A painful cutaneous laser stimulus evokes responses from single neurons in the human thalamic principal somatic sensory nucleus ventral caudal (Vc).

Authors: Kobayashi, K.\(^1\), Winberry, J.\(^1\), Liu, C.C.\(^1\), Treede, R.D\(^2\), Lenz FA\(^1\)
Department of \(^1\)Neurosurgery, Johns Hopkins Hospital
Baltimore MD 21278
\(^2\)Chair of Neurophysiology, University of Heidelberg at Mannheim, D-68167 Mannheim, Germany

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Address all correspondence and proofs to:
Frederick A. Lenz
Department of Neurosurgery,
Johns Hopkins Hospital
Meyer Building 8-181
600 North Wolfe Street
Baltimore, MD 21287-7713
Telephone - 410-955-2257
FAX - 410-287-4480
Email - flenz1@jhmi.edu

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Abstract

Cutaneous application of painful radiant heat laser pulses evokes potentials (laser evoked potentials, LEPs) which can be recorded from scalp or intracranial electrodes. We have now tested the hypothesis that the response of thalamic neurons to a cutaneous laser stimulus occurs at latencies predicted by the conduction delay between the periphery and the thalamus. We have carried out recordings from human thalamic neurons in the principal sensory nucleus (ventral caudal, Vc) in patients undergoing awake surgery for the treatment of tremor.

The results demonstrate that many neurons respond to the laser with early and/or late latency peaks of activity, consistent with conduction of the response to the laser stimulus through pathways from A\(\delta\) and C fibers to the thalamus. These peaks were of short duration perhaps due to the somatotopic and modality specific arrangements of afferent pathways to the thalamus. The responses of these thalamic neurons to the laser stimulus sometimes included low threshold spike (LTS) bursts of action potentials, consistent with previous studies of different painful stimuli. A prior study has demonstrated that spike trains characterized by common LTS bursts such as the intermediate (I) category change their category more commonly than do those without LTS bursts (NG – nongrouped category), spontaneously during changes in the cognitive task. Spike trains of laser responsive neurons were more common in the I category, while those of laser non-responsive neurons were more common in the NG category. Therefore, neuronal spike trains in the I category may mediate shifts in endogenous or cognitive pain-related behavior.
**Introduction**

Although imaging studies have dramatically enhanced our understanding of the pain pathway from the periphery to Vc and then to primary somatic sensory cortex, they do not provide direct evidence of neuronal activation by painful stimuli (Bushnell et al. 1999; Apkarian et al. 2005; Casey and Bushnell 2001). Cutaneous application of a painful laser beam leads to neuronal activation which generates a negative potential (N2) and then a positive potential recorded from the scalp vertex (Cz) electrode (P2) (laser evoked potentials, LEPs) (Carmon et al. 1976). LEPs recorded from the scalp or the cortical surface or subcortical structures are a complicated vector sum of responses in multiple cortical and subcortical generators (Tarkka and Treede 1993; Peyron et al. 2002; Ohara et al. 2004b). Scalp LEPs are degraded further by muscle and blink artifacts, by temporal and spatial filtering at the level of the scalp, skull, and CSF (Pfurtscheller and Cooper 1975) (Cooper et al. 1965; Gevins et al. 1994), and by large inter-electrode distances (Gevins et al. 1994). Scalp studies have reported that directed attention increases the LEP P2 peak but not the LEP N2 peak (Becker et al. 1993; Kanda et al. 1996; Miltner et al. 1989; Towell and Boyd 1993; Zaslansky et al. 1996; Zaslansky et al. 1995; Siedenberg and Treede 1996).

Cortical or subcortical recordings of LEPs can minimize many of these sources of error (Lenz et al. 1998c; Frot et al. 1999; Kanda et al. 2000). However, the interpretation of these recordings is still complicated by multiple different generators (Vogel et al. 2003), and by endogenous processes such as novelty and directed attention (Ohara et al. 2004a; Ohara et al. 2004c).

The contribution to LEPs of conduction delays, and of endogenous factors is unclear. This uncertainty could be clarified by an understanding of inputs to the cortex as revealed by recordings of neuronal responses along the pathway from the periphery to the somatic sensory cortex. Although there have been recordings of responses to painful laser pulses from human primary afferents (see
(Bromm et al. 1984)(Treede et al. 1995)), there have been no recordings from neurons located in the primate spinal cord, thalamus or cortex, to our knowledge.

We have now tested the hypothesis that thalamic neuronal responses to a painful cutaneous laser heat stimulus will occur at the latency predicted by the conduction of the response to the laser from the periphery to the thalamus. We have carried out recordings of the responses of human thalamic neurons in the principal sensory nucleus (ventral caudal, Vc) to painful cutaneous laser stimuli in patients undergoing awake surgery for the treatment of tremor. The results demonstrate the presence of many thalamic neurons that respond to the laser at latencies consistent with conduction of that response via Aδ and C fiber pathways to the thalamus.

MATERIALS AND METHODS

These studies were carried out in seven patients at the Johns Hopkins Hospital (2005-2008) during the physiologic exploration of the thalamus which preceded implantation of deep brain stimulating (DBS) electrodes in Vim for treatment of essential tremor (ET)(Koller et al. 2001). ET is a mono-symptomatic illness characterized by a 4 to 7 Hz postural or action tremor which is often familial, and which can be ameliorated by ingestion of ethanol (Deuschl et al. 1998). In this study, patients were excluded who had neurologic symptoms in addition to the tremor characteristic of ET including: patients with both essential and Parkinson’s tremor, or patients with ET tremor in addition to either torticollis or a cerebellar syndrome (Jankovic 2002). A neurologist specializing in movement disorders confirmed the diagnosis of ET and identified patients to be excluded.

The protocol for these studies was reviewed and approved annually by the Institutional Review Board of the Johns Hopkins University. All patients signed an informed consent document for these
Intraoperative procedures

All patients were taken off tremor medications for 18 hours prior to the procedure. The thalamic exploration was performed as a stereotactic procedure using the Leksell frame. First, the frame coordinates of the anterior (AC) and posterior (PC) commissures were measured by magnetic resonance imaging. These coordinates were then used to estimate the nuclear locations. Physiological corroboration of nuclear location was then performed under local anesthesia (i.e., subject fully conscious) by using single unit recording. Single unit recordings were made using a high impedance platinum microelectrode with an impedance of approximately 0.5 megohms (Neuroguide, Alpha Omega, Nazareth, Israel).

The microelectrode was advanced through a burrhole 2.5 cm off the midline just anterior to the coronal suture. The initial trajectories were always focused on Vc because the response of neurons in this area to somatosensory stimulation provides the most reliable landmark to guide the procedure (Lenz et al. 1995; Garonzik et al. 2002). Therefore, neuronal responses to stimulation confirmed the location of the anterior border of Vc, as determined by the imaging study. Surface electromyograms (EMG) were recorded from flexors and extensors of the wrist and elbow. The signal from a foot pedal was used to indicate the timing of sensory stimuli or verbal commands.

Study of Thalamic Activity:

When a neuron was first isolated, spontaneous activity was recorded briefly. The activity of that neuron was then studied to identify neurons responding to light touch, tapping or pressure to skin...
(cutaneous sensory neurons), or to deep stimuli such as pressure to muscles or ligaments and passive joint movement (deep sensory neurons). These neurons were characterized by a neuronal receptive field (RF). The activity of neurons was also examined as patients carried out movements such as making a fist, flexing or extending the wrist or elbow, pointing, etc. The anterior and inferior borders of Vc were identified by the most anterior and the most inferior deep or cutaneous neuron in the region where the majority of neurons responded to either deep or cutaneous stimuli (Ohara and Lenz 2003; Lee et al. 2005). As in our previous studies of human thalamus, we used the atlas maps and nomenclature described by Hassler (Schaltenbrand and Bailey 1959).

In each patient, the appropriate atlas map was positioned over the operative map of neuronal location to the same scale by fitting the atlas map both to the location of the ACPC line and to the locations of neurons. From these overlaid maps, we estimated the locations of neurons in Vc and adjacent nuclei including: ventral intermediate (Vim) and Vcpo (ventral caudal portae)(Figure 4).

During the laser studies, the patients wore protective glasses and were reclined with their eyes open, quietly wakeful. Cutaneous heat stimulation was delivered by Thulium YAG laser (Neurotest, Wavelight, Starnberg, Germany). Stimuli were applied contralateral to side of the thalamic recordings on the neck, mandible/cheek or forearm depending upon the neuronal RF. To avoid sensitization or fatigue of primary nociceptive afferents (Meyer et al. 1994) the laser beam was moved at random to a slightly different position for each stimulus. Laser pulses with different energy levels (300 to 500 mJ) were applied in this way to avoid the risk of burns, since approximately 500 laser pulses were applied in each patient. No burns occurred with this protocol. As the laser stimulus was applied the subject was asked to either count the stimuli (counting) or rest quietly with eyes open (noncounting). A total of 40-80 laser pulses were applied during one run with a pseudorandom inter-pulse interval of 2 sec.
and inter-run interval of 30 sec. After each run the patient was asked to rate the pain stimulus over the run using a VAS intensity scale from 0—no pain, to 10—the most intense pain imaginable.

**Data Collection and Analysis:**

The signals recorded digitally (Neuroguide, Alpha Omega, Nazareth, Israel) during the procedure included: the foot pedal indicating events during the examination, the microelectrode signal, electromyograms (EMGs), and the audio channel describing instructions to the patient, application of stimuli, etc.

Digitally recorded microelectrode signals were analyzed using template-matching of action potential waveforms (Spike2 software, CED, Cambridge, UK). Subsequent analyses of spike characteristics such as the firing rate (FR) and interspike interval duration (ISI), including burst detection, were carried out using MATLAB (Mathworks, Natick, MA). Analysis of bursting was carried out on spontaneous activity with eyes open and with the arm at rest. The absence of tremor was confirmed by visual inspection and by EMG signals.

**Spike Train Characteristics:**

As a bias free measure of neuronal bursting activity, we constructed plots of the ISI before an action potential in a spike train (n) versus the ISI after that action potential (n+1), designated as a n versus n+1 plot (Figure 3). This plot provides a graphical display of the characteristics of the spike train overall as reflected by clusters of points in the plot (Kim et al. 2009). In the plot of n versus n + 1, the cluster at the lower right indicates a long ISI (approximately 200 to 1000ms, see Figure 3, upper left panel) followed by a short ISI (< 6 ms), the defining characteristic of the first spike in an LTS burst. In general, the lower left and upper right clusters indicate the ISIs within and between bursts,
respectively. In this case, clusters at the upper left indicate a short ISI (< approximately 16 ms) followed by a bimodal long ISI (40 to 100 ms, and 300 to 1000 ms), which indicate that the post-burst and intraburst ISIs are bimodal.

Spike trains in the nongrouped (NG) category showed high frequency spontaneous firing during the spike train (Figure 4, bottom left panel) resulting in n versus n+1 plots characterized by a central cluster. This distribution is consistent with the lack of LTS bursts as indicated by the absence of clusters at the four corners of the n versus n+1 plot, particularly the cluster in the lower right (Figure 4, top left panel).

Spike trains in the intermediate (I) category had irregular spike firing, and common LTS bursts (Figure 4, top and bottom right). The clusters in the I category (Figure 3, right top and bottom) were approximately consistent with the lower right and lower left clusters of the G category (Figure 3, left upper). The I category of spike trains also had a central cluster (Figure 3, right upper and lower), similar to that found for the NG category (figure 3 left bottom).

Individual LTS bursts in the spike train were identified by criteria used in studies of awake cynomologous monkeys which have been confirmed by intracellular studies in thalamic slices from the same species (Ramcharan et al. 2000a; Ramcharan et al. 2000b). The criteria (50-6-16) to select these bursts from the spike train were as follows: the inter-spike interval (ISI) preceding the first action potential in the burst had a duration > 50 ms, the ISI following the first action potential in the burst had a duration of < 6 ms, and all following action potentials are considered as part of the burst if their ISI increased by no more than 2 ms for each succeeding action potential, up to a maximum ISI of 16 ms. All bursts meeting these criteria, including bursts composed of two action potentials, were included in the burst rate. We have repeatedly validated, illustrated and published these techniques and these burst criteria in previous studies (Lee et al. 2005; Lenz et al. 1998a; Lenz et al. 1994).
We also measured the primary event rate (PR) which includes all spikes occurring outside bursts plus the first spike in each burst, as defined by the criteria described above. Therefore, the PR is a measure of the rate of spikes occurring between bursts (McCarley et al. 1983; Cox and Lewis 1966). We calculated burst rate/primary event rate (BR/PR) as an indicator of the relationship between burst firing and ongoing neuronal firing, reflected by the PR. If the difference in burst rates between two neuron types is lost when the ratio of BR/PR is calculated, then the difference in bursting is dependent on the PR, and perhaps on the degree of membrane depolarization. A measure of neuronal firing which is related to the BR/PR is the percentage of action potentials occurring in bursts (BP). The number of action potentials occurring in bursts included bursts of two action potentials.

RESULTS

Microelectrode and LFP recordings were made in seven patients with ET including three women (72, 69, and 82 years old; patient numbers 548, 589, and 591) and four men (62, 66, 71, and 62 years old; 542, 582, 570, and 587). All subjects were right handed and the left thalamus was explored in all subjects; neurons were recorded along 1 trajectory in four cases and 2 trajectories in 2 (582 and 591). A total of 27 neurons were studied, of which 22 were studied as the patient counted the stimuli. The remaining 5 neurons were studied as the patient rested quietly with eyes open. Stimulation-related changes in FRs were observed in 12 neurons.

Neuronal response to the laser stimulus.

Figure 1 shows the results for a neuron with a response to both light manual touch of the skin in the RF indicated and to laser stimulation in the chin and upper neck. The histogram in Figure 1B shows an early and a late response to the laser stimulation. The significance of the early peak was
established by determining the likelihood that a given response (number and height of bins) was significantly different from prestimulus activity. The onset of a peak was defined to be the first bin ≥ the mean plus 3SD level before the maximum. The end of a peak was the first bin ≤ the mean plus 3SD level after the maximum, and the duration was the difference between these two points in time. The two bins in the first peak in Figure 1B both had a height of greater than mean plus 3SD of baseline activity. The likelihood that any single bin would be this high or higher was P=0.00135. Therefore, the chance of two adjacent bins ≥ mean plus 3SD among fifty post-stimulus bins was unlikely at random ((P=0.0000836, Binomial distribution). Therefore, this analysis demonstrates that the neuron in figure 1 had a significant early latency change in firing in the post-stimulus period with respect to the baseline period, and so was designated as a laser responsive neuron.

In addition to the neuron in Figure 1 we identified three laser responsive neurons with RFs on the face. The neuron in Figure 1 had a short latency peak at 40 ms and a duration of 40ms (Figure 1), and a later peak of activity at 210ms with a duration of 100ms. Among two other laser responsive neurons with facial RFs, the latency (duration) of peaks of activity occurred at 212ms (50) and 280ms (50). Therefore, the latency among late peaks for neurons with facial RFs was estimated to be 231 ± 43ms.

Among nine laser responsive neurons with RFs on the upper extremity, three had a short latency peaks at 70ms (40), 60 (25), and 70 (40)(Figure 2B). One of these three had a later peak at 390 (20ms). The other neurons with upper extremity (UE) RFs had peaks of 375 (75), 200 (50), 325 (50), 225 (60), 280 (80), and 313 (75). The onset of the earliest peak among neurons with facial RFs
was shorter (40ms) than that for the three neurons with upper extremity RFs (67 +/- 6ms) by over 5SD as estimated from the UE statistic; this difference could not be tested statistically.

The later peak for neurons with UE RFs occurred at 301 +/- 71 ms, much higher than the onsets for neurons with facial RFs (231 ± 43ms). Durations were not significantly different between neurons with RFs on the face or UE. Durations were short for early (53 ± 10 ms) and late components (62 ± 22 ms) combined across neurons with face and UE RFs. These timing variables may be related to the conduction velocities, and to the somatotopic and modality specific arrangement of afferent pathways to the thalamus.

Neuronal spike train characteristics

The firing pattern of these neurons was then examined in terms of the G, NG and I categories (see Methods: Neuronal spike train characteristics)(Kim et al. 2009). Statistical independence of neuronal measures of spike train category and of laser responsiveness can be questioned on the basis of the small number of neurons and subjects. Although these same limitations apply to many studies of nociceptive responsive cells in the primate forebrain (Perl and Whitlock 1961; Casey 1966; Apkarian and Shi 1994; Kenshalo Jr. et al. 1980; Bushnell et al. 1993; Lee et al. 1999) we have presented the present results in descriptive terms and not in statistical terms. The majority of laser responsive neurons had I category spike trains (11/12) while none of these neurons were included in the NG category spike trains (0/7). The single G category spike train was found in a laser responsive neuron. In summary, laser responsive neurons commonly had spike trains in the I category, while laser non-responsive neurons exclusively had spike trains in the NG category.

The proportion of NG category spike trains was similar between the counting (6/22) versus the non-counting condition (1/5). The proportion of I category spike trains in the counting condition
The number of laser responsive neurons was similar between neurons studied during the counting (10/22) versus the non-counting condition (2/5). Thus, laser responsive neurons were more strongly associated with spike train category than with the counting versus the non-counting condition.

The only neuronal spike train in the G category (Figure 3, upper left, 582-25.132) had a significant increase in the rate of LTS in response to the laser (Figure 2A). The histogram of a neuron in the I category (Figure 3B, upper right, 591-26-742) had a short spike histogram peak of mean plus 4 SD (350ms bin, Figure 2B upper) and a LTS burst histogram peak at 6 SD (200ms bin), respectively. The significance of these results is demonstrated by the probability that a single bin of greater than mean plus 4SD (P=0.000032) will occur once among 20 poststimulus bins (Figure 2B, P=0.000032 x 20 or 0.00064). In summary, the response of neuronal spike trains in the I and G categories to the laser can include increases in the spike rate, the burst rate, or both.

The parameters of individual bursts were then determined by the criteria for identification of bursts as described in the Methods (Neuronal spike train characteristics). Among neurons having the I pattern, the laser responsive neurons showed a strong trend towards lower FRs (4.2, 1.9- 6.0: median, 10th and 90th confidence intervals) versus laser non-responsive neurons (7.9, 2.3 -18.5: P=0.059, Mann-Whitney U test). Similarly, the principle event rate was significantly lower among laser responsive neurons versus laser non-responsive neurons (4.9, 1.9-6.0 versus 7.9, 2.6-17.9, P=0.03). Burst rates were not significantly different between the laser responsive neurons versus non-responsive neurons (0.22, 0.03-0.42 versus 0.12, 0.0-0.89, P=0.85). None of the other variables in this analysis were significantly different between neurons responding to the laser versus those not responding.
These data also suggest that the variability of the rate data was much less for laser responsive neurons versus laser non-responsive neurons including FR (R 7.8 vs NR 15.8: 90% CI minus 10% CI), primary rate (7.3 vs 15.8), and burst rate (0.68 vs 1.2). Overall, these results suggest that the neurons which respond to the laser have lower, less variable firing rates than those which do not respond.

Neuronal location

The number of neurons responding to the laser stimulus was higher among those located below the AC-PC line (6/7) than among those above (7/20). This result and examination of Figure 4 suggest that the neurons responding to the laser were preferentially located at or below the inferior border of Vc. All NG spike trains were recorded from neurons located in Vc above the AC-PC line (7/20) and none were located below (0/7). The number of neuronal spike trains in the I category was similar between neurons located above the AC-PC line (13/20) versus those below the ACPC line (6/7). In summary NG neurons were more commonly located above the ACPC line, while all laser responsive neurons were located below.

DISCUSSION

The onset latencies of laser stimulation are very short. This may be due to the use of the thulium Yag laser that activates peripheral receptors without the receptor activation delay that characterizes carbon dioxide lasers (Bromm and Treede 1984). The conduction velocity for the early peak is the difference in the length of the pathway from the forearm minus the face (0.6 minus 0.1 = 0.5m) divided by the difference in latency for early latency activity between neurons with upper
extremity (UE) minus facial RFs (67 minus 40ms = 27ms). This quotient yields a peripheral conduction velocity of 18m/sec which is within the reported range for nociceptive A\textsubscript{δ} fibers activated by the cutaneous laser in primates (Kakigi et al. 1991; Treede et al. 1995; Magerl et al. 1999).

For the late latency peak the difference in the pathway (0.5m) divided by the difference in the latencies between neurons with UE minus those with facial RFs (356 minus 231 = 125ms) leads to an estimated peripheral conduction velocity of 4m/sec, which is within the range of prior estimates of C fiber velocity following activation by a laser (Treede et al. 1995; Magerl et al. 1999). These results suggest that the peaks of neuronal firing are related to inputs from A\textsubscript{δ} and C fiber pathways when activated by a cutaneous laser in humans and monkeys. These responses are also consistent with the published onset (inflection point) of the subdural LEPs in response to laser stimulation of the forearm, which can occur as early as 70ms (Figure 1 in (Lenz et al. 1998c) and (Ohara et al. 2004b)). Finally, they are similar to the reported latency of an early component recorded from the scalp during attention to the laser (Valeriani et al. 2000).

The present conduction velocity measured between the forearm and the face is similar to that for the subdural LEP N2s measured between the hand dorsum and the face (12m/s (Lenz et al. 1998b)). In the same study, the velocity calculated from the subdural LEP P2 is much faster than C fiber conduction velocities. It is more likely that the latency of the P2 peak is the result of cortical processing of an earlier barrage of nociceptive inputs rather than to C fiber related conduction delays (Becker et al. 1993; Kanda et al. 1996; Miltner et al. 1989; Towell and Boyd 1993; Zaslansky et al. 1996; Zaslansky et al. 1995; Siedenberg and Treede 1996).

In the present study, both early and late peaks had short durations in the range of 50ms, particularly in comparison with the duration of the subdural N2 wave which ranges from 100-140ms (Lenz et al. 1998b). This may be consistent with evidence suggesting that there are small anatomic
elements having somatotopic or modality specificity within the human Vc thalamus (Lenz et al. 1988; Patel et al. 2006; Jones et al. 1982). The properties of the elements related to pain and temperature sensation suggest that thalamocortical neurons may receive projections from as few as 1 STT fiber among the small number of STT fibers projecting to the thalamus (Mehler 1962; Brodal 1981). Therefore, the short duration of peaks of laser evoked thalamic neuronal activity may be related to the small amount of jitter produced by the small number of afferent fibers, and to the precision of the laser stimulus.

The location of neurons responsive to the painful laser stimulus is more commonly in and adjacent to Vcpc, corresponding to monkey VPI (Figure 4). Neurons with responses to noxious mechanical stimuli and heat stimuli other than laser are found in human Vcpc (Lee et al. 1999). Similar neuronal responses are found in studies of anesthetized and awake monkeys and suggest that neurons responsive to noxious stimuli are located in VPI and VP (Perl and Whitlock 1961; Casey 1966; Casey and Morrow 1983; Apkarian and Shi 1994). These results are consistent with the location of primate spinothalamic terminations within the core as well as posterior and inferior to the core of Vc ((Willis Jr. et al. 2001; Graziano and Jones 2004) cf (Craig 2004)).

The response of neurons in these nuclei to painful and nonpainful thermal and mechanical stimuli is characterized by low threshold spike (LTS) bursts as well as single action potentials (Lee et al. 2005). The behavioral state is well known to influence thalamic LTS bursting activity, so that LTS bursting increases during drowsiness or sleep (Domich et al. 1986; Steriade et al. 1997). In addition LTS bursting can be increased in some kinds of neurological diseases, such as absence epilepsy (Coulter et al. 1989; Linas et al. 1999). There is now evidence that thalamic LTS bursting can be related to sensory stimulation, or to motor and cognitive events (Martinez-Conde et al. 2002; Ramcharan et al. 2000a; Lee et al. 2005). In the present data, spike trains of laser responsive
neurons were commonly in the I category, but were not observed among neuronal spike trains in the NG category. I category spike trains have frequent LTS bursts, and a much greater tendency to change category spontaneously, or at the transition between spontaneous eyes open to mental arithmetic (Kim et al. 2009). In view of the response of neurons with spike trains in the I category to the painful laser stimulus, the present results suggest that I category spike trains may mediate changes in endogenous or cognitive pain related behavior.
**Figure Legends**

**Figure 1.** Activity of a neuron (591.1.27.247) in Vc responding to the painful laser stimulus. A, The RF for this neuron is shown in the left upper panel, and the shape of action potentials recorded from this neuron are shown in the left lower panel. The right panels show the location of the neuron (arrow) relative to the positions of the trajectories, nuclear boundaries, and other recorded neurons. Neurons indicated by a triangle had deep RFs, while those indicated by circles had cutaneous RFs. The orientation of the anterior commissure posterior-commissure (AC-PC) line is indicated by the dark horizontal line; the right end of this line indicates the posterior commissure (PC). The trajectory is shown by the oblique line (left-anterior, up-dorsal). The right panel shows the nuclear locations as approximated from the position of the AC-PC along which the trajectory has been shifted until the most anterior neuron with a cutaneous RF is aligned with the anterior border of Vc.

B. Raster and histogram of the response to the laser pulses delivered at time zero. The scales for the axes for all recordings and histograms are indicated in each panel. The solid horizontal line is the mean and the dashed horizontal line is mean plus 3SD.

**Figure 2.** Raster and histograms for the responses of two thalamic neurons to the laser stimulus. A. recordings from a neuron (582.1.25.132) with a significant response in the histogram (upper panel) of spikes but not in the histogram of LTS bursts (lower panel). B. recordings from a neuron (591.1.26.742) with responses shown in both the histograms for all action potentials (upper panel) and for LTS bursts (lower panel), composed of one bin at mean plus 4 and 6 SD respectively. The solid horizontal line is mean prestimulus level of activity, and the two dashed lines are mean plus 2 and 4 SD. Other conventions are as in Figure 1.
**Figure 3. Examples of N versus N+1 plots.** Plots for spike trains in the Grouped (G) spike train category (left top), the Non-grouped (NG) category (left bottom), and intermediate (I) category (right top and bottom).

**Figure 4. Locations of neurons responding or not responding to the laser stimulus.** The locations of these neurons are shown relative to the map of thalamus, estimated as described in the methods.
Reference List


A

B

Spikes per bin

Vop, Vim, Vc

1 mm

P

C

0.2 ms

1 mm

PC

0-0.5 0.5 1.0 (s)

15 10 5 0

VimVop Vc

PC

1 mm

0.2 ms

Spikes per bin

0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80

-0.5 0 0.5 1.0 (s)