutaneous stimuli after microdialysis of strychnine into the primate spinal cord (Lin et al. 1994). To our knowledge, the effects of i.t. strychnine on LTM neurons either within or projecting to lateral thalamus have not been investigated. Because the majority of low-threshold input to thalamus is carried via the dorsal column-medial lemniscal pathway, which has synapses in the dorsal column nuclei but not the spinal cord, our initial hypothesis was that spinally administered strychnine would have no effect on this input to thalamus and consequently that LTM neurons in VPL thalamus would not be affected by i.t. strychnine. We further conjectured that NS and WDR neurons would be expected to be influenced by i.t. strychnine because input to these thalamic cells from spinal second-order neurons is likely to be modified by strychnine.

To test these hypotheses and to gain a better understanding of how i.t. strychnine alters sensory input to the thalamus, extracellular single-unit recordings were conducted in the VPL thalamus of anesthetized rats, and the effects of i.t. strychnine on unit responses to cutaneous stimuli were determined. The characterization of thalamic units was undertaken at a time when allodynia was present, allowing the relationship between unit responses and overt symptoms of allodynia to be investigated. Preliminary data from the current study have been presented in abstract form (Sherman et al. 1996).

**METHODS**

Male Wistar rats (Charles River, St.-Constant, PQ, Canada) weighing 342 ± 44 g (mean ± SD) at the time of the experiment were used for all procedures. Animals were housed in an animal care facility with a 12-h light/dark cycle and free access to rat chow and tap water. All experiments were conducted in accordance with the "Guidelines of the Canadian Council on Animal Care" and were approved by the University of Toronto Animal Care Committee.

**Implantation of i.t. catheters**

Rats were fitted with i.t. catheters prepared from stretched polyethylene tubing (PE-10, Becton Dickenson, Sparks, MD). General anesthesia for this procedure was provided by either halothane (1–3% in oxygen; Fluothane; Wyeth-Ayerst, Montreal, PQ, Canada) or methohexital sodium (Brietal, Eli Lilly, Toronto, ON Canada; 70 mg/kg ip). As previously described (Sherman and Loomis 1994), the catheters were filled with sterile saline, inserted through the cisterna magna into the spinal subarachnoid space, and guided 8.5 cm caudally (L1 termination). The rostral 3–5 cm of the catheter was threaded through the skin on the back of the neck and sealed with a stainless steel plug. Animals were permitted to recover for ≥3 days after surgery, and only those without signs of neurological impairment were used in the experiments. All animals received a single injection of buprenorphine (Temgesic, Rickett & Colman, Hull, England; 0.3 mg/kg sc) at the time of surgery. Chlorhexidine acetate cream (Hibitane, Ayerst Laboratories, Montreal, PQ, Canada; antibacterial/antifungal) was applied daily to the incision for the next 3 days.

**Testing of i.t. catheters**

After recovery from surgery, but ≥24 h before the acute experiment, the patency of the i.t. catheter and its approximate position in the spinal subarachnoid space were tested using an i.t. injection of lidocaine. All animals received 7 μL of preservative-free, sterile 5% lidocaine hydrochloride (Xylocaine spinal, Astra Pharma, Mississauga, ON, Canada) flushed through the i.t. catheter with 10 μL of sterile saline. Animals that failed to exhibit signs of brief, reversible, unilateral or bilateral paralysis of the hindlimbs were not used for experimentation.

**Acute anesthetized animal preparation**

On the day of the experiment, surgical anesthesia was induced with halothane, the left jugular vein was cannulated, and anesthesia was maintained with intravenous ethyl carbamate (urethan, 10% wt/vol in saline; Sigma Chemical, St. Louis, MO). The initial urethan dose (1.1 g/kg) was infused slowly during 5–10 min as the effect of halothane declined. Throughout the experiment, anesthesia was supplemented with intravenous urethan as required. A tracheal tube was implanted in all animals and most (14 of 19) had the left carotid artery cannulated for continuous monitoring of blood pressure. Instantaneous heart rate was derived from the blood pressure tracing off-line using a computer program. Body temperature was maintained at 37°C using a thermostatically regulated heating blanket and a colonic probe. Atropine (0.5–1.0 mg/kg ip; Ormond Veterinary Supply, Ancaster, ON, Canada) was administered to reduce respiratory secretions. All incisions and contact points with the stereotaxic frame were infiltrated with 2% lidocaine to reduce basal sensory input.
Extracellular single-unit recordings

The animal was placed in a stereotaxic frame, a craniotomy (~3 x 4 mm) was performed in the region overlying lateral thalamus, and the dura mater was removed from the exposed region of cortex. Thalamic recordings were conducted contralateral to the site of the i.t. catheter as determined by an i.t. lidocaine injection (which was given >24 h before, see Testing of i.t. catheters).

Extracellular recording was performed using commercially available, Parylene-coated tungsten electrodes (WB300310A and WB300308A; Micro Probe, Clarksburg, MD) plated with gold and platinum to a final impedance between 0.2 and 2.5 MΩ. Standard extracellular, single-unit recording techniques were employed. Action potentials were amplified, isolated with a dual-window discriminator and observed on an oscilloscope. Unit firing rate and blood pressure were digitized and monitored on-line with a CED 1401 data acquisition system and SPIKE-2 software (Cambridge Electronic Design, Cambridge, England). A digital recording device (VB-100-B; Instrutech, Great Neck, NY) in combination with a video cassette recorder was used for data storage.

Vertical electrode penetrations through lateral thalamus were carried out using an electronic microdrive (Burrell Instruments, Fishers, NY). After entering the thalamus (4–5 mm below the cortical surface), the electrode was advanced in small steps (5–20 μm) while brush, touch, joint movement, and pinch stimuli were delivered to find an appropriate unit in VPL. To investigate adequately the effects of spinal strychnine on a thalamic cell, the unit needed to be discriminated clearly from other units, have a stable action potential amplitude for ≥1 h (no electrode drift), and have a receptive field that included cutaneous regions expected to be affected by i.t. strychnine (i.e., the hindquarters).

Once a suitable unit was found, control responses were determined. If the unit’s response to pinch did not differ in magnitude from the response to brush (i.e., if the unit was a LTM cell), no further pinch stimuli were applied. For NS and WDR units, pinches were applied to a few cutaneous sites, but no attempt was made to completely map the receptive field for pinch. The effects of radiant heat were also tested on two units. This stimulus was applied for 15 s with a 50-W projector bulb at a fixed distance of 5 cm from the stimulation site. After the effects of noxious stimuli were determined, brush stimuli were applied in a systematic pattern over the trunk and hindquarters.

The effects of an air jet stimulus also were assessed in 13 animals. Air jet stimulation consisted of 20 air puffs (each of 50-msec duration) delivered through a 19-gauge needle at 2 Hz. The frequency and duration of the air puffs were controlled with a stimulator (Grass Instruments, Quincy, MA) and an electronically controlled valve. The driving pressure of 30 psi (≈207 kPa) was provided with a regulated supply of compressed air. This stimulus was applied approximately parallel to the surface of hairy skin and had sufficient force to deflect the pelage for 1–2 cm from the tip of the needle. Unlike the brush stimulation, which was moved continuously while mapping the low-threshold receptive field, the air jet stimulus was focused on a single site throughout the experiment, thus allowing comparison of response magnitudes before and after i.t. strychnine. When the air jet stimulus was applied to multiple sites on the same animal (e.g., Fig. 8), a separate air jet stimulator was fixed in place at each site rather than moving the stimulator from site to site.

Assessment of the effects of i.t. strychnine

As in our recent paper (Sherman et al. 1997), i.t. saline was not given routinely as a control for i.t. strychnine as the risk of losing the unit was increased by handling the short segment of i.t. catheter just behind the recording site. However, saline injections (15 μL i.t.) did not have any effect on unit responses when tested in a small group of animals and never produced any allodynia-like responses when tested in several previous studies (Sherman and Loomis 1994–1996).

After control responses were determined, each animal was given a single injection of 40 μg of strychnine. Strychnine hemisulfate (Sigma Chemical) was dissolved in 0.9% sterile saline (Astra Pharma), injected with a hand-held Hamilton microliter syringe, and flushed through the i.t. catheter with 10 μL of saline. The concentration of the strychnine solution was 10 mg/ml (near saturation) to minimize the volume of the injection and consequently reduce the rostro-caudal spread of strychnine in the spinal subarachnoid space.

To ensure that changes in receptive fields and response properties were attributable to the actions of i.t. strychnine and not the result of sensitization from multiple pinches or heat stimulation, control brush stimulation always was applied to the hindquarters after responses to pinch or heat were assessed and immediately before i.t. strychnine administration. After i.t. strychnine, noxious stimuli were not applied until after (strychnine-dependent) unit responses to brush could be adequately characterized.

Brush stimuli were applied frequently after i.t. strychnine. A normal stimulation consisted of sequentially brushing 10 different sites on the hindquarters and tail (e.g., Fig. 2) during a period of 1–2 min. Although more rostral sites were tested in a few animals, the present results are consistent with those of previous studies demonstrating that the effects of i.t. strychnine were localized segmentally near the site of strychnine delivery in the lumbar spinal cord. Either brush or air jet was applied at 2- to 3-min intervals for ~30 min after i.t. strychnine. If strychnine-dependent, brush-evoked responses persisted after this point, the stimulations were continued, but their frequency was reduced.

Reconstruction of recording sites

On completion of the experiment, an electrolytic lesion was made at the recording site by applying an anodal current of 10–15 μA for ~15 s. The animal then was deeply anaesthetized with urethane and perfused transcardially with normal saline followed by 10% formalin (~200 ml of each). Brains were postfixed in 10% formalin for 2 days, cut in 100-μm transverse sections with a freezing microtome, and stained with cresyl violet. The position of the recording site was confirmed by coordinating depth measurements and track locations with histologically recovered lesions. Data from individual experiments were pooled on thalamic transverse sections based on the atlas of Paxinos and Watson (1986).

Data analysis

For each unit, the mean spontaneous firing rate was calculated during the 20-s interval immediately before each cutaneous stimulation. A unit was considered to have a significant response to a particular stimulus if two or more consecutive 1-s bins in the event histogram exceeded the prestimulus mean firing rate by >2 SD. The mean spontaneous firing rates are depicted as solid horizontal lines on the event histograms, whereas the two times standard deviation cutoffs are depicted as dotted lines.

For comparison of the mean unit responses to air jet stimulation before and after i.t. strychnine, the data were normalized (as percent of maximum) to reduce interunit variability and subjected to a two-way repeated measures analysis of variance. Significant differences between pairs of means were identified using a Newman-Keuls test.

Blood pressure and heart rate were monitored in 14 of the 19 animals in this study, and the means of the maximum brush-evoked increases in mean arterial pressure and heart rate were compared before and after i.t. strychnine, as previously described (Sherman and Loomis 1996). A paired t-test was used to identify significant changes in these parameters relative to the prestrychnine control.
The effects of i.t. strychnine were determined on 19 units in 19 animals. As shown in Fig. 1, all of the units were in lateral thalamus: 17 in the VPL thalamic nucleus and 1 in the posterior thalamic nucleus, and the recording site for the remaining unit could not be determined with certainty (however, the stereotaxic coordinates and the location of the electrode track suggest that this unit was also in lateral thalamus, either within VPL or immediately adjacent to this nucleus). The majority of units examined were LTM (14), but the effects of i.t. strychnine also were assessed on three NS and two WDR neurons. i.t. strychnine affected the responses of all of the units tested.

Effects of i.t. strychnine on thalamic LTM neurons

The effects of i.t. strychnine on the responses of thalamic LTM neurons to cutaneous stimuli are shown in Figs. 2–4. Before i.t. strychnine, the unit depicted in Fig. 2 responded to brush stimulation of the contralateral hindquarters or ipsilateral hind leg, but (predictably) did not respond to air jet at a site outside of the brush receptive field (Fig. 2A). After 40 μg of i.t. strychnine, the receptive field expanded to include the ipsilateral flank and the base of the tail (Fig. 2B). Brush stimulation also triggered motor withdrawal reflexes when applied to several sites on the hindquarters (not shown). At 4 min after the i.t. injection of strychnine, application of the air jet stimulus to a site where it had previously not been effective in eliciting a unit response now evoked a motor withdrawal reflex and a significant unit response with an afterdischarge (Fig. 2B). By 20 min poststrychnine, the effects of the drug were no longer evident, and responses to air jet and brush approached control levels. While the size of the brush receptive field returned to its prestrychnine dimensions, the magnitude of the brush-evoked unit responses were reduced to below the control level.

A second example of the effects of i.t. strychnine on a LTM unit is shown in Fig. 3. This cell was excited by brush stimulation applied to the hind leg or trunk (contralateral to the thalamic recording site) but not to other regions of the hindquarters. Before i.t. strychnine, brush stimulation did not affect heart rate or blood pressure and did not elicit any type of motor reflex (Fig. 3A). In contrast, after 40 μg of i.t. strychnine, similar brush stimulation evoked a pressor response (Fig. 3B), a gradual increase in heart rate (Fig. 3B), and motor withdrawal reflexes (not shown). The spontaneous firing rate of the thalamic unit increased and the cutaneous receptive field expanded relative to the prestrychnine control. The latter effect became more clearly evident between 15 and 30 min after drug administration, and, at its maximum extent, the receptive field included a large part of the flank region and a portion of the tail.

The responses of thalamic LTM neurons to air jet and the effects of i.t. strychnine on these responses are summarized in Fig. 4. While recording from 10 LTM units in 10 different animals, air jet stimuli were applied repeatedly to 36 different cutaneous sites both before and after i.t. strychnine. As shown in Fig. 4A, i.t. strychnine did not significantly affect the magnitude of the unit response to air jet at sites where air jet evoked significant unit responses before strychnine. However, new cutaneous sites, which had been unresponsive to air jet before i.t. strychnine, were recruited and responded to air jet after i.t. strychnine.

Effects of i.t. strychnine on thalamic WDR neurons

An example of the effects of i.t. strychnine on the response characteristics of a WDR neuron is shown in Fig. 5. This unit had a small receptive field on the contralateral hind paw and responded incrementally to mechanical stimuli of increasing intensity. Radiant heat was also effective in evoking a significant unit response. As with the LTM units, expansion of the brush receptive field was the most prominent effect of i.t. strychnine on this thalamic WDR neuron (Fig. 5D and the figure in Fig. 5F). Brushing also triggered an abrupt motor withdrawal reflex (not shown) when applied to the hind paw, hind leg, or trunk (contralateral to the recording site in VPL), which coincidentally were the sites of maximal brush-evoked unit responses. Responses to radiant heat appeared to be reduced slightly after i.t. strychnine, whereas the incremental responses to brush and pinch were relatively unaffected (Fig. 5, E and F). Air jet stimuli applied bilaterally to several different sites on the hindquarters failed to elicit a significant unit response either before or after i.t. strychnine (Fig. 8).

Effects of i.t. strychnine on thalamic NS neurons

An example of the effects of i.t. strychnine on a NS thalamic neuron is shown in Fig. 6. Before i.t. strychnine, this unit responded only to noxious pinch, whereas innocuous brush stimulation failed to alter heart rate, blood pressure, or the unit firing pattern. In contrast, after 40 μg of i.t. strychnine, similar brush stimulation evoked a pressor response, a gradual increase in heart rate, motor withdrawal reflexes, and a significant increase in the firing rate of the cell (Fig. 6B). The spontaneous firing rate of the unit, the basal heart rate, and the blood pressure were elevated after i.t. strychnine.

Figure 7 illustrates a more dramatic example of the effects of i.t. strychnine on an NS neuron in VPL. This unit
FIG. 2. Example of a LTM unit in VPL thalamus. During the prestrychnine control period, an air jet stimulus applied to the ipsilateral trunk (open oval on rat figure) failed to elicit a significant unit response (A, top). Brushing significantly increased the unit firing rate when applied to the contralateral hindquarters or to the ipsilateral hind leg (A, bottom and shading on figure). After 40 μg of i.t. strychnine, the brush receptive field expanded to include the ipsilateral flank and the base of the tail (B, shading on figure and bottom). Brush stimulation also triggered a motor withdrawal reflex (not shown) when applied to either flank or to the trunk region contralateral to the thalamic recording site. Air jet stimulation (shaded oval on rat figure) also evoked a motor withdrawal response and a significant unit response with an afterdischarge (B, top). By 20 min poststrychnine (C) responses to air jet and brush approached control levels. Location of the recording site in the VPL thalamic nucleus is shown (A) on a schematic diagram of the rat brain in cross-section (D). Times in B and C indicate the time poststrychnine. (Note that the receptive field boundary is based on statistical criteria as outlined in METHODS, so that a single 1-s bin crossing the cutoff line does not constitute a response: e.g., ipsilateral flank in A.)

Examples of cardiovascular responses to cutaneous stimulation, in the presence or absence of i.t. strychnine, are illustrated in Figs. 3 and 6. Before i.t. strychnine, noxious pinch evoked both a pressor response and tachycardia (Fig. 6A), whereas brush stimulation applied to the rat hindquarters or trunk had little effect on heart rate or blood pressure and did not elicit any type of motor reflex. (Figs. 3A and 6A). In contrast, after 40 μg of i.t. strychnine, similar brush stimulation evoked a pressor response (Figs. 3B and 6A), a gradual increase in heart rate (Fig. 6A), and motor withdrawal reflexes (not shown) in response to this normally innocuous stimulus.

Before i.t. strychnine, brush stimulation did not significantly alter the mean heart rate or mean arterial blood pressure. However, after i.t. strychnine, similar stimulation resulted in 4.8- and 6.2-fold increases in the mean brushing-evoked elevations in heart rate and mean arterial blood pressure, respectively, which were significantly greater than their respective prestrychnine controls (for heart rate, \( P = 0.0040; \)})
FIG. 3. Example of the effects of intrathecal strychnine (40 μg) on cardiovascular and thalamic neuronal responses to innocuous cutaneous brush stimulation. Before intrathecal (i.t.) strychnine, brush stimulation applied to the rat hindquarters or trunk failed to alter heart rate or blood pressure (A). Firing rate of a LTM neuron in VPL thalamus was increased in response to brush stimulation applied to the contralateral hind leg or trunk but not to other cutaneous sites (A). After 40 μg of i.t. strychnine (B), the spontaneous firing rate of the thalamic unit increased, and the unit response to brush stimulation appeared to be enhanced modestly relative to the prestrychnine control. Maximum brush-evoked increase in blood pressure occurred 13 min after i.t. strychnine (B) and is shown here. During this brush stimulation, motor withdrawal reflexes were evoked by brushing the contralateral hind paw, the base of the tail, and the ipsilateral flank and hind leg (not shown). In this particular case, basal heart rate was elevated after i.t. strychnine but could not be further increased by brush stimulation. Location of the recording site in VPL is shown (C) on a schematic diagram of the rat brain in cross-section (C).

Overall effects of i.t. strychnine

Figure 8 provides a summary of i.t. strychnine-induced changes in receptive fields and unit responses for thalamic neurons. Most of the units had unilateral brush receptive fields before drug administration, but after i.t. strychnine developed large bilateral receptive fields. Note that the poststrychnine receptive fields for brush shown in Fig. 8 are composites and include all cutaneous regions where brush stimulation evoked a significant unit response at any time point after i.t. strychnine. Because the receptive fields changed continuously after strychnine administration, these composite receptive fields depict the maximum boundaries of the brush receptive field throughout the drug’s time course and thus do not correspond exactly to receptive fields shown at specific time points in previous figures. In one case (Fig. 8, 2nd row, last column), thalamic single-unit recording could not be maintained for a long enough period after i.t. strychnine to adequately map the brush receptive field. However, application of several air jet stimuli provided evidence of receptive field expansion relative to the prestrychnine control.

For the 18 animals in Fig. 8 in which the receptive field for brush could be determined, the results are based on a total of 84 applications of the brush stimulus applied to the hindquarters within 45 min after i.t. strychnine. During 57 of these stimulus applications, the unit exhibited a novel response that had not been observed in the absence of i.t. strychnine. In 10 cases, NS units began to respond to brush stimulation. During recordings from 13 LTM neurons, 42 stimulations resulted in a novel response outside of the original receptive field. Similar receptive field expansion was observed during seven applications of the brush stimulus while recording from two WDR neurons. Evidence of allodynia, in the form of cardiovascular and/or motor responses to low-threshold stimulation, was observed in 18 of the 19 animals during 42 applications of the brush stimulus. Thirty of these stimulations resulted in a novel unit response concurrently with allodynia.

Table 1 illustrates the temporal relationship between brush-evoked allodynia and unit responses for 17 of the 19 animals. (For the reasons explained in the previous paragraphs, there was insufficient data to assess this relationship for the remaining 2 animals). Note that for the majority of animals (9/17), the onset of symptoms of allodynia occurred during the same stimulation as the onset of changes in the thalamic unit response to brush. In contrast, the offset of symptoms of allodynia usually occurred before the termination of the brush-evoked unit response, and only in one
the ability of repetitive, natural, innocuous brush stimulation to cause wind-up of rat dorsal horn neurons after iontophoretic strychnine. Wind-up normally is observed only with repetitive activation of C fibers (Dickenson and Sullivan 1987, 1990; Mendell 1966). Strychnine-dependent, brush-induced wind-up was most evident with LTM spinal neurons, but also was observed with about one-half of the WDR units tested. After microdialysis of strychnine (1 mM) into the cat spinal dorsal horn, Sorkin and Puig (1996) reported enhanced responses of some neurons to hair deflection, enlargement of low-threshold receptive fields, and increased afterdischarges. These 18 cells (of 26), which exhibited a heightened responsiveness to brush (>30% increase in the evoked unit response), included 10 WDR, 6 LTM, and 2 high-threshold neurons (Sorkin and Puig 1996). In a different study, microdialysis of strychnine (2 mM) into the pri- mate spinal dorsal horn significantly increased background activity of 25 WDR and 2 high-threshold spinal nociceptive tract (STT) cells as well as enhancing unit responses to brush and pinch, but not noxious heat (Lin et al. 1994). A similar study in the rat found an increase in background activity and enhanced responses of spinal WDR neurons to brush, pressure, and pinch after microdialysis of strychnine (2 mM) in close proximity (<750 μm) to the recording sites (Peng et al. 1996).

The most surprising results of the present study were the marked increases in the receptive fields and evoked responses of LTM neurons because these were unexpected on anatomic grounds. The majority of low-threshold input to the rat VPL arises from the dorsal column nuclei (Feldman and Kruger 1980), which derive a substantial portion of their input directly from low-threshold primary afferents without a synapse in the spinal cord (Giufrida and Rustioni 1992; Willis and Coggeshall 1991). Thus it was not expected that all of the LTM units examined in the current study would be so markedly affected by spinal strychnine. These current findings suggest that, at least in the rat, there is significant low-threshold input to LTM neurons in VPL, which arises from the spinal cord. There are several pathways by which such information could reach the thalamus. The dorsal column nuclei receive an important input from second order spinal neurons to cutaneous stimulation. The present study is case outlasted the strychnine-induced changes in the unit response to brush.

DISCUSSION

Local injection of strychnine into the lumbar spinal cord produced profound changes in the responses of lateral thalamic neurons to cutaneous stimulation. The present study is the first to demonstrate changes in response properties of neurons in the VPL thalamus concomitantly with overt symptoms of strychnine-induced allodynia. All of the LTM and WDR neurons tested in these experiments developed expanded cutaneous receptive fields after i.t. strychnine, whereas all of the NS neurons became responsive to low-threshold brush or air jet stimuli. The effect of i.t. strychnine on NS neurons in lateral thalamus resembles our previous observations in rat medial thalamus, where NS neurons became responsive to low-threshold cutaneous stimuli at a time when strychnine-induced allodynia was evident (Sherman et al. 1997). The current results are of particular interest because they show that during strychnine-induced allodynia, the functional properties of both nociceptive and nonnoci- ceptive neurons change markedly.

As there is strong evidence that the direct, receptor-mediated actions of i.t. strychnine occur at the spinal cord level (Sherman and Loomis 1994, 1995; Sivilotti and Woolf 1994), the changes we observed in the thalamus resulted from strychnine-induced alterations in the response properties of spinal cord neurons. Indeed, there is considerable evidence indicating that strychnine alters the functional properties of some spinal NS, WDR, and LTM neurons. Wilcox and associates (1996) recently have demonstrated the ability of repetitive, natural, innocuous brush stimulation to cause wind-up of rat dorsal horn neurons after iontophoretic strychnine. Wind-up normally is observed only with repetitive activation of C fibers (Dickenson and Sullivan 1987, 1990; Mendell 1966). Strychnine-dependent, brush-induced wind-up was most evident with LTM spinal neurons, but also was observed with about one-half of the WDR units tested. After microdialysis of strychnine (1 mM) into the cat spinal dorsal horn, Sorkin and Puig (1996) reported enhanced responses of some neurons to hair deflection, enlargement of low-threshold receptive fields, and increased afterdischarges. These 18 cells (of 26), which exhibited a heightened responsiveness to brush (>30% increase in the evoked unit response), included 10 WDR, 6 LTM, and 2 high-threshold neurons (Sorkin and Puig 1996). In a different study, microdialysis of strychnine (2 mM) into the pri- mate spinal dorsal horn significantly increased background activity of 25 WDR and 2 high-threshold spinal nociceptive tract (STT) cells as well as enhancing unit responses to brush and pinch, but not noxious heat (Lin et al. 1994). A similar study in the rat found an increase in background activity and enhanced responses of spinal WDR neurons to brush, pressure, and pinch after microdialysis of strychnine (2 mM) in close proximity (<750 μm) to the recording sites (Peng et al. 1996).

The most surprising results of the present study were the marked increases in the receptive fields and evoked responses of LTM neurons because these were unexpected on anatomic grounds. The majority of low-threshold input to the rat VPL arises from the dorsal column nuclei (Feldman and Kruger 1980), which derive a substantial portion of their input directly from low-threshold primary afferents without a synapse in the spinal cord (Giufrida and Rustioni 1992; Willis and Coggeshall 1991). Thus it was not expected that all of the LTM units examined in the current study would be so markedly affected by spinal strychnine. These current findings suggest that, at least in the rat, there is significant low-threshold input to LTM neurons in VPL, which arises from the spinal cord. There are several pathways by which such information could reach the thalamus. The dorsal column nuclei receive an important input from second order spinal neurons to cutaneous stimulation. The present study is case outlasted the strychnine-induced changes in the unit response to brush.
FIG. 5. Example of a WDR unit in VPL thalamus. Before intrathecal strychnine the receptive field for brush-evoked responses was limited to 1 hind paw, contralateral to the recording site (A and shading on the figure in C). Brush stimulation applied at any other site on the trunk or the hindquarters was ineffective in eliciting a significant unit response (as defined in METHODS section). This unit responded incrementally to mechanical stimuli of increasing intensity as demonstrated for brush and pinch (B). Radiant heat also evoked a significant unit response (C). After 40 μg of i.t. strychnine, the brush receptive field expanded to include bilateral regions of the trunk, the base of the tail, and most of the contralateral hindquarters (D and shading on the figure in F). Brush stimulation also triggered an abrupt motor withdrawal reflex (not shown). Responses to radiant heat appeared to be slightly reduced after i.t. strychnine (F), whereas the incremental responses to brush and pinch were relatively unaffected (E). Location of the recording site in VPL thalamus is shown (G) on a schematic diagram of the rat brain in cross-section (H). Note that the receptive field boundary is based on statistical criteria as outlined in METHODS, so that a single 1-s bin crossing the cutoff line does not constitute a response, regardless of its magnitude: e.g., contralateral trunk in A.

Five of the LTM units characterized in the present study exhibited bilateral receptive fields, and after i.t. strychnine, the receptive fields of the majority of the units expanded to include a portion of the ipsilateral body. LTM neurons with large, complex, bilateral receptive fields have been described in the lateral region of the rat VPL, which Emmer (1965) termed the ‘‘SII’’ thalamus (Emmers 1984; Sugitani 1979; Tomasulo and Emmers 1970). Because this region is in close proximity to the contralateral hindlimb projection area (Emmers 1965) and the present study only examined units with receptive fields that included the hindquarters, it is likely that some of the units characterized in the current study were in this thalamic region. There are at least two sources of ipsilateral input to VPL, which might have contributed to the appearance of bilateral receptive fields under strychnine, the lateral cervical nucleus and ipsilateral component of the STT. In the rat lateral cervical nucleus, it has been reported that 40% of the neurons have bilateral inputs (Giesler et al. 1979b), so that projections to thalamus from this nucleus could contribute to the observed bilateral receptive fields. Alternatively, Tomasulo and Emmers (1970) have described a low-threshold ipsilateral input to thalamus that runs through the ventral quadrant of the spinal cord, which may be composed of ipsilaterally projecting STT cells. Enhancement of these ipsilateral inputs at the spinal level after i.t. strychnine would result in strengthening this input, which, in many cases, may be subliminal under normal conditions and might explain the appearance of large bilateral receptive fields.

An important study by Kirk and Denny-Brown (1970) used behavioral responses to pinprick and stroking to map cutaneous dermatomes and found that primates given subcutaneous strychnine (0.25–0.5 mg/kg) exhibited a substantial reduction in the region of sensory loss after dorsal root lesions. These authors suggested that strychnine unmasked previously ineffective inputs from the denervated cutaneous regions, a concept that is supported by other studies. For example, observations in the cat spinal cord have shown that
FIG. 6. Example of a NS neuron in the Po thalamic nucleus. This unit exhibited a significant increase in firing rate in response to pinch (A) at several different cutaneous sites, including the contralateral flank (C, × on the rat figure). Noxious pinch also evoked apressor response and tachycardia (A). Before i.t. strychnine, innocuous brush stimulation failed to alter heart rate, blood pressure, or the unit firing pattern (A). In contrast, after 40 μg of i.t. strychnine, similar brush stimulation evoked a pressor response, a gradual increase in heart rate, motor withdrawal reflexes (not shown), and a significant increase in the firing rate of the cell (B). Spontaneous firing rate of the unit, the basal heart rate, and the blood pressure were elevated after i.t. strychnine. E: receptive field depicted for brush-evoked unit responses after i.t. strychnine. Location of the recording site in the Po thalamus is marked (●) on a schematic diagram of the rat brain in cross-section (D).

Iontophoretically applied glycine caused shrinkage of the cutaneous receptive field (Zieglgansberger and Herz 1971), whereas strychnine (by microdialysis) produced receptive field expansion (Sorkin and Puig 1996). The current results extend these observations by demonstrating that i.t. strychnine-induced changes at the spinal level can influence the boundaries of neuronal receptive fields at supraspinal sites and indicate that glycine is important in limiting the spatial extent of input to thalamic neurons.

The responses to brushing appeared larger during i.t. strychnine than before, but we did not attempt to quantify this increase because we could not be sure that the stimulus was exactly the same in the two conditions. However, with the air jet stimulus, it was possible to quantify the unit response, and this revealed that at sites where the air jet was effective before i.t. strychnine there was no additional increase after strychnine. In contrast, at sites where the air jet was ineffective before i.t. strychnine, whether within or outside the brush-evoked receptive field (see Fig. 8), the air jet produced a response after strychnine. These results suggest that the major effect of i.t. strychnine is to enhance (presumably by disinhibition) normally subliminal inputs to the cells. It has been well established that dorsal horn neurons have subliminal inputs (Woolf and King 1989), and Sivilotti and Woolf (1994) have speculated that these inputs would become suprathreshold after increased excitability of the neuron as would occur after strychnine. There is evidence that receptive field expansion can occur in the absence of significant changes in response magnitude (Cook et al. 1987; Semba et al. 1985). When exciting the neuron by brushing the receptive field, the brush normally would pass through the subliminal surround region. Thus the overall response of the neuron to the brush stimulus would be larger after i.t. strychnine because these previously subliminal inputs now would excite the neuron. These increased responses to a brush stimulus that moves across the skin also may explain why the allodynia is observed best with the use of a moving brush stimulus (Sherman and Loomis 1994; Yaksh 1989). This kind of stimulus will produce a greater response in a larger number of neurons and thus presumably provide sufficient activity in nociceptive neurons to evoke a nociceptive response.

The ability of low-threshold mechanical stimuli to activate NS thalamic neurons after i.t. strychnine can be explained by the previously reported findings that spinal strychnine can lower the threshold of some dorsal horn NS neurons (Sorkin and Puig 1996). Another possibility is that the thalamic NS neurons normally receive convergent input from WDR or LTM neurons but that the magnitude of the input produced by the low-threshold stimuli is normally not suffi-
FIG. 7. Example of a NS neuron in VPL thalamus. Pinch stimulation applied to either hind paw evoked a significant increase in the firing rate of this unit (A, X on rat figure), but pinch was ineffective when applied on the flanks or tail (X on rat figure). Before i.t. strychnine, innocuous air jet (open ovals on rat figure) or brush stimulation applied to the rat hindquarters failed to alter the unit firing rate (A). In contrast, after 40 μg of i.t. strychnine, similar brush or air jet stimuli (shaded ovals) evoked significant increases in the firing rate of the cell (B). Receptive field for brush-evoked unit responses is indicated by shading on the rat figure. Effects of i.t. strychnine were reversible because innocuous stimuli (air jet shown as an example) were no longer effective in evoking unit responses by 29 min after the i.t. strychnine injection. Location of the recording site in VPL thalamus is marked (●) on a schematic diagram of the rat brain in cross-section (D).

cient to excite them. This proposal is supported by the observation that, after i.t. strychnine, spinal WDR and LTM neurons respond much more intensely to low-threshold inputs (Sorkin and Puig 1996) and thus might be capable of exciting the thalamic neurons. These inputs could arise directly from NS, WDR, and LTM STT neurons (Hodge and Apkarian 1990; Katter et al. 1996) or via polysynaptic ascending pathways such as the postsynaptic dorsal column pathway and spinocervical tract (Cliffer and Giesler 1989; Giesler et al. 1979b, 1988). Although we did not delineate the rostral extent of these changes in detail, careful examination revealed that the decrease in threshold and/or receptive field expansion was limited to dermatomes caudal to upper thoracic levels, and this is consistent with the extent of the direct action of i.t. strychnine (Sherman and Loomis 1995).

The changes in properties of thalamic NS and WDR neurons after strychnine are consistent with their involvement in mediating allodynia. In addition to the lowered threshold of NS neurons, the expansion of the low-threshold receptive fields of WDR neurons is also likely to contribute to touch-evoked allodynia and to hyperalgesia. Receptive field expansion leads to an increase in the overlap of adjacent receptive fields, and, as a result, stimulation at any one cutaneous site activates a larger number of neurons than before receptive field expansion. It has been suggested that this increase in afferent input evoked by a given stimulus results in abnormally exaggerated sensations such as allodynia or hyperalgesia (Dubner and Basbaum 1994). However, in spite of this empiric evidence linking changes in thalamic units to allodynia, it must be remembered that the cardiovascular and motor endpoints used as indices of allodynia in the current study probably involve only spinal or brain stem reflexes, not thalamo-cortical pathways.

Despite the strong similarities between the changes observed in lateral thalamic neurons in the current experiments (Sorkin and Puig 1996) and thus might be capable of exciting the thalamic neurons. These inputs could arise directly from NS, WDR, and LTM STT neurons (Hodge and Apkarian 1990; Katter et al. 1996) or via polysynaptic ascending pathways such as the postsynaptic dorsal column pathway and spinocervical tract (Cliffer and Giesler 1989; Giesler et al. 1979b, 1988). Although we did not delineate the rostral extent of these changes in detail, careful examination revealed that the decrease in threshold and/or receptive field expansion was limited to dermatomes caudal to upper thoracic levels, and this is consistent with the extent of the direct action of i.t. strychnine (Sherman and Loomis 1995).

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However, the electrophysiological studies (Lin et al. 1994; Peng et al. 1996; Sorkin and Puig 1996; Wilcox et al. 1996) used different routes of strychnine administration and different types of cutaneous stimuli. To our knowledge, all previous studies demonstrating strychnine-induced allodynia administered the drug by the i.t. route (Beyer et al. 1985, 1988; Onaka et al. 1996; Sherman and Loomis 1994–1996; Sosnowski and Yaksh 1989; Yaksh 1989). However, in the electrophysiological studies (Lin et al. 1994; Peng et al. 1996; Sorkin and Puig 1996; Wilcox et al. 1996) strychnine was delivered by iontophoresis or microdialysis, and thus the data are not directly comparable due to differences in concentrations and spatial distribution. Furthermore, no previous study attempted to correlate changes in spinal (or thalamic) unit responses and receptive fields with evidence of behavioral allodynia. A second reason why the results of the current study could not be predicted by extrapolation from studies of the effects of strychnine on spinal neurons is that the input to the thalamus arises from only a subset of neurons, and these may have different properties. Thus the effects of strychnine on spinal units would not necessarily be mirrored at the thalamic level.

There was a reasonably good temporal correlation between the effects of i.t. strychnine on thalamic units (receptive field expansion/decreased threshold) and overt symptoms of allodynia (abnormal brush-evoked cardio-
The critical observation is that all of thalamic neurons investigated in the present study exhibited dramatic functional changes within minutes of i.t. strychnine administration and that the majority of these changes occurred during the time course of strychnine-dependent allodynia. Thus these observations are consistent with the involvement of the VPL thalamus in the manifestation of strychnine-dependent allodynia, but the possibility that these changes contribute to other types of sensory dysfunction such as vascular and motor reflexes. The onset of allodynia was coincident with the onset of novel unit responses for just over half of the animals, and of the 57 brush stimulations eliciting abnormal unit responses, 30 evoked cardiovascular or motor reflexes characteristic of allodynia. Because a large population of neurons is required to elicit gross motor and cardiovascular responses, the particular unit monitored in some experiments might not have been recruited for the overall population response to strychnine.

### Table 1

<table>
<thead>
<tr>
<th>Unit Type</th>
<th>$n$</th>
<th>Unit Changes Produced by i.t. Strychnine</th>
<th>Number of Units Affected</th>
<th>Onset of Allodynia Relative to Onset of Unit Change</th>
<th>Offset of Allodynia Relative to Offset of Unit Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before Same After</td>
<td>Before Same After</td>
</tr>
<tr>
<td>LTM</td>
<td>12</td>
<td>Receptive field expansion</td>
<td>12</td>
<td>4 5 3</td>
<td>6 5 1</td>
</tr>
<tr>
<td>NS</td>
<td>3</td>
<td>Response to brush</td>
<td>3</td>
<td>0 2 1</td>
<td>3 0 0</td>
</tr>
<tr>
<td>WDR</td>
<td>2</td>
<td>Receptive field expansion</td>
<td>2</td>
<td>0 2 0</td>
<td>1 1 0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td></td>
<td>17</td>
<td>4 9 4</td>
<td>10 6 1</td>
</tr>
</tbody>
</table>

i.t., intrathecal; LTM, low-threshold mechanoreceptive; NS, nociceptive specific; WDR, wide dynamic range.
paresthesia or poor localization of cutaneous stimuli cannot be ruled out on the basis of the current results.

In summary, the findings of this study have indicated that i.t. strychnine results in profound changes in the responses of nociceptive and nonnociceptive neurons in the VPL thalamus of the rat. These changes are consistent with our previous observations in medial thalamus and probably result from abnormal input from the spinal cord dorsal horn mediated by several ascending pathways projecting to thalamus. The profound effects of i.t. strychnine on both tactile and nociceptive input provide support for the important role of glycine played in modulation of somatosensory information under normal circumstances. Changes in thalamic units after i.t. strychnine are no doubt responsible for mediating some of the behavioral changes induced by this drug. It is also likely that similar mechanisms are responsible, in at least some cases, for mediating the touch-evoked allodynia and paraesthesia frequently associated with neuropathic pain in humans.

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