Response Characteristics of Spinothalamic Tract Neurons That Project to the Posterior Thalamus in Rats

Xijing Zhang and Glenn J. Giesler, Jr
Department of Neuroscience, University of Minnesota, Minneapolis, Minnesota

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Zhang, Xijing and Glenn J. Giesler Jr. Response characteristics of spinothalamic tract neurons that project to the posterior thalamus in rats, J Neurophysiol 93: 2552–2564, 2005; doi:10.1152/jn.01237.2004. A sizeable number of spinothalamic tract axons terminate in the posterior thalamus. The functional roles and precise areas of termination of these axons have been a subject of recent controversy. The goals of this study were to identify spinothalamic tract neurons (STT) within the cervical enlargement that project to this area, characterize their responses to mechanical and thermal stimulation of their receptive fields, and use microantidromic tracking methods to determine the nuclei in which their axons terminate. Forty-seven neurons were antidromically activated using low-amplitude (≤30 μA) current pulses in the contrateral posterior thalamus. The 51 points at which antidromic activation thresholds were lowest were surrounded by ineffective tracks indicating that the surrounded axons terminated within the posterior thalamus. The areas of termination were located primarily in the posterior triangular, medial geniculate, posterior and posterior intralaminar, and suprageniculate nuclei. Recording points were located in the superficial and deep dorsal horn. The mean antidromic conduction velocity was 6.4 m/s, a conduction velocity slower than that of other projections to the thalamus or hypothalamus in rats. Cutaneous receptive fields appeared to be smaller than those of neurons projecting to other areas of the thalamus or to the hypothalamus. Each of the examined neurons responded exclusively or preferentially to noxious stimuli. These findings indicate that the STT carries nociceptive information to several target nuclei within the posterior thalamus. We discuss the evidence that this projection provides nociceptive information that plays an important role in fear conditioning.

INTRODUCTION

Several lines of evidence, dating back >40 yr, indicate that the posterior thalamus plays a prominent role in nociceptive processing. The seminal observation was made by Poggio and Mountcastle (1960), who found that a large number of neurons recorded in the posterior thalamus of cats were activated by noxious mechanical stimuli. In subsequent years, neurons within the posterior thalamus have been shown to respond to noxious mechanical, thermal, and electrical stimulation of the skin within frequently large, discontinuous bilateral receptive fields (Benedek et al. 1997; Bordi and LeDoux 1994; Brinkhaus et al. 1979; Calma 1965; Casey 1966; Craig et al. 1994; Curry 1972; Curry and Gordon 1972; Gauriau and Bernard 2004b; Guilbaud et al. 1977, 1980; Nyquist and Greenhoot 1974; Perl and Whitlock 1961; Whitlock and Perl 1961). Neurons in the posterior thalamus can also be activated by visceral noxious stimuli such as intra-articular injection of bradykinin (Guilbaud et al. 1977) and noxious distention of visceral organs including the bladder, esophagus, duodenum, and colon (Carstens and Yokota 1980; Horn et al. 1999). Cells in the posterior thalamus are also activated by electrical stimulation of the pelvic nerve (Bruggeman et al. 1994) and tooth pulp (Matsumoto et al. 1988). Two studies noted that the percentage of nociceptive neurons that were encountered in the posterior thalamus appeared to be much greater than were present in the ventral posterior lateral thalamic nucleus (VPL) of cats (Guilbaud et al. 1977; Poggio and Mountcastle 1960).

In humans, nociceptive units have also been recorded in areas posterior and inferior to the ventrocaudal thalamic nucleus (Lenz et al. 1993b, 1994). These neurons seem likely to be located within the posterior thalamus. Lenz and colleagues also found that small-amplitude stimulation pulses delivered through recording electrodes in this area elicit painful sensations in awake humans (Lenz et al. 1993a).

There is currently considerable controversy surrounding the termination of the spinothalamic tract (STT) within the posterior thalamus. A number of early anatomical studies in which either degeneration or anterograde labeling techniques were used showed that many STT axons terminate within several nuclei within the posterior thalamus including the medial region of the medial geniculate (MGm), the suprageniculate nucleus (SG), and the posterior nucleus (Po) (Apkarian and Hodge 1989; Boivie 1979; Mehler 1969; Mehler et al. 1960; Ralston and Ralston 1992). However, Craig et al. (Craig 2004; Craig et al. 1994) suggested that many axons from marginal zone neurons projected specifically to a previously unrecognized nucleus that is rich in calbindin-immunoreactive fibers. They named this area the posterior portion of the ventral medial nucleus (VMpo). The evidence supporting the existence of VMpo was challenged in a review by Willis et al. (2002) and in an accompanying commentary by Jones (2002). In addition, Graziano and Jones (2004) recently re-examined the projections of the STT in cats. They concluded that the nociceptive, calbindin-rich zone described by Craig and colleagues was not, in fact, located within the posterior thalamus but was located within the medial portion of the ventral posterior medial nucleus. Graziano and Jones (2004) also found that fibers that were anterogradely labeled by injections into the marginal zone of trigeminal nucleus caudalis were distributed widely throughout the posterior thalamus, supporting the older anatomical studies. Graziano and Jones (2004) also demonstrated that none of the labeled trigeminothalamic fibers were immunoreactive for calbindin, refuting the suggestion by Craig et al. that...
STT and trigeminothalamic fibers contained calbindin. Recently, Gauriau and Bernard (2004a) examined the projections of the STT in rats using injections of the anterograde tracer PHA-L into several specific regions of the spinal gray matter including the marginal zone. They found dense projections within the posterior thalamus in several nuclei including the posterior triangular nucleus (PoT), the posterior intralaminar nucleus (PIL), and Po.

In several previous physiological studies, spino- or trigeminothalamic neurons have been antidromically activated from the posterior thalamus (Dostrovsky and Craig 1996; Hirata et al. 2000; Trevino et al. 1973). However, anatomical and other physiological studies have shown that many STT axons pass through the posterior thalamus in route to more rostral areas such as the ventral posterior lateral nucleus (VPL) and the hypothalamus (Apkarian and Hodge 1989; Applebaum et al. 1979; Boivie 1979; Dado et al. 1994a; Mehler 1969; Mehler et al. 1960; Zhang et al. 1995). Therefore it is not clear whether the axons that were antidromically activated in the posterior thalamus in previous studies passed through the area or terminated within it.

In a previous study of spinohypothalamic tract axons in which microantidromic activation methods were used, we (Dado et al. 1994a) noted that several axons could not be followed into levels rostral to posterior thalamus. The primary goal of the present study was to pursue these initial observations and determine the functional characteristics of STT axons that terminate in the posterior thalamus in rats. We used the electrophysiological method of microantidromic activation techniques to identify the subnuclei within the posterior thalamus in which their axons terminate.

Methods

Male Sprague-Dawley rats weighing 300–450 g were anesthetized with urethan (1.3 g/kg ip), artificially ventilated, and paralyzed with a continuous infusion of flaxedil (20 mg/h). The external jugular vein was cannulated for drug administration. End-tidal CO2 and core temperature were monitored and maintained at normal levels. The cervical enlargement of the spinal cord was exposed by laminectomy. The dura was retracted, and a pool of warm mineral oil was formed over the exposed area of the spinal cord. A craniotomy exposed the brain over the diencephalon and mesencephalon bilaterally to allow the insertion of stainless steel monopolar stimulating electrodes.

Single units in the cervical enlargement of the spinal cord (C5–C4) were recorded using stainless steel (5–10 MΩ) or tungsten microelectrodes (2–5 MΩ). Cathodal current pulses (500 μA, 200 μs, 10 Hz) delivered through a stimulating electrode placed in the contralateral posterior thalamus served as search stimuli. The criteria used for antidromic activation included: responses occurred at a constant latency (<0.2 ms variability), responses were able to follow high-frequency stimuli (≥333 Hz), and putative antidromic action potentials collided with orthodromic action potentials (Lipski 1981).

To determine the area of termination for each axon, microantidromic mapping was done in the diencephalon as previously described (Dado et al. 1994a; Zhang et al. 1995). Briefly, the stimulating electrode was moved rostrally throughout the hypothalamus and thalamus in a series of electrode penetrations across the mediolateral extent of the brain. If the neuron was antidromically activated in the hypothalamus or in rostral levels of the thalamus, the unit was discarded. Antidromic tracking for each axon was begun at a level near the rostral pole of the diencephalon. After multiple tracks were made across the most rostral level, the stimulating electrode was moved 0.5–1.0 mm caudally, and the procedure was repeated at the new level. Within an anterior-posterior plane, tracks were separated by 300–500 μm mediolaterally. Within each track the electrode was lowered from the dorsal to the ventral surface and antidromic thresholds were determined at 200-μm intervals. A series of electrode tracks was made until a point was located at which the antidromic threshold was ≤30 μA (low-threshold point). All examined units were antidromically activated using currents ≥30 μA in the posterior thalamus; none could be activated at greater latencies at levels rostral to posterior thalamus. Current pulses ≤30 μA have been shown to activate spinohypothalamic tract axons at a distance of ≤400 μm from the stimulating electrode (Burstein et al. 1991; Dado et al. 1994a). To locate the apparent terminal areas of the axons, we surrounded penetrations containing the most rostral low threshold point dorsally, ventrally, rostrally, medially, and laterally with penetrations in which the axon could not be activated antidromically with ≥500 μA. Failure to activate the axon antidromically with a 500-μA pulse is interpreted as evidence that the axon did not pass through the stimulated areas (Zhang et al. 1995).

The boundaries of cutaneous receptive fields and the response characteristics of recorded cells were determined using innocuous and noxious mechanical stimuli. Units were classified as low-threshold (LT), wide-dynamic-range (WDR), or high-threshold (HT) neurons according to their responses to graded mechanical stimulation of their receptive fields (see Dado et al. 1994b for details).

Thermal stimuli were applied to the area of the cutaneous receptive fields in which mechanical stimuli produced the highest frequency responses. Thermal stimuli were delivered using a pelter-type stimulator with a contact area of 9 × 9 mm (Wilcox and Giesler 1984). Stimulus duration was 30 s, and the interstimulus interval was 180 s. The stimulating surface of the skin was maintained at 35°C during interstimulus intervals. The mean response frequency to each stimulus was defined as the mean frequency during stimulus minus the mean firing frequency in the 30 s before each stimulus. An ascending series of heat stimuli of 39, 41, 45, 50, 55, and 58°C were applied followed by a descending series of cooling stimuli of 20, 10, and 1°C. Skin temperature was increased at 10°C/s and decreased at 2°C/s.

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At the end of each experiment, electrolytic lesions were made at the tip of the stimulating electrode at low-threshold points in the brain (25 μA DC for 40 s) and at the tip of the recording electrode (25 μA DC for 15 s). Rats were perfused with 0.9% saline followed by 10% formalin containing 1% potassium ferrocyanide (Prussian blue reaction). The areas of the brain and spinal cord containing lesions were cut transversely at 50 μm using a freezing microtome. Sections were counterstained with Neutral red and examined under a microscope. The locations of lesions were reconstructed with the aid of an attached camera-lucida drawing tube. Recording locations were categorized as being located within the superficial (i.e., marginal zone or substantia gelatinosa) or deep (i.e., nucleus proprius or lateral reticulated area) dorsal horn. The atlas of Paxinos and Watson (1986) was used to help identify the locations of lesions within the brain.

Results

An example of a spinal cord neuron that was antidromically activated from the contralateral midbrain and posterior thalamus is illustrated in Fig. 1. The unit was initially antidromically activated from a low-threshold point within the anterior pretectal nucleus (B and C) at a latency of 2.4 ms (b1). The unit was antidromically activated at a second low-threshold point located in the PoT (A and C) at a latency of 2.7 ms. The antidromic threshold at this point was 10 μA. Stimulus pulses of 10 μA have been shown to activate axons a distance of not >170 μm (Burstein et al. 1991; Dado et al. 1994a; Ranck 1975). The ability of the neuron to follow high-frequency (333 Hz) antidromic stimulus pulses and collision of antidromic with orthodromic action potentials are illustrated (a, 2 and 3).
The unit was also antidromically activated from a low-threshold point in the upper cervical white matter, and antidromic action potentials from this point collided with those elicited in the midbrain or posterior thalamus (not illustrated) (see Dado et al. 1994a), demonstrating that the same unit was activated from both locations in the brain. This neuron could not be
antidromically activated using high-amplitude (500 μA) stimulus pulses delivered throughout the dorsal-ventral extent of stimulation tracks located medial, rostral, and lateral to the most rostral low-threshold point (C). This unit was recorded in the deep dorsal horn (D), had a small receptive field on the lateral toes (E), and was activated only by noxious mechanical and heat stimuli (F).

Figure 2 illustrates an example of an STT neuron in the marginal zone that projected to the posterior triangular nucleus of thalamus. A single lowest-threshold point for antidromic activation was seen in the posterior thalamus. The low-threshold point was surrounded medially, laterally, or rostrally by antidromic stimulation tracks. Within VPL, the area immediately rostral to the low threshold point (A, −4 mm from...
bregma), stimulus pulses with amplitudes between 100 and 500 μA activated the neuron at the same antidromic latency as that seen at the low-threshold point, indicating that activation from the rostral points resulted from spread of the high-amplitude pulses caudally to the low-threshold point. The unit could not be activated with high-amplitude pulses throughout any of the remaining stimulus tracks (A and B). The unit was recorded in the marginal zone (C), had a small receptive field on the forelimb (D), and was classified as a WDR neuron (E).

Figure 3 depicts an STT neuron that was recorded in the marginal zone and projected to the posterior thalamus. The stimulating electrode was initially placed in the posterior thalamus level, −5 mm posterior to bregma (A). A single lowest-threshold point was found in a track that was 3.2 mm lateral to

FIG. 3. An example of a neuron in C8 with an axon that projects to the contralateral posterior thalamus. A: cross-sections showing the multiple levels through which the stimulating electrode passed in the contralateral diencephalon. The amplitude of current required to activate the neuron antidromically is indicated. The low-threshold point was in the contralateral posterior thalamus and was surrounded medially, laterally, and rostrally by ineffective stimulation tracks, indicating that the axon ended in the posterior thalamus. a1: 3 overlapping traces of antidromic responses of recorded neuron. The amplitudes of stimulus artifacts were reduced for clarity of presentation. B: representation of a dorsal view of the diencephalon. Locations of antidromic thresholds within stimulating tracks are indicated. The minimum antidromic threshold in each penetration is represented by a symbol (inset). C: responses of this neuron to cutaneous stimuli. D: recording site in marginal zone of C8. E: receptive field on ipsilateral leg.
the midline. The antidromic threshold was 12 μA and the latency was 6.5 ms (a1). The low-threshold point was located in the medial geniculate nucleus of the posterior thalamus (A). The low-threshold point was surrounded with stimulation tracks in which the unit could not be antidromically activated by high-amplitude current pulses (B), indicating that the axon terminated in the region surrounding the lowest-threshold point (A). The neuron was recorded in the marginal zone of C₈ (D). Its cutaneous receptive field was small and located on the ipsilateral forelimb (E). The unit responded with low-frequency discharges only to noxious stimulation of its cutaneous receptive field (C).

Antidromic activation within the posterior thalamus

Each of the 47 identified neurons were antidromically activated from low-threshold points (≤30 μA) in the contralateral posterior thalamus that were surrounded medially, laterally, and rostrally by tracks in which current pulses of 500 μA did not activate the neuron antidromically. Seventeen low-threshold points were located within the PoT, 13 were located in the medial geniculate nucleus (MG), 7 in Po, 6 in the PIL, 5 in SG, 2 in the optic tract (OT), and 1 in the lateral geniculate nucleus (Table 1, Fig. 4). In four cases, spinal neurons were antidromically activated at two distinct latencies from two low-threshold points within the posterior thalamus, suggesting that some spinal neurons send branches into more than one area of the posterior thalamus. In one case, low-threshold points were encountered in SG and Po, in another they were located in MG and PIL, in a third in PoT and MG, and in the fourth in PoT and OT. The mean current for antidromic activation from 51 low-threshold points in the contralateral posterior thalamus was 21.4 ± 1.3 (SE) μA. Photomicrographs of lesions at the low threshold points within the PoT, MG, PIL, SG, and Po are illustrated in Fig. 5C–G.

Recording sites

The locations of lesions marking 43 of the recording sites of neurons are shown in Fig. 6. Four lesions were not recovered. Twenty-eight recording sites (65%) were located in the superficial dorsal horn (SDH). Fifteen recording sites were located in the deep dorsal horn (DDH). Within the DDH, 11 recording sites were located in the nucleus proprius and 4 in the lateral reticulated area. Twenty-eight neurons were recorded in C₇, 19 in C₈. Photomicrographs of lesions at the recording site of a neuron in the SDH and another in the DDH are shown in Fig. 5, A and B.

TABLE 1. Areas of termination in posterior thalamus response categories and recording locations of recorded neurons

<table>
<thead>
<tr>
<th>Recording Location</th>
<th>Response Category</th>
<th>PoT</th>
<th>MG</th>
<th>Po</th>
<th>PIL</th>
<th>SG</th>
<th>OT</th>
<th>LGN</th>
<th>Totals</th>
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<td>1</td>
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<td>0</td>
<td>2</td>
<td>0</td>
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<td>5</td>
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<tr>
<td></td>
<td>HT</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>NC</td>
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<td>4</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
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</tr>
<tr>
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<td>12</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
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<td>3</td>
</tr>
<tr>
<td></td>
<td>HT</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>9</td>
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<tr>
<td></td>
<td>NC</td>
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<td>1</td>
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<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>17</td>
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<tr>
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</table>

Recording locations are based on locations of lesions in all but four cases. In these, three were recorded at depths <100 μm and are included as superficial dorsal horn (SDH) neurons; one was recorded at a depth of >400 μm and is included in deep dorsal horn (DDH). PoT, nucleus triangular of the posterior thalamus; MG, medial geniculate nucleus; Po, posterior thalamus nucleus; PIL, posterior intralaminar thalamus nucleus; SG, suprageniculate thalamus nucleus; LGN, lateral geniculate nucleus; WDR, wide dynamic range; HT, high threshold; NC, not classified.
Cutaneous receptive fields

Attempts were made to locate the receptive fields of 40 of the examined neurons. Thirty-three had cutaneous receptive fields that were restricted to the ipsilateral forepaw, forelimb, or shoulder. (Fig. 7). The receptive fields of seven (18%) neurons could not be located despite repeated applications of innocuous and noxious mechanical, thermal and electrical stimuli over much of the limbs and trunk. With the exception of neurons in the lateral spinal nucleus (unpublished observations), we have encountered very few projection neurons that were unresponsive to all forms of stimulation in previous studies in rats (Burstein et al. 1991; Dado et al. 1994b; Kostarczyk et al. 1997; Zhang et al. 1995).

Classification by responses to cutaneous mechanical stimuli

Twenty-five units (76%) responded only to noxious stimuli and were classified as HT neurons (Table 1, Figs. 1 and 3). Sixteen HT neurons were recorded in the SDH and nine in the DDH. Eight (24%) of 33 tested units responded to innocuous mechanical stimulation but responded at higher frequencies to increasingly intense noxious stimulation. These units were classified as WDR neurons (Fig. 2). Five WDR neurons were recorded in the SDH, three in the DDH. The mean responses of all examined neurons to the pinch and squeeze stimuli were $4.5 \pm 0.8$ and $8.2 \pm 1.1$ spikes/s. The mean responses of SDH neurons to these stimuli were $5.0 \pm 1.0$ and $8.6 \pm 1.5$ spikes/s; the mean responses of DDH neurons were $3.6 \pm 1.1$ and $7.6 \pm 1.6$ spikes/s.

Responses to cutaneous thermal stimuli

Thirteen neurons were tested for their responses to an ascending series of heat stimuli. Nine examined neurons (69%) responded to heat stimuli applied to their cutaneous receptive fields. The individual and overall mean responses ($\pm$SE) are shown in Fig. 8. Eight heat-response neurons were classified HT and one as WDR. Four neurons did not respond to heat stimuli; three were classified as HT and one as WDR. These 13 neurons were also tested with a series of innocuous cooling and noxious cold stimuli, and only 1 neuron responded to noxious cold stimuli (10 and 1°C). This neuron projected to Po. Among nine cells that responded to heat stimuli, four projected to PoT.
two to MG, and two to Po. One neuron projected to both PoT and MG.

The mean stimulus-response function for neurons that responded to heat stimuli is plotted on the double-natural logarithmic scale in Fig. 9. The function was fit with a line that had a slope of 2.3 ($r^2 = 0.81$, $P < 0.02$). The approximate mean response threshold (48.1°C) to heat stimuli was extrapolated from the stimulus-response function.

**Conduction velocities**

The mean antidromic latency from the low-threshold points in the contralateral posterior thalamus to the recording sites in the cervical enlargement for 47 examined neurons was $7.9 \pm 1.0$ (SE) ms (range, 2.5–40.0 ms). Assuming that the axons crossed in the spinal cord and ascended in a straight path to the thalamus, the estimated mean conduction velocity was $6.4 \pm 0.5$ m/s (range, 0.9–14.0 m/s; Fig. 10). The mean conduction velocity of neurons located in the SDH ($5.1 \pm 0.4$ m/s) was significantly slower than that of cells in the DDH ($8.9 \pm 0.8$ m/s; $P < 0.001$, t-test). Two STT neurons had conduction velocities within the range of unmyelinated fibers; these conducted at 0.9 and 1.0 m/s.

**Discussion**

In these experiments, microantidromic stimulation methods were used to identify STT neurons that project to the posterior thalamus and to determine the nuclei to which they project. We also determined the responses of these neurons to innocuous and noxious mechanical and thermal cutaneous stimuli. Each unit examined in this study was antidromically activated from the posterior thalamus using stimulus pulses of $\leq 30$ (mean = $21 \mu$A). Current pulses $= 30 \mu$A have been shown to activate spinal axons in the brain at a distance of $\leq 400 \mu$m from the stimulating electrode (Burstein et al. 1991; Dado et al. 1994a).

We surrounded lowest-threshold points medially and laterally with stimulating tracks within which the axon could not be activated antidromically with high-amplitude, 500-µA stimuli.
within the posterior thalamus and did not simply course through the area in route to other targets.

The method of antidromic activation has been used to identify and characterize axons within a number of ascending and descending projection systems including the spinohypothalamic, spinomesencephalic, spinothalamic, reticulospinal, rubrospinal, vestibulospinal, and corticospinal tracts (Applebaum et al. 1979; Burstein et al. 1991; Dado et al. 1994; Katter et al. 1996; McMahon and Wall 1985; Shinoda et al. 1976, 1977; Zhang et al. 1995). Axons of several of these systems have been examined subsequently using intra-axonal dye injections (Dick et al. 1988; Shinoda et al. 1982a,b; Yen et al. 1991). These studies have shown that microantidromic activation methods can provide an accurate, but less detailed, description of the path and termination of axons within the CNS.

Applebaum et al. (1979) also used microantidromic activation methods to examine the projections of functionally identified primate STT axons. The bulk of the examined axons were found to terminate in VPL. However, a single HT axon from a neuron recorded in the marginal zone was followed to its termination medial to the MG. The authors describe the termination point as “near the posterior thalamus,” although based on their Fig. 6A, it is possible that it was located within the mesencephalic reticular formation. In our earlier study in rats, we (Dado et al. 1994a) antidromically activated four axons from low-threshold points within and adjacent to the PoT. None of these could be activated from rostral levels within the diencephalons, indicating that they terminated within the PoT.

In the present study, more STT axons were found to terminate within PoT (36%) than within any other area of the posterior thalamus. A recent anatomical anterograde tracing study (Gauriau and Bernard 2004a) also indicated that a large percentage of STT fibers projected to the PoT in rats. Our findings suggest that the overwhelming majority of axons that form this large projection to PoT carry information regarding nociceptive mechanical and heat stimuli. STT neurons that project to PoT were located within both the marginal zone and DDH. Gauriau and Bernard (2004a) found that injections of PHA-L that involved the marginal zone produced rich labeling of fibers and terminals within PoT, and injections that were restricted to the DDH produced only sparse labeling within PoT. In the present study, neurons that were found to project to PoT were found almost equally within the marginal zone and deep dorsal horn, suggesting that the contribution from the DDH might be larger than originally believed.

In addition to PoT, the SG, MGm, Po, and PIL were each found to receive direct nociceptive STT projections. These findings confirm previous anatomical results (Boivie 1979; Cliffer et al. 1991; Dado and Giesler 1990; Gauriau and Bernard 2004a; Graziano and Jones 2004; Jones and Burton 2005).

![Figure 9](http://jn.physiology.org/)

**FIG. 9.** Stimulus-response function for posterior thalamus projecting neurons that responded to a series of innocuous and noxious heat stimuli plotted on double-natural logarithmic scales. Regression analysis of the stimulus–response function yielded a curve with a slope of 2.3 ($r^2 = 0.81$). Response threshold was defined as a mean firing frequency $\pm 1.5$ spikes/s above background; $\downarrow$, the calculated response threshold for heat stimuli.

![Figure 10](http://jn.physiology.org/)

**FIG. 10.** Histogram of conduction velocities of examined axons between recording sites and low-threshold points in the contralateral posterior thalamus. The mean conduction velocity of axons of SDH (■) neurons was significantly slower than that of DDH neurons (□).
for all examined neurons (recorded in SDH and DDH) to pinch.

1960), it is likely that a considerable convergence of informa-

tion occurs on posterior thalamic neurons. Because nociceptive neurons in the posterior thalamus generally have large complex, discontinuous bilateral receptive fields (Guilbaud et al. 1977, 1980; Matsumoto et al. 1988; Peschanski et al. 1981). Our results indicate that STT axons contribute prominently to the responses of neurons in this region. Our findings indicate that a far larger percentage of the STT projection to the posterior thalamus originates from neurons in the marginal zone than is the case for projections to other regions of the diencephalon in rats. Nearly two-thirds of the neurons (65%) that project to the posterior thalamus were located within the superficial dorsal horn (SDH). In contrast, only 16–32% of rat spinohypothalamic tract neurons were located in the SDH (Dado et al. 1994b; Kostarczyk et al. 1997; Zhang et al. 1995). Giesler et al. (1976) reported that injections of HRP into the medial thalamus of rats failed to label any neurons in the SDH. Our results also suggest that neurons that project to the posterior thalamus have smaller-diameter, more slowly conducting axons than do other spinal projection neurons in rats. The mean conduction velocity of posterior thalamus projecting axons was found to be 6.4 m/s. The majority of axons in this study had conduction velocities between 2 and 8 m/s, suggesting that they had thinly myelinated axons. This conduction velocity is only about one-third of that for spinohypothalamic tract neurons also recorded in the cervical enlargements of rats (Dado et al. 1994b; Kostarczyk et al. 1997; Zhang et al. 1995). Giesler et al. (1976) recorded from STT neurons in the lumbar enlargement of rats. These neurons, backfired from the area of the medial lemniscus and VPL, had conduction velocities between 14 and 26 m/s. Two axons in this study had conduction velocities of ∼1 m/s. Craig and colleagues (Andrew and Craig 2001a,b; Craig and Dostrovsky 1991, 2001) have shown that many STT neurons in the marginal zone of cats have unmyelinated axons. To our knowledge, this is the first report of any type of spinal cord projection neurons in rats with unmyelinated axons. None have been demonstrated in monkeys.

The receptive fields of neurons projecting to the posterior thalamus had small or medium sized receptive fields that were located in the ipsilateral forepaw, forelimb, or shoulder. In contrast, Dado et al. (1994b) reported that almost half of spinohypothalamic tract neurons recorded in the cervical enlargement of rats have large or complex receptive fields. Based on the sizes of the receptive fields of neurons in this study, it appears that STT neurons that project to the posterior thalamus are capable of providing information on the location of nociceptive stimuli. Because nociceptive neurons in the posterior thalamus generally have large complex, discontinuous bilateral receptive fields (Guilbaud et al. 1980; Poggio and Mountcastle 1960), it is likely that a considerable convergence of information occurs on posterior thalamic neurons.

An additional distinctive feature of many STT neurons that project to the posterior thalamus is their low-frequency discharges to noxious stimuli. We found that the mean responses for all examined neurons (recorded in SDH and DDH) to pinch and squeeze <10 spikes/s. We re-examined responses to these same stimuli from previous studies of spinohypothalamic tract neurons in our lab (Dado et al. 1994; Kostarczyk et al. 1997; Zhang et al. 1995). The mean responses of spinohypothalamic tract neurons to pinch (37.9 ± 3.3 spikes/s) and squeeze (48.8 ± 4.2) were roughly four times greater than those of STT neurons that project to the posterior thalamus and differed significantly (P < 0.001, t-test). We and others have also seen similar higher-frequency responses to comparable stimuli by primate STT neurons projecting to VPL (Dougherty et al. 1992; Owens et al. 1992; Palecek et al. 1994a,b; Zhang et al. 2000). The low-frequency responses to noxious stimuli by STT neurons that project to the posterior thalamus are reminiscent of those seen in nociceptors (Willis and Coggeshall 2004). There are several possible reasons why STT neurons that project to posterior thalamus appear to respond to noxious cutaneous stimuli with lower-frequency discharges. It is conceivable that such neurons are more susceptible to suppressive influences of the urethan anesthesia than are spinohypothalamic tract neurons. It is also possible that the inherent cellular properties (e.g., density of potassium channels, etc.) of posterior thalamic projecting cells limit the frequency of their responses. Each of these possibilities could be examined in future studies. Another possibility is that posterior thalamus-projecting neurons receive and convey input from a comparatively small number of nociceptors. The fact that these neurons have smaller receptive fields supports this idea.

Almost 70% of STT neurons that project to the posterior thalamus responded to noxious heat stimuli. These thermally responsive cells terminated in the PoT, MG, and Po. The examined neurons had accelerating responses to increasingly intense noxious heat. They are capable of producing clear increases in their firing rates in response to small changes in the intensity of noxious heat stimulus. The mean response was described by a power function with a slope of 2.3. Human psychophysical studies indicate that the stimulus-response functions that describe the human sensory experience of noxious heat have slopes of 2.0–3.0 (Price 1988). The calculated threshold for examined neurons that responded to heating was 48.1°C, a temperature that is considerably higher than the threshold for pain in humans and other animals (44–45°C) (Hardy et al. 1950). It appears that these neurons receive a specific input from high-threshold thermal afferent receptors (e.g., Aδ mechanohot nociceptors) (Willis and Coggeshall 2004) and mainly transmit information related to intense heat stimuli to posterior thalamic targets.

Nociceptive and fear-producing stimuli alter blood pressure and heart rate and produce “freezing behavior” in rats (Iwata et al. 1986). The repeated pairing of a neutral auditory or visual stimulus [conditioned stimulus (CS)] with noxious foot shock [unconditioned stimulus (US)] causes presentation of the CS alone to produce autonomic responses (Jarrel et al. 1986; LeDoux et al. 1986a, 1986b, 1990a). This form of learning is called fear or emotional conditioning. Considerable evidence indicates that the posterior thalamus and the amygdala play important roles in the production of fear conditioning (Iwata et al. 1986; Jarrell et al. 1986; LeDoux et al. 1990a). Lesions of the central and lateral nuclei of the amygdala (LeDoux et al. 1986; 1990a; Shi and Davis 1999) prevent rats from successfully acquiring fear conditioning (LeDoux 1990b). Neurons in these nuclei that have been implicated in fear conditioning...
have been shown to respond to noxious as well as auditory and visual stimuli (Romanski et al. 1993). Previous findings indicate that neurons within the central and lateral nuclei of the amygdala receive a direct noxious input from posterior thalamic nuclei including the PoT, SG, PIL, and Po (Bordi and LeDoux 1994; Burton and Jones 1976; LeDoux et al. 1985; 1990b; Ottersen and Ben-Ari 1979). These nuclei of the posterior thalamus also send large projections to a number of cortical areas including perirhinal, primary auditory, visceral, ventral temporal association, second and third somatic sensory, and insular and frontal cortices (Burton and Jones 1976; Friedman and Murray 1986; Gauriau and Bernard 2004b; Graybiel 1972; Heath and Jones 1971; Jones and Leavitt 1973; Kurokawa et al. 1990; Ledoux et al. 1985; Linke 1999; Linke and Schwegler 2000). Many of these cortical regions in turn project to the amygdala, suggesting that, in addition to direct inputs, the amygdala receives input from the posterior thalamus via thalamo-cortico-amygdaloid projections (Linke and Schwegler 2000). Lesions in posterior thalamus can also block the acquisition of fear conditioning (LeDoux et al. 1986a,b; Shi and Davis 1999). Additional evidence for an important role of posterior thalamic nuclei in fear conditioning was reported by Cruikshank et al. (1992), who showed that conditioned fear responses can be produced using low-amplitude stimulation within the PIL nucleus as a US instead of foot shock, indicating that activation within the posterior thalamus produces sufficiently aversive emotional responses that can be used as the US in fear-conditioning paradigms. Our findings indicate that the STT sends a large nociceptive projection to several nuclei within the posterior thalamus. It is reasonable to conclude therefore that at least a portion of the nociceptive information that is necessary for fear conditioning is provided by this STT projection. In addition, the STT projection to the posterior thalamus is capable of providing nociceptive input to a sizeable number of cortical regions via the widespread projections of neurons within the posterior thalamus.

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