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Laterodorsal Nucleus of the Thalamus: A Processor of Somatosensory Inputs

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Abstract

The laterodorsal (LD) nucleus of the thalamus has been considered a “higher order” nucleus that provides inputs to limbic cortical areas. Although its functions are largely unknown, it is often considered to be involved in spatial learning and memory. Here we provide evidence that LD is part of a hitherto unknown pathway for processing somatosensory information. Juxtacellular and extracellular recordings from LD neurons reveal that they respond to vibrissa stimulation with short latency (median = 7 ms) and large magnitude responses (median = 1.2 spikes/stimulus). Most neurons (62%) had large receptive fields, responding to six and more individual vibrissae. Electrical stimulation of the trigeminal nucleus interpolaris (SpVi) evoked short latency responses (median = 3.8 ms) in vibrissa-responsive LD neurons. Labeling produced by anterograde and retrograde neuroanatomical tracers confirmed that LD neurons receive direct inputs from SpVi. Electrophysiological and neuroanatomical analyses revealed also that LD projects upon the cingulate and retrosplenial cortex, but has only sparse projections to the barrel cortex. These findings suggest that LD is part of a novel processing stream involved in spatial orientation and learning related to somatosensory cues.

Keywords

vibrissae; trigeminal; barrel cortex; limbic cortex; spatial orientation; rat

Sensory information in any given modality is processed along multiple parallel streams. For example, at each level of the somatosensory system there are several groups of spatially segregated neurons that share physiological and anatomical properties and that relay information upstream to similarly segregated neuronal populations (see Dykes, 1983). Parallel processing of somatosensory information has been studied extensively in the rodent trigeminal vibrissae-to-cortex system, where the anatomical correlates of neuronal groupings are most evident (reviewed by Woolsey, 1996). Here, stimuli are transduced by mechanoreceptors associated with the vibrissae and relayed along trigemino-tectal and trigemino-thalamic pathways (Cohen and Castro-Alamancos, 2007; Hemelt and Keller, 2007).

Most studies have focused on the trigemino-thalamic pathway and its role in relaying vibrissal inputs to the neocortex. Three parallel trigemino-thalamic pathways have been identified (reviewed by Pierret et al., 2000; Deschenes et al., 2005; Yu et al., 2006): 1) A *lemniscal* pathway that arises from the principal trigeminal nucleus (PrV) and relays information through the ventroposterior medial (VPM) thalamic nucleus to the primary somatosensory cortex (SI,

barrel cortex); 2) A *paralemniscal* pathway from the trigeminal nucleus interpolaris (SpVi), through the posterior medial thalamic nucleus (POm) to various cortical areas including SI; 3) An *extralemniscal* pathway from SpVi through the ventrolateral segment of VPM (VPMvl) to SII and to the dysgranular zone of the barrel cortex. Although these pathways are not strictly parallel—there exist interactions among their components (e.g., Timofeeva et al., 2005)—accumulating evidence suggests that each of these pathways is involved in unique aspects of sensorimotor processing (Derdikman et al., 2006; Yu et al., 2006).

In the course of studying response properties of VPM and POm neurons, we fortuitously discovered a fourth trigemino-thalamic pathway. This pathway originates primarily from SpVi and processes vibrissae-related information through the laterodorsal nucleus of the thalamus (LD). Because LD neurons project preferentially to limbic cortical areas and to the posterior parietal cortex (Jones and Leavitt, 1974; Robertson, 1977; Spiro et al., 1980; Robertson and Kaitz, 1981; Sripanidkulchai and Wyss, 1986; Thompson and Robertson, 1987a; Schmähmann and Pandya, 1990; van Groen and Wyss, 1992; Reep et al., 1994; Shibata, 2000), we propose that this LD pathway may be involved in spatial orientation and learning involving vibrissae information.

MATERIALS AND METHODS

Surgical procedures

Experiments were conducted using 24 female Sprague–Dawley rats weighing 220–280 g. Urethane anesthesia (1.5 g/kg) was used and the animals were maintained at stages III/3–4 (Friedberg et al., 1999) by monitoring electro-corticograms and by testing reflexes to pinch and cornea stimulation. Supplemental doses of urethane (0.15 g/kg) were given if necessary. Body temperature was maintained at 37°C with a servo-controlled heating blanket. All procedures strictly adhered to institutional and federal guidelines.

Juxtacellular recording and labeling

To reliably identify the location of recorded units, some of the vibrissa responsive neurons were recorded and labeled juxtacellularly with biocytin (Pinault, 1996). Briefly, a glass micropipette (tip diameter 2 μ m, impedance 15–25 M Ω) was filled with 2% biocytin (Invitrogen, Eugene, OR) in artificial cerebrospinal fluid and was connected to an intracellular amplifier (Neuro Data IR183A, Cygnus Technology, Delaware Water Gap, PA). The micropipette was advanced slowly to depth of 4–4.5 mm from the pial surface, while stimulating the vibrissae (see below). When a responsive unit was identified, positive current pulses of 1–10 nA, 200 ms, were applied through the amplifier's bridge circuit, while slowly advancing the pipette until a "loose seal" configuration was obtained. The neuron's responses to vibrissa stimuli were then recorded. After recording, positive current pulses (1–10 nA, 200 ms, 2 Hz) producing modulation in the neuron's firing rate were delivered for 10–30 minutes to obtain reliable labeling. At least 2 hours later the animal was deeply anesthetized with pentobarbital (60 mg/kg) and perfused with 0.1 M phosphate buffer (PB, pH 7.4) followed by 4% buffered paraformaldehyde and 0.5% of glutaraldehyde. The fixed brains were sectioned at 60 μ m in the coronal plane and standard procedures were performed to visualize the biocytin-filled neurons (Gottlieb and Keller, 1997).

Extracellular recordings

Extracellular recordings of single unit action potentials were made using quartz-insulated platinum-tungsten electrodes (filament stock diameter 80 μ m). Recording electrodes had impedance between 2–4 M Ω . Spike data from each neuron were acquired using the Plexon (Dallas, TX) data acquisition system and sampled at 40 kHz.

At the end of each experiment, electrolytic lesions (5–10 μ A, 20 seconds) were made to confirm the recording sites. Then animals were deeply anesthetized with sodium pentobarbital (60 mg/kg) and perfused with 0.1 M PB followed by 4% buffered (pH 7.4) paraformaldehyde. The fixed brains were sectioned at 80 μ m in the coronal plane and recording sites were identified in Nissl-stained sections.

Vibrissa stimulation

We used three different methods for vibrissa stimulation. All recorded LD neurons were tested for responses to vibrissae displacements with air-puffs delivered with Pi-cospritzer (General Valve, Fairfield, NJ) through a plastic tube (0.5 mm diameter) with a pressure of 60 psi. The frequency of stimulation was 0.5 Hz and stimulus duration was 50 ms. Several vibrissae were deflected simultaneously with this approach and the position of the tube was adjusted for each cell to elicit the shortest latency and largest magnitude response. We regularly calibrated the stimulator and determined the time lag between the trigger and the arrival of the air-puff at the vibrissae. Response latencies were corrected for this delay.

A home-made speaker-based stimulator was used for single vibrissa deflections (as described by Miasnikov and Dykes, 2000). An individual vibrissa was inserted into a glass micropipette (1 mm diameter) that was attached to the membrane of a miniature speaker. Application of current pulses to the speaker membrane deflected the micropipette $\approx$ 0.5 mm in the anterior–posterior direction. Stimulation frequency was 0.5 Hz and stimulus duration was 50 ms.

To determine the receptive field size of individual LD neurons manual stimulation of individual vibrissae was performed with cotton swabs.

Electrical stimulation

To study interactions between LD and the trigeminal nucleus interpolaris (SpVi) or the somatosensory (barrel) cortex (SI) we used electrical microstimulation. Placement of stimulating electrodes was guided by recordings of multiunit responses to manual stimulation of the vibrissae. Cortical stimulating electrodes were targeted to layers IV/V of the barrel cortex. SpVi was stimulated with a monopolar tungsten electrode (tip size 10–15 μ m; 0.1 M Ω) and SI was stimulated with a bipolar tungsten electrode (tip size 10–15 μ m; tip separation 0.5–1 mm; 0.1 M Ω). Stimuli were delivered through a constant-current stimulus isolator (PSIU6, Grass Technologies, Warwick, RI) driven by a pulse generator (S88 Stimulator, Grass Technologies), and consisted of 200- μ s long pulses at 0.03–1 mA. At the end of each experiment we made electrolytic lesions (10 μ A, 20 seconds) to mark the stimulation sites.

Data analysis

Recorded units were sorted offline with Plexon's Offline Sorter. Time stamps from each well-isolated unit were exported to Matlab (MathWorks, Natick, MA) and analyzed with custom-written routines. Peristimulus time histograms (PSTHs) were plotted with a 1-ms bin size for responses to vibrissa stimulation and 0.5-ms bin size for responses to electrical stimulation. Onset latency was defined as the first two consecutive bins of stimulus-evoked spikes that significantly exceeded (99% confidence interval) spontaneous activity (100-ms period preceding the stimuli). Response offset was defined as three consecutive bins that did not significantly exceed spontaneous activity. Magnitudes of responses were calculated as number of spikes during significant response duration. Data are presented as median, mean $\pm$ standard deviation, and range.

Anterograde and retrograde tracing

Experiments were conducted using female Sprague–Dawley rats weighing 220–280 g. All survival surgery was performed under sterile conditions and under ketamine (100 mg/kg) and xylazine (8 mg/kg) anesthesia, maintaining body temperature at 37°C using a thermostatically regulated heating pad. We placed the rats in a stereotaxic device and created a craniotomy over the barrel cortex, the LD nucleus, or SpVi. Tracer injections were guided by stereological coordinates and by recording multiunit responses to vibrissae stimuli. We anterogradely labeled efferents from SpVi with *Phaseolus vulgaris* leucoagglutinin (PHA-L; 3.5% in PB; Vector Laboratories, Burlingame, CA) ejected through a glass pipette (50 µm tip diameter) by applying positive current pulses (7 µA, 7 seconds on/off, 40 minutes) supplied by a constant current stimulus isolator (Grass Product Group). We retrogradely labeled afferents to LD, and, in separate experiments, afferents to SI barrel cortex with FluoroGold (2% in water, Fluorochrome, Denver, CO) ejected through a glass pipette (20 µm tip diameter) connected to a 0.5 µL Hamilton syringe (Hamilton, Reno, NV). Injection volumes were 40–60 µL for LD, and 100–200 µL for barrel cortex.

After a 1-week postsurgery survival period the animals were deeply anesthetized and perfused with aldehydes, as described above. We cut coronal sections (50 µm thick) and processed them for immunocytochemistry with antibodies to PHA-L (1:5,000, Vector Labs) or FluoroGold (1:50,000, Fluorochrome) using the ABC-DAB procedure, as in our previous studies (Keller et al., 1985). We then mounted the sections on gelatin-coated slides and counterstained them with Neutral Red. The slides were then dehydrated, defatted, and coverslipped.

Digital images were obtained with a Microfire charge coupled device (Optronics, Goleta, CA) mounted on an Olympus (Japan) BX50 microscope. Images were stored and processed on a Macintosh computer using Photoshop (San Jose, CA). Image manipulations were restricted to resizing, cropping, and linear adjustments.

RESULTS

Response properties of LD neurons

We recorded from 36 well-isolated single units that responded to vibrissa stimulation. The borders of the LD are readily identified in Nissl-stained sections (Fig. 1). It lies ventral to the hippocampus and its fimbria and immediately dorsal to the ventroposterior and posteromedial thalamic nuclei. Whereas the core of LD contains a high density of relatively small cells, its ventral and ventromedial borders are ringed by a cell-sparse zone, demarcating its border with adjacent thalamic nuclei (arrowheads in Fig. 1A).

We confirmed that all cells were in LD by juxtacellular labeling with biocytin or by producing electrolytic lesions (see Materials and Methods). We excluded from analysis recordings from sites in or immediately adjacent to other thalamic nuclei. Figure 1B illustrates the locations of all recorded cells. They were dispersed throughout the LD nucleus, including its medial and lateral parts. However, we found that vibrissa responsive cells were preferentially arranged in 100–300 µm long clusters along the axis of penetration. Examples of juxtacellularly labeled neurons are depicted in Figure 3B, and an example of a lesion site is shown in Figure 2A.

We tested the responses of LD neurons to vibrissae stimulation with the use of air puffs to displace multiple vibrissae. We also tested responses to displacements of individual vibrissae with the use of a speaker-based stimulator (see Materials and Methods). Typical responses of a LD neuron are shown in Figure 2. When we stimulated multiple vibrissae this cell had a robust (2.7 ± 0.9 spikes/stimulus) and short latency response (7 ms; Fig. 2B). This cell responded robustly also to stimulation of six individual vibrissae (Fig. 2C). Responses ranged in magnitude from 1.6 ± 1.2 spikes/stimulus to 0.6 ± 0.7 spikes/stimulus. Analyses of responses

to individual vibrissae show that the magnitude of responses to vibrissa A1, A2, and A3 were statistically indistinguishable, but significantly larger than responses to the other vibrissae tested (ANOVA followed by Newman-Keuls post hoc test, $P < 0.05$). Response latencies to all vibrissae were similar (5–6 ms), except for responses to A4 (8 ms) that also evoked responses with the lowest response magnitude. Thus, this LD neuron does not have a distinguishable principal whisker, like that of neurons in other stations of the vibrissato-cortex pathway (Simons, 1985). We performed similar analyses of responses from four LD neurons: two of them were similar to the neuron described above in that they had no distinguishable principal whisker. The remaining two responded preferentially to a single vibrissa.

We tested receptive field size by manually deflecting individual vibrissae (see Materials and Methods). Of the 21 cells tested this way, 13 neurons responded to six or more vibrissae, two responded to one or two vibrissae, and the remaining six cells did not respond to single vibrissa deflections, preferring multi-whisker stimulation.

Among all recorded neurons, onset latency to vibrissae stimulation ranged from 5–58 ms, with a median of 7 ms (mean = 12.7 ± 13.7 ; Fig. 3A). Responses were often robust, ranging in magnitude from 0.3–3.69 spikes/stimulus (median = 1.2; mean = 1.4 ± 0.9). Response duration ranged from 12–61 ms (median = 26; mean = 31.4 ± 14.5). Thus, most neurons responded to at least the first half of the 50 ms stimulus.

Most vibrissa-responsive LD neurons (81%; 29/36) were spontaneously active. Spontaneous activity rates ranged from 0–6.2 Hz (median = 0.8; mean = 1.3 ± 1.5).

The heterogeneity of responses is depicted in Figure 3B, which shows examples of three LD neurons that were juxtacellularly labeled. Cell B1 had short latency (6 ms) responses to vibrissae stimulation, and a large receptive field encompassing mystacial vibrissae A1, A2, A3, B1, B2 and two supraorbital vibrissae. Cell B2 also had short latency responses (6 ms), but a small receptive field, consisting only of two vibrissae. Cell B3 displayed low-magnitude and long-latency responses (24 ms), and responded only to co-activation of multiple vibrissae. We found no correlation between the location, morphology, or functional properties of the recorded neurons, suggesting a continuum in the properties of neurons throughout the nucleus.

Inputs from SpVi trigeminal nucleus

The short latency responses to vibrissa stimulation suggest that LD neurons might receive direct inputs from the trigeminal nuclei, the target of vibrissa primary afferents (Arvidsson, 1982). To test this hypothesis we injected, in two animals, a retrograde tracer (FluoroGold) into LD to label LD-projecting neurons in the brainstem nuclei. Retrogradely labeled cells were found mostly in the trigeminal nucleus interpolaris SpVi (Fig. 4A), suggesting that SpVi provides direct vibrissa-related inputs to LD neurons. There were also a small number of labeled cells in the trigeminal nucleus principalis (PrV) and nucleus oralis (SpVo). In addition, we found labeled cells in the medial vestibular nucleus and in the spinal vestibular nucleus, in agreement with previous reports (Doi et al., 1997). To confirm that SpVi innervates LD neurons, we injected an anterograde tracer (PHA-L) in SpVi. As depicted in Figure 4B, PHA-L-labeled axons were found in the ventral part of LD, where they formed large en passant and terminal boutons, whose morphologies resembled those of “driver afferents” described in other thalamic nuclei (Sherman and Guillery, 1998; Guillery et al., 2001). As reported previously (Peschanski et al., 1984; Chiaia et al., 1991), we also found labeled SpVi axons and terminals in VPM and POm.

To determine if these anatomical relationships might account for the short latency responses in LD, we recorded responses of vibrissa-responsive LD neurons to electrical stimulation of SpVi. Figure 4C shows responses recorded from a representative LD neuron. This cell

responded to vibrissae stimulation with a latency of 6 ms (Fig. 4C1), and to electrical stimulation of SpVi with a latency of 3.5 ms (Fig. 4C2). We recorded similar responses to SpVi stimulation in each of eight LD neurons tested. Their response onset latencies ranged from 2.0–14.5 ms (Fig. 4D), with a median of 3.8 ms (mean = 5.3 ± 4.0). Thus, both the anatomical and electrophysiological findings are consistent with the hypothesis that LD contains neurons whose responses to vibrissae are mediated by direct inputs from SpVi.

Connections with barrel cortex

Vibrissa-related information is relayed directly to the vibrissa representation in the somatosensory cortex—the “barrel cortex” (Woolsey and Van der Loos, 1970)—from both VPM and POM (Keller et al., 1985; Lu and Lin, 1993; Bureau et al., 2006), the previously identified thalamic nuclei that process vibrissa information. To determine if LD neurons also project to the barrel cortex we injected PHA-L in LD in two animals. We found no labeled afferents in the barrel cortex, or in the second somatosensory cortex. We did identify dense projections to the cingulate and retrosplenial cortex, consistent with previous reports on targets of LD efferents (Jones and Leavitt, 1974; Spiro et al., 1980; Kaitz and Robertson, 1981; Robertson and Kaitz, 1981; Sripanidkulchai and Wyss, 1986; Thompson and Robertson, 1987^{a,b}; van Groen and Wyss, 1990, 1992). To confirm this finding we injected the retrograde tracer FluoroGold in barrel cortex in two separate animals. Injection sites were 1×0.6 mm in diameter. We found a large number of neurons in both VPM and POM, consistent with the known projections from these nuclei to the barrel cortex (see above). However, we found only very sparse distribution of retrogradely labeled cells in LD. These were preferentially located in the ventral aspect of this nucleus (Fig. 5A).

We also tested the interactions between LD and the barrel cortex with the use of electrical microstimulation and extracellular recordings. We recorded the responses of 10 vibrissa-responsive neurons in LD to electrical stimulation of the barrel cortex (see Materials and Methods). In only two of these neurons, we found antidromic responses to barrel cortex stimulation (Fig. 5B,D, gray bar). A representative neuron is shown in Figure 5B, which depicts its responses to vibrissae stimulation (Fig. 5B1, latency = 5 ms), antidromic responses to stimulation of S1 (Fig. 5B2, latency = 1.7 ms), and collision test (Fig. 5B3, collision interval = 3.4 ms). Thus, both anatomical and physiological data suggest that LD projections to barrel cortex are relatively sparse.

In contrast to the antidromic responses, stimulation of barrel cortex evoked orthodromic responses in 9 of the 10 LD neurons tested. A representative example is shown in Figure 5C. This LD neuron had short latency responses to vibrissae stimulation (Fig. 5C1, 8 ms), and robust responses to barrel cortex stimulation, with a latency of 4.5 ms (Fig. 5C2). As a group, orthodromic responses ranged from 4.5–12 ms (median = 7 ms; mean = 7.2 ± 2.3 ; Fig. 5D). The relatively long latency of these responses may suggest that these orthodromic responses are polysynaptic, reflecting indirect barrel cortex inputs relayed to LD through other cortical and subcortical sites.

DISCUSSION

We demonstrate a hitherto unknown pathway for processing trigeminal somatosensory information. This pathway originates from the SpVi, projects to the LD, and from there targets primarily limbic cortical areas. The pathway conveys to these cortical areas short latency and robust responses to stimulation of multiple mystacial vibrissae. Thus, LD contains a population of vibrissae-responsive neurons whose anatomical location and physiological properties are distinct from those of neurons in adjacent thalamic nuclei.

The discovery of this pathway increases to four the number of somatosensory pathways that relay information from the vibrissae through the thalamus. The other three pathways are the lemniscal (via the VPM), paralemniscal (via the POM), and the extralemniscal pathway (via the VPMvl) (Pierret et al., 2000; Yu et al., 2006).

Trigeminal inputs to LD

Several lines of evidence support the conclusion that direct inputs from the trigeminal nuclei shape vibrissal responses of LD neurons. Anterograde and retrograde neuroanatomical tracing demonstrate direct inputs from these nuclei—primarily SpVi—to LD (Fig. 4A,B). A small number of neurons in PrV and in SpVo also project to LD. Consistent with these direct projections, the response latencies of LD neurons to vibrissal stimulation are relatively short (median = 7 ms; 12 ± 13.7 ms). These latencies are similar to those of neurons in the superior colliculus, whose responses are also thought to be mediated by direct inputs from SpVi (median 6.2 ms; 6.5 ± 0.6 ; Hemelt and Keller, 2007). The response latencies of LD neurons are similar also to those of VPM neurons, which receive direct inputs from PrV (Ito, 1988: median = 7 ms; Diamond et al., 1992: mean = 7 ms; Friedberg et al., 2004: mean = 7.33 ± 0.36 ms).

Electrical stimulation of SpVi evokes reliable, short latency (mean = 5.3 ± 4.0 ms), suprathreshold responses in LD neurons (Fig. 4C,D). This latency range is consistent with monosynaptic inputs from SpVi to LD, as it overlaps with latencies of monosynaptic responses to electrical stimulation of trigeminal inputs to other thalamic nuclei: POM responses to SpVi stimulation (4.9 ± 3.1 ms; Chiaia et al., 1991), and VPM responses to PrV stimulation (4.1 ± 2.8 ms; Chiaia et al., 1991).

Thus, both anatomical and electrophysiological evidence are consistent with the hypothesis that LD contains neurons that are driven to spike threshold by direct inputs from the trigeminal nuclei, primarily SpVi.

The relatively large variance in the response latency of LD neurons reflects the heterogeneity of their response kinetics (Fig. 3). For example, 6 of 36 neurons had response latencies greater than 20 ms, suggesting that they do not receive direct, driving inputs from SpVi. Three of these six neurons were also distinguished from other LD neurons by their low-magnitude responses ($0.34 - 0.86$ spikes/stimulus) and low spontaneous activity ($0 - 0.2$ Hz, mean = 0.07 Hz). In addition, these neurons did not respond to single vibrissa stimulation, but only to multi-vibrissae activation (see Fig. 3B3). The vibrissae responses of these neurons may be mediated by inputs from the superior colliculus (Thompson and Robertson, 1987a; Kolmac et al., 1998), or from higher-order cortical areas such as the posterior parietal cortex or limbic cortex (Kaitz and Robertson, 1981; Yeterian and Pandya, 1985; Thompson and Robertson, 1987a).

Previous studies have suggested that LD may receive visual and somatosensory inputs *indirectly* via the pretectal nuclei, the superior colliculus, or the ventral geniculate nucleus (Robertson et al., 1980, 1983; Thompson and Robertson, 1987b; Kolmac et al., 1998). To our knowledge, this is the first description—in any species—of *direct* trigeminal or any other somatosensory inputs to LD (Jones, 2007; D.N. Pandya and E.H. Yeterian, pers. commun.).

Interactions with the cerebral cortex

Our electrophysiological and neuroanatomical data suggest that there are relatively sparse direct connections between LD and the barrel cortex (Fig. 5; Negyessy et al., 2000). Our data are consistent with previous findings, in several species, demonstrating that LD has reciprocal connections with the limbic cortical areas, including the cingulate, retrosplenial, and subicular cortex (Jones and Leavitt, 1974; Spiro et al., 1980; Kaitz and Robertson, 1981; Robertson and Kaitz, 1981; Sripanidkulchai and Wyss, 1986; Thompson and Robertson, 1987a; van Groen and

Wyss, ¹⁹⁹⁰,1992;Shibata, 2000). There are also reports that LD interacts with the posterior parietal cortex (Robertson, 1977;Yeterian and Pandya, 1985;Schmahmann and Pandya, 1990;Reep et al., 1994). In addition, LD receives inputs from the visual cortex (areas 17 and 18, Thompson and Robertson, 1987a;Negyessy et al., 2000;Shinkai et al., 2005) and the second motor cortex (Shibata and Naito, 2005).

Electrical stimulation of SI revealed relatively long latency orthodromic responses (mean = 7.2 ± 2.3 ms). These latencies are longer than those recorded in POm (Landisman and Connors, 2007: mean = 5.6 ± 3.1 ms) or VPM (Landisman and Connors, 2007: mean = 4.0 ± 1.6 ms; Beierlein et al., 2002: mean = 3.4 ± 0.9 ms) in response to SI stimulation. Since these data were obtained from brain slices of young animals, response latencies in POm and VPM neurons in adult animals are likely even shorter. These findings suggest that the orthodromic responses that we recorded in LD to electrical stimulation of barrel cortex are polysynaptic. They may reflect connections between SI and the retrosplenial cortex, which projects heavily upon LD (see above).

Receptive field structure

Heterogeneity also characterizes the receptive field size of LD neurons. Most LD vibrissa-responsive neurons had large receptive fields, consisting of six or more vibrissae. Thalamic VPMvl neurons, whose response properties are also mediated by inputs from SpVi (Williams et al., 1994; Pierret et al., 2000) also have large receptive fields, consisting of 7.2 ± 2.8 vibrissae (median = 7, H. Bokor and M. Deschenes, pers. commun.). Neurons in the superior colliculus, which is also innervated by SpVi, have similarly large receptive fields (13.8 ± 0.1 vibrissae; Hemelt and Keller, 2007). The receptive field size of neurons in these three structures is comparable to that of SpVi neurons, which have receptive fields consisting of four or more vibrissae (Woolston et al., 1982; Jacquin et al., 1989; Timofeeva et al., 2004), further supporting the conclusion that the responses of LD neurons are shaped by direct SpVi inputs.

In contrast, VPM neurons—innervated primarily by the PrV—typically respond to a smaller number of vibrissae (1.2 ± 0.5 vibrissae: Rhoades et al., 1987; 2.9 ± 0.9 vibrissae: Timofeeva et al., 2005). Two of the LD neurons recorded here (5.6%) had a small receptive field of 1 or 2 vibrissae; the responses of these neurons occurred at short latencies (see Fig. 3B2). It is possible that the receptive fields of these neurons, like those of their counterparts in VPM, are shaped by inputs from PrV.

Finally, a small number of LD neurons (5 of 36, 13.9%) responded only when multiple vibrissae were stimulated (see Fig. 3B). Thus, the response properties of these neurons resemble those of POm neurons, which rarely respond to stimulation of individual vibrissae (Trageser and Keller, 2004;Lavalley et al., 2005;Masri et al., 2006).

In conclusion, LD appears to contain neurons with heterogeneous response properties, resembling those found in the three hitherto described vibrissae-related thalamic nuclei: POm, VPM, and VPMvl.

LD: A first-order or higher-order nucleus?

It is generally accepted that thalamic nuclei can be divided into two classes (see Sherman and Guillery, 2005). First-order nuclei are concerned with relaying to the cortex information from subcortical afferents. Examples include the lateral geniculate nucleus (LGN) in the visual system and the VPM nucleus in the somatosensory system. Higher-order nuclei are concerned with relaying information from one cortical area to another. In the visual system the pulvinar is thought to be involved in this relay. The somatosensory correlate is represented by POm. First- and higher-order nuclei are defined by both morphological and physiological criteria.

Physiologically, first-order nuclei contain neurons whose receptive field properties are determined by ascending inputs. That is, ascending inputs (e.g., from the brainstem) drive these cells to firing threshold. Higher-order nuclei contain neurons whose drivers are descending cortical inputs and whose receptive fields are determined by these descending projections. Morphologically, driver afferents—whether cortical or subcortical—are characterized by thick and highly branched (“type II”) axons with large terminals (Guillery, 1966; Sherman and Guillery, 1998; Guillery et al., 2001)

According to these morphological and physiological criteria, LD might be defined as a first-order nucleus: It receives driving inputs from trigeminal nuclei and is innervated by type II efferents from these nuclei (Fig. 4B). However, a significant minority of its neurons has response properties that resemble those of higher-order POM neurons (see above). Another ambiguity arises from the fact that LD receives inhibitory inputs from the zona incerta (ZI), a structure that is thought to exclusively target higher-order thalamic nuclei (Bartho et al., 2002; Mitrofanis, 2005; Trageser et al., 2006). If the existence of ZI inputs defines higher-order nuclei, LD would appear to violate this rule. This ambiguity might be resolved by expanding the definition of higher-order thalamic nuclei. We and others have recently shown that some POM neurons can function as first-order neurons when the inhibitory inputs from ZI are suppressed (Trageser and Keller, 2004; Lavalley et al., 2005). Thus, LD—and other “higher-order” nuclei—may function also as first-order nuclei during certain behavioral states (Trageser et al., 2006).

Functional role of LD

LD is often described as part of the anterior thalamic nuclear complex (ATN), both because of LD’s anatomical location and because its connections with the neocortex are similar to those of other nuclei in the ATN complex (Robertson and Kaitz, 1981; Jones, 2007). The prevailing theory is that LD, through its interactions with the hippocampus, is involved in learning and memory, particularly of spatial tasks (Shibata, 2000; Shibata and Naito, 2005). Lesions in LD (as well as in other ATN nuclei) result in impairments in spatial memory (Mizumori et al., 1994; Warburton et al., 1997; Wilton et al., 2001; van Groen et al., 2002). That LD is involved in spatial tasks is supported also by the existence in LD of head-directional cells—neurons that fire preferentially when the head is aligned in a particular direction in space (Mizumori and Williams, 1993). The inputs to LD from visually related structures, and the dependence of some head-directional cells on visual inputs, suggests that LD may be involved in spatial navigation using visual inputs (Mizumori and Williams, 1993; van Groen et al., 2002).

Our present data demonstrate the existence of neurons in LD that receive direct inputs from the trigeminal nuclei and respond to vibrissae inputs at short latencies and with large magnitude responses. These findings support the hypothesis (Robertson et al., 1980; Thompson and Robertson, 1987a) that LD is a multisensory nucleus that integrates multimodal information, including trigeminal/somatosensory inputs, for spatial orientation and learning tasks.

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Fig. 1.

A: Photomicrograph of a Nissl-stained coronal section (AP level 2.56 mm) depicting the relationship between the laterodorsal (LD) thalamic nucleus and the adjacent posteromedial (POm) and ventroposterior lateral (VPL) thalamic nuclei. Arrowheads demarcate the cell-sparse region outlining the borders between LD and adjacent thalamic nuclei. **B:** Anatomical location of all recorded cells, indicated by white circles. LD is shaded for emphasis. Anatomical borders for VPM (ventroposterior medial), POm, VA (ventroanterior), VL (ventrolateral), and VPL thalamic nuclei are shown. Line drawings adapted from Paxinos and Watson (1988).

Fig. 2.

Typical responses of an LD neuron. **A:** Nissl-stained coronal section showing an electrolytic lesion (arrow) at a recording site in LD, whose borders are outlined. **B:** Peristimulus time histogram (PSTH) of responses to multi-vibrissae stimulation. **C:** PSTHs to displacements of six individual vibrissae; the identity of the stimulated vibrissa is indicated above each PSTH.

Fig. 3.

Heterogeneity among LD neurons. **A:** Distribution of onset latencies to multi-vibrissae stimulation for all recorded LD cells ($n = 36$). **B:** Three examples of juxtacellularly labeled neurons and their responses to vibrissae stimulation. First column (panels labeled 'a') shows low-power images of Nissl-stained coronal sections containing biocytin-labeled neurons (black arrowheads). LD borders are indicated with white arrowheads. Middle column (panels labeled 'b') depicts the labeled neurons at higher magnification. Right column (panels labeled 'c') shows PSTHs computed from the response of the labeled cells to vibrissae stimulation. Stimulation of individual vibrissae evoked large magnitude and short latency responses in cells B1 and B2. Receptive field size consisted of seven vibrissae for B1 and two vibrissae for B2. Cell B3 responded only to simultaneous deflection of multiple vibrissae and its responses were of low magnitude and long latency.

Fig. 4.

Anatomical and electrophysiological evidence for direct inputs to LD from SpVi. **A1**: Nissl-stained horizontal section through the brainstem, depicting retrogradely labeled cells in SpVi following FluoroGold injection into LD. Right panel (**A2**) is a higher magnification of the image on the left; asterisk serves as a fiduciary point. **B1**: Nissl-stained coronal section depicting anterogradely labeled axons within LD, VPM, and POM thalamic nuclei, following PHA-L injection into SpVi. In the right panel (**B2**) a higher-magnification of the image on the left is shown. Arrows point to labeled axons and terminals. **C**: PSTH of responses of an LD neuron to multi-vibrissae stimulation (**C1**) and electrical stimulation of SpVi (**C2**). **D**: Distribution of latencies to electrical stimulation of SpVi.

Fig. 5.

Connections with barrel cortex. **A1**: Confocal microscope image showing sparse distribution of retrogradely labeled cells in LD following FluoroGold injection in SI. Note dense labeling in VPM. Right panel (A2) is a higher magnification of the image on the left. Labeled neurons in LD are indicated by arrows. **B**: PSTHs of responses of an LD neuron to vibrissae stimulation (B1), and electrical stimulation of barrel cortex (B2,3). An antidromic response (arrow, B2) is evoked when SI is stimulated 6.2 ms before a spontaneous spike occurs. When the collision interval is reduced to 3.4 ms the antidromic spike is abolished (B3). **C**: PSTHs computed from a different LD neuron in response to vibrissae stimulation (C1), and SI stimulation (C2). The variable latency of the response to electrical stimulation suggests that it is evoked

orthodromically. **D:** Distribution of latencies in response to stimulation of SI. Only two cells (gray bar) responded antidromically, as determined by a collision test.