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Different Topography of the Reticulothalmic Inputs to First- and Higher-Order Somatosensory Thalamic Relays Revealed Using Photostimulation

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Lam YW, Sherman SM. Different topography of the reticulothalmic inputs to first- and higher-order somatosensory thalamic relays revealed using photostimulation. *J Neurophysiol* 98: 2903–2909, 2007. First published September 19, 2007; doi:10.1152/jn.00782.2007. The thalamic reticular nucleus is a layer of GABAergic neurons that occupy a strategic position between the thalamus and cortex. Here we used laser scanning photostimulation to compare in young mice (9–12 days old) the organization of the reticular inputs to first- and higher-order somatosensory relays, namely, the ventral posterior lateral nucleus and posterior nucleus, respectively. The reticulothalamic input footprints to the ventral posterior lateral nucleus neurons consisted of small, single, topographically organized elliptical regions in a tier away from the reticulothalamic border. In contrast, those to the posterior nucleus were complicated and varied considerably among neurons: although almost all contained a single elliptical region near the reticulothalamic border, in most cases, they consisted of additional discontinuous regions or relatively diffuse regions throughout the thickness of the thalamic reticular nucleus. Our results suggest two sources of reticular inputs to the posterior nucleus neurons: one that is relatively topographic from regions near the reticulothalamic border and one that is relatively diffuse and convergent from most or all of the thickness of the thalamic reticular nucleus. We propose that the more topographic reticular input is the basis of local inhibition seen in posterior nucleus neurons and that the more diffuse and convergent input may represent circuitry through which the ventral posterior lateral and posterior nuclei interact.

INTRODUCTION

The thalamic reticular nucleus is a thin layer of GABAergic cells adjacent to the relay nuclei of the (dorsal) thalamus. Most axons connecting the thalamus and cortex in either direction pass through the thalamic reticular nucleus and innervate neurons there with collaterals; reticular neurons, in turn, provide strong inhibition to thalamic relay cells. Due to its strategic location between the thalamus and cortex, the thalamic reticular nucleus is often suggested as a key player in regulating the relay of information through the thalamus to cortex (Crick 1984; Guillery and Sherman 2002; Guillery et al. 1998; McAlonan et al. 2006; Pinault 2004; Sherman and Koch 1986; Yingling and Skinner 2007). Understanding the functional organization of inputs from the thalamic reticular nucleus to thalamic relay nuclei, and how it varies among these nuclei, is of obvious importance.

Thalamic nuclei have been classified into first- and higher-order relays: the first-order relays receive their driver inputs, which represent the main information to be relayed, from subcortical sources, whereas the higher-order relays receive their driver inputs from layer 5 of the cortex as part of a cortico-thalamo-cortical route (Guillery and Sherman 2002). The somatosensory thalamic examples of these different relays are the ventral posterior (lateral or medial) nucleus (1st order) and much or all of the posterior nucleus (higher order), and these are the subjects of the present investigation.

Differences in organization between first- and higher-order relays are beginning to emerge (see DISCUSSION) (Sherman and Guillery 2006). One difference is suggested by prior retrograde tracing studies in cats and rats: the higher-order posterior nucleus was reported to have a more diffusely organized reticulothalamic input compared with the first-order ventral posterior nucleus (Crabtree 1996; Crabtree et al. 1998). Labeling of a large number (>100) of cells juxtacellularly in the somatosensory and other sections of the thalamic reticular nucleus of rats, however, has led to a somewhat different conclusion, namely, that the axonal arbors of reticular neurons are mostly circumscribed, quite topographically organized and usually contained within a single thalamic nucleus (Pinault and Deschênes 1998; Pinault et al. 1995).

The possible difference in the functional organizations of the reticulothalamic inputs between first- and higher-order relays is important because it indicates different underlying regulatory mechanisms of information processing for these nuclei. Therefore we sought to understand and compare the topography of the first- and higher-order reticulothalamic pathway by using laser-scanning photostimulation (Lam and Sherman 2005; Lam et al. 2006; Shepherd et al. 2003) to map the reticular inputs to the ventral posterior lateral nucleus (1st order) and posterior nucleus (higher order) in a mouse somatosensory thalamocortical slice preparation (Agmon and Connors 1991; Reichova and Sherman 2004). We found that, although the reticular inputs to the ventral posterior lateral nucleus were topographic and specific, the inputs to the posterior nucleus neurons were more variable: some were relatively topographic, but others were diffuse, often complex in organization, and varied from relay neuron to relay neuron.

METHODS

Preparation of brain slices

All animal procedures followed the animal care guidelines of the University of Chicago. All experiments were performed on thalamocortical slices taken from young BALB/c mice (Harlan, 9–12 days postnatal). To obtain the slices, each animal was deeply anesthe-

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tized by inhalation of isoflurane, and its brain was quickly removed and chilled in ice-cold artificial cerebrospinal fluid (ACSF), which contained (in mM) 125 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 25 $NaHCO_3$, and 25 glucose. Tissue slices (500 μm) were cut using a vibrating tissue slicer in the plane appropriate for an intact thalamocortical slice (Agmon and Connors 1991; Reichova and Sherman 2004), transferred to a holding chamber containing oxygenated ACSF, and incubated at 30°C for ≥1 h prior to recording.

Physiological recording

Whole cell recordings were performed using a visualized slice setup (Cox and Sherman 2000; Lam et al. 2005). Recording pipettes were pulled from borosilicate glass capillaries and had tip resistance of 4–8 MΩ when filled with solution (termed hereafter the pipette solution), which contained (in mM) 117 Cs-gluconate, 13 CsCl, 2 $MgCl_2$, 10 HEPES, 2 Na_2-ATP, and 0.3 Na-GTP. We typically recorded in voltage-clamp mode and maintained the cell at 0 mV holding potential for easy detection of inhibitory postsynaptic currents (IPSCs). The K^+ channel blocker, Cs^+, was used in the pipette solution to suppress I_{K_leak} and help hold the cell at 0 mV. The pH of the pipette solution was adjusted to 7.3 with CsOH or gluconic acid, and the osmolality was 280–290 mOsm.

A few threads of nylon filaments, attached to a platinum wire slice holder, were used to secure the slices in the bath during experiment. The distance between these filaments was large, and they were always carefully placed to avoid the area of recording and photostimulation (Fig. 1*B*, *inset*). We recorded from the first- and higher-order somatosensory thalamic relays (respectively, the ventral posterior lateral and the posterior nuclei). The ventral posterior lateral nucleus could be identified in the slices by its dark and fibrous appearance and its proximity to the thalamic reticular nucleus. All of the neurons in this nucleus were recorded within 350 μm of the border with the thalamic reticular nucleus. Neurons of the posterior nucleus were selected from the region lighter in color and medial to the ventral posterior lateral nucleus (i.e., >650 μm from the reticulothalamic border). Often, we simultaneously recorded from two thalamic relay cells using an Axopatch 200B and Axoclamp 2A (Axon Instruments, Foster City, CA). The amplitudes of IPSCs were maximized by holding the cells at 0 mV (Cox and Sherman 2000; Lam et al. 2005). The access resistance of the cells was constantly monitored throughout the recordings (>1 h for most experiments), and recordings were limited to neurons with a stable access of <30 MΩ throughout the experiment.

Photostimulation

We used our previously described methods for photostimulation (Lam and Sherman 2005; Lam et al. 2006). Data acquisition and photostimulation were controlled by a program written in Matlab (MathWorks, Natlick, MA) developed in the laboratory of Karel Svoboda (Shepherd et al. 2003). Nitroindolinyl (NI)-caged glutamate (Canepari et al. 2001) (Sigma-RBI) was added to recirculating ACSF to a concentration of 0.39 mM during recording. Focal photolysis of the caged glutamate was accomplished by a pulsed UV laser (355 nm wavelength, frequency-tripled Nd:YVO4, 100-kHz pulse repetition rate, DPSS Laser, San Jose, CA). Figure 1*A* shows a schematic illustration of the optics: the laser beam was directed into the side port of a double-port tube (U-DPTS) on top of an Olympus microscope (BX50WI) using UV-enhanced aluminum mirrors (Thorlabs, Newton, NJ) and a pair of mirror galvanometers (Cambridge Technology, Cambridge, MA) and then focused onto the brain slice using a low-magnification objective (4 × 0.1 Plan, Olympus). Angles of the galvanometers were computer controlled and determined the position stimulated by the laser. The optics was designed to generate a nearly cylindrical beam in the slice so as to keep the mapping two dimensional (Shepherd et al. 2003). The Q-switch of the laser and a shutter (LS3-ZM2, Vincent Associate, Rochester, NY) controlled the timing of the laser pulse for stimulation. A variable neutral density wheel (Edmund, Barrington, NJ) controlled the power of the laser at different levels during experiments by attenuating the intensity of the laser. A thin microscope coverslip in the laser path reflected a small portion of the laser onto a photodiode. The current output from this photodiode was amplified, acquired by the computer and used to monitor the laser intensity during the experiment. Photodiode output was calibrated to laser power at the back focal plane of the objective when we set up the optical equipment. The laser power was measured using a power meter (Thorlabs).

The standard stimulation pattern for mapping the reticular input consisted of positions arranged in a 16 × 8 or 24 × 8 array, with 50 μm between adjacent rows and columns (Fig. 1*B*, red circles). To avoid receptor desensitization, local caged-glutamate depletion, and excitotoxicity, stimulation of these positions were arranged in a sequence that maximized the distance between consecutive trials. The light stimulus was 2 ms long, which consisted of 200 laser pulses. The time interval between photostimuli was 5 s. The laser power used (measured at the back-focal plane of the objective) ranged from 8 to 40 mW. The transmittance of the objective was ~40% at 355 nm wavelength, so the actual power of the laser reaching the slices was actually less than half of these values. We did not see any change of

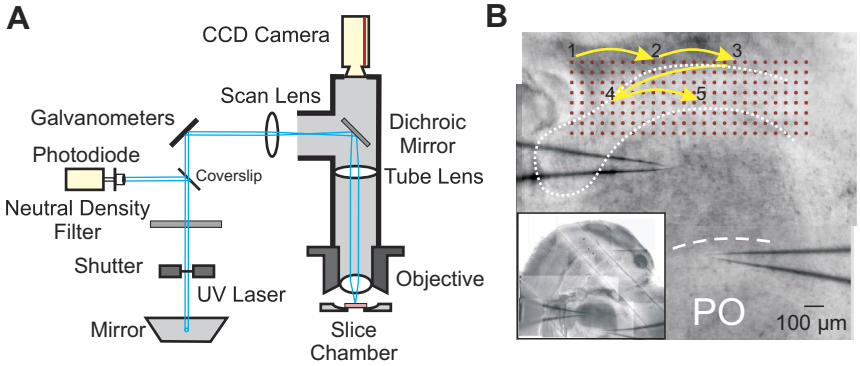

FIG. 1. Method for photostimulation. *A*: schematic diagram of the optics of the laser-scanning photostimulation setup. *B*: photomicrograph of the slice preparation superimposed with a diagram of photostimulation pattern. The photomicrograph shows an experiment in which we simultaneously recorded two neurons, one from each of the ventral posterior lateral and the posterior nucleus. Each red circle in the rectangular array indicates a location at which the laser is focused during the mapping trials. The spot locations were stimulated in a distributed manner, and the positions of the first five trials are indicated (see METHODS for details). The white dotted lines in this and other photomicrographs outline the border of the thalamic reticular nucleus; the white dashed line indicates the border between ventral posterior (lateral and medial) and posterior nuclei. *Inset*: photomicrograph of a somatosensory thalamocortical slice (from another experiment) at a smaller magnification. The semitransparent bands seen in the inset and other photomicrographs are threads used to tether the slice.

the recording quality during experiments that suggested damage from phototoxicity.

Data analysis

Responses were analyzed using programs written in Matlab. For presentation of the data, traces of the 150 ms recording immediately after the photostimulation were superimposed on a photomicrograph of the slice and recording pipettes (see RESULTS). The photomicrographs were taken without using differential infrared-contrast optics, and therefore brain regions including an extensive fiber representation, such as the internal capsule or ventral posterior lateral nucleus, appeared dark due to the high contrast settings of the video camera. The abovementioned traces were arranged into a 16 × 8 or 24 × 8 array and placed where the laser was focused during the stimulation, thus the reticular area that projected to the recorded neuron could be visualized as the region with a large upward current (IPSCs) in these traces.

The areas of the thalamic reticular nucleus in the slices providing afferents to the recorded thalamic relay neuron are referred as their "input fooprints." For better visualization of their shapes and size, the response traces to photostimulation were smoothed (1 ms moving average), and the peak IPSCs of the smoothed traces were plotted in pseudocolor displays.

The footprint shapes and locations were determined by visual inspection. Spontaneous IPSCs were uncommon and the responses to photostimulation could be easily detected by their short latency and the presence of similar IPSC responses in adjacent stimulation locations. For quantitative comparison, the footprint areas were estimated by counting the total number of traces that have peak IPSCs >100 pA and calculated using the following equation: number of traces × 50 μm × 50 μm.

For consistency and comparison between figures, the same color scale was used in all the pseudocolor plots. The threshold used for area calculations (100 pA) is indicated by a red arrow next to the color bar. Differences between the areas of the footprints of different thalamic cell populations (e.g., the ventral posterior lateral nucleus vs. the posterior nucleus) were compared using the Wilcoxon signed-rank test. Differences of footprint shapes and locations were compared using the χ^2 test.

RESULTS

Reticular input footprints to neurons of the ventral posterior lateral nucleus

As an extension and confirmation of our previous results in rats (Lam and Sherman 2005), we recorded from 14 relay neurons from the ventral posterior lateral nucleus and again found that photostimulation only in a small, elliptical area of the thalamic reticular nucleus elicited IPSCs in these neurons. Figure 2 illustrates a typical example. Recordings of the 150-ms period immediately after the photostimulation are superimposed on a photomicrograph of the slice in which the experiment was performed. The borders of the thalamic reticular nucleus are indicated by a pair of white dotted lines, and the location of the recorded neuron is marked by a red star. The recordings within a rectangular region in which photostimulation successfully elicited IPSCs are enlarged and shown in the top right. The peak IPSCs were also calculated and plotted in a pseudocolor display (bottom right). Other figures below showing responses to photostimulation are organized like Fig. 2.

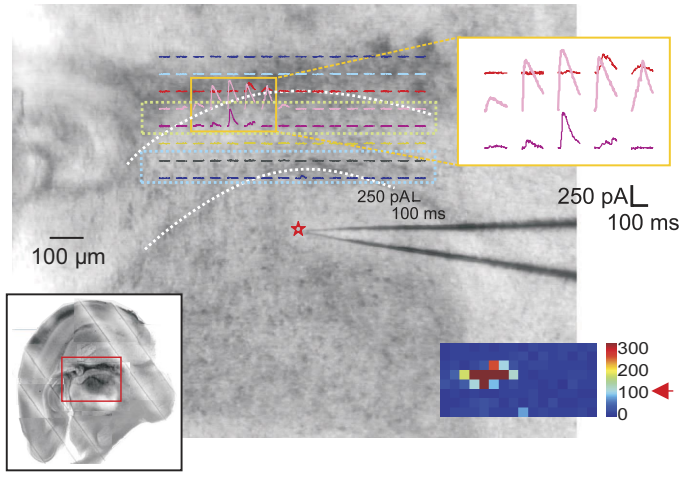

FIG. 2. An example of the reticulothalamic input footprint to ventral posterior lateral nucleus neurons. The red star indicates the location of the neuron. The approximate location where we recorded from is indicated by a red rectangle in the photomicrographs of a thalamocortical slice shown lower left (taken during another experiment). The dotted rectangles in this figure and Figs. 3–5 indicate where we designated as the "inner" (blue) and "outer" (green) tiers of the thalamic reticular nucleus during data analyses. Voltage-clamp responses to photostimulation at 128 locations, arranged in a 16x8 rectangular array, are shown superimposed on a photomicrograph taken during the experiment. The 150-ms recording period immediately after the photostimulation is shown at each stimulation site. The region where inhibitory postsynaptic currents (IPSCs) could be evoked was roughly elliptical and is bounded by a yellow rectangle in the figure. The responses in this region are also shown in a larger scale in the upper right. The peak IPSCs of smoothed traces were calculated and plotted as a pseudocolor display (*bottom right*). The color bar next to the pseudocolor plot indicates the scale, and the red arrow indicates the threshold used during the calculation of footprint areas (see METHODS). The experiment was conducted using a laser power of 35 mW.

Reticular input footprints to neurons of the posterior nucleus

We found that the reticulothalamic input footprints to the posterior nucleus are larger and often more diffusely organized than those to the ventral posterior lateral nucleus. The shapes of these footprints varied among posterior nucleus neurons and can be roughly divided into three categories. For the first, the reticulothalamic input footprint consisted of a single elongated area, usually quite large in size and adjacent to the reticulothalamic border. Figure 3*A* shows an example with the same organization as Fig. 2. For the second, responses could be evoked from more than one separate area of the thalamic reticular nucleus. Figure 3*B* illustrates an example in which photostimulation evoked IPSCs in two separate, elliptical regions of the thalamic reticular nucleus; one region was located near the border between the thalamic reticular nucleus and internal capsule, and the other occupied a larger area near the reticulothalamic border. The shape of the footprints can be seen from the response traces overlaid on top of the photomicrograph of the slice (areas encircled by the yellow and black rectangles) and is more clearly shown in the pseudocolor plot of the peak IPSCs (Fig. 3*B*, *bottom right*). Selected response traces from both regions are also displayed more clearly in larger scales (Fig. 3*B*, top and middle right). For the third, IPSCs were evoked in posterior nucleus neurons throughout the whole thickness of the thalamic reticular nucleus (thickness of the footprint ≥200 μm). Figure 4*B* shows an example.

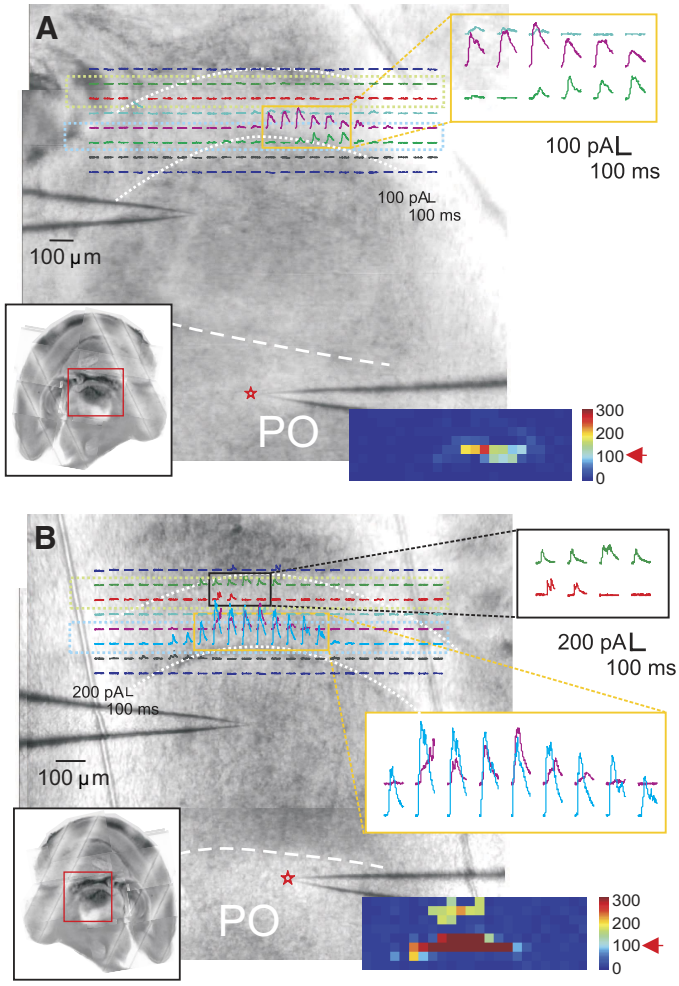

FIG. 3. Two examples of the reticulothalamic input footprints to posterior nucleus neurons; conventions as in Fig. 2. In both examples, the responses to photostimulation at 192 locations, arranged in a 24 × 8 rectangular array, are shown superimposed on a photomicrograph taken during the experiment. The red stars indicate the locations of the posterior nucleus neurons. The white dashed lines indicate the approximate borders between ventral posterior (lateral and medial) and posterior nuclei in both *A* and *B*. *A*: reticulothalamic input footprint that consists of a single elliptical area located near the reticulothalamic border. The laser power used was 37 mW. *B*: example in which IPSCs could be evoked in 2 discontinuous regions of the thalamic reticular nucleus. One was located near the border of the thalamic reticular nucleus and the internal capsule (black rectangle) and the other was adjacent to the reticulothalamic border (yellow rectangle). The power of the laser used was 18 mW.

Comparison of reticulothalamic footprints to the ventral posterior lateral and the posterior nuclei

IPSC AMPLITUDE. We performed 12 dual recording experiments during which we simultaneously recorded a pair of relay cells, one from each of the ventral posterior lateral and the posterior nuclei. We successfully evoked IPSCs in both neurons in 11 experiments, and Fig. 5 summarizes the results (the 1 in which we failed was discontinued, and the data were not used in any analyses). The maximum peak IPSCs from all response traces evoked by photostimulation were measured for each cell and we found no significant difference between the peak IPSCs elicited in neurons of the ventral posterior lateral nucleus [751 ± 668 (SD) pA] and the posterior nucleus (564 ± 365 pA; Wilcoxon signed-rank test, $P = 0.328$).

FOOTPRINT TYPES. Figure 4, *A* and *B*, illustrates examples of the reticulothalamic footprints determined for two relay cells in a dual recording experiment, one in the ventral posterior lateral nucleus (Fig. 4*A*), and the other in the posterior nucleus (Fig. 4*B*). As indicated, the footprint in Fig. 4*A* is much more compact than is that in Fig. 4*B*, suggesting that the differences of the reticulothalamic footprints to these two nuclei were not some artifact of different preparations or technical details of stimulation/recording.

Overall, all of the 14 ventral posterior lateral nucleus neurons had a single elliptical input footprint limited to a portion of the thickness of the thalamic reticular nucleus. Of the 22 recorded neurons from the posterior nucleus, 7 (32%) had a single elliptical input footprint limited to a portion of the thickness of the thalamic reticular nucleus, 12 (55%) had evoked IPSCs responses from more than one contiguous region, and 3 (14%) had especially large, footprints extending throughout the thickness of the thalamic reticular nucleus. These differences in footprint shapes between the two thalamic

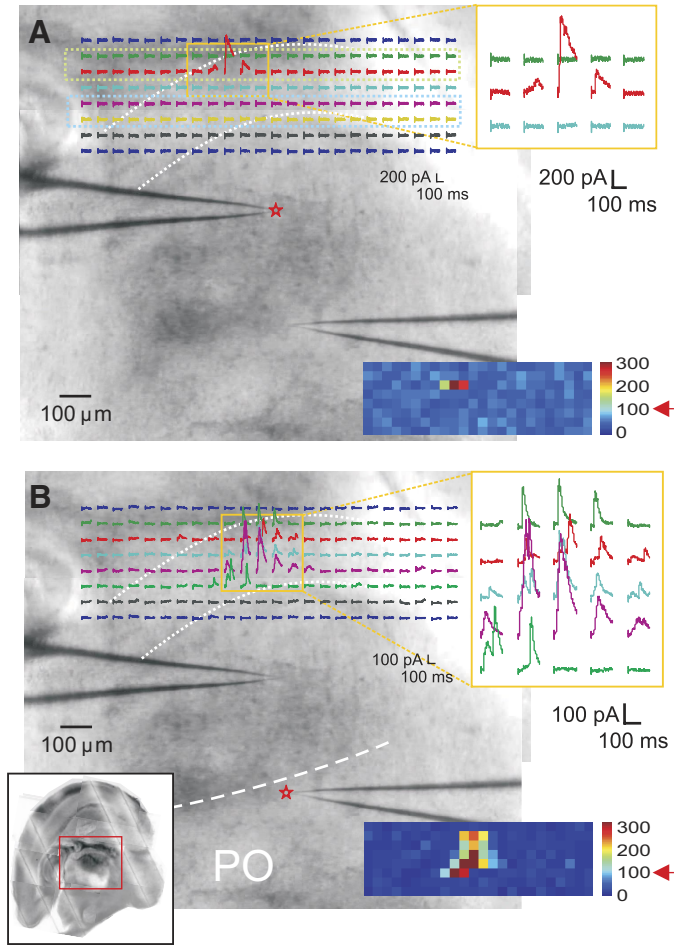

FIG. 4. Results from a dual-recording experiment; conventions as in Fig. 2. The red stars indicate the neurons of which the data are shown. The white dashed line indicates the approximate border between ventral posterior (lateral and medial) and posterior nuclei. The power of the laser used was 12 mW. *A*: reticulothalamic input footprint to a neuron in the ventral posterior lateral nucleus. IPSCs were evoked only in a small elliptical area near the border of the thalamic reticular nucleus and internal capsule. *B*: reticulothalamic input footprint to a simultaneously recorded neuron in the posterior nucleus. IPSCs could be evoked in a relatively nontopographic area spread throughout the whole thickness of the thalamic reticular nucleus.

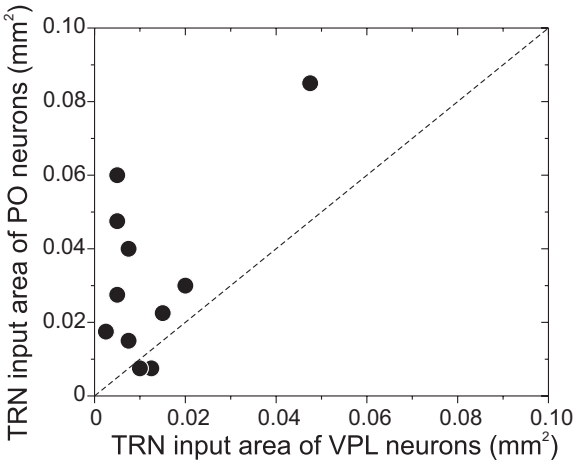

FIG. 5. Comparison of footprint areas of the reticulothalamic input to neurons in the posterior nucleus and ventral posterior lateral nucleus from 11 dual-recording experiments. These footprint areas are plotted against each other. - - -, slope of 1. The posterior nucleus neurons have significantly larger reticulothalamic input footprints (Wilcoxon signed-rank test, $P < 0.005$).

relay nuclei are statistically significant ($\chi^2 = 18.3$, df = 2, $P < 0.0001$).

FOOTPRINT LOCATIONS. We also found differences in footprint locations between the reticulothalamic inputs to these two thalamic nuclei. We designated the area within 100 μm from the reticulothalamic border as the "inner tier" and that between 150 to 250 μm from the border as the "outer tier." Most posterior nucleus neurons (21 of 22; 95%) received some of their reticular input from the inner tier of the thalamic reticular nucleus. We classified the reticulothalamic footprints of the posterior nucleus neurons according to their anatomical locations. Five of the 22 footprints (23%) were within the inner tier of the thalamic reticular nucleus. Most footprints (16 of 22; 73%), however, consisted of more than one area or were nontopographic and occupied both the inner and outer tiers. The footprint of one posterior nucleus neuron (4%) was located inside the outer tier. The footprints of all 14 ventral posterior lateral nucleus neurons were located inside the outer tier of the thalamic reticular nucleus. These differences in locations are statistically significant ($\chi^2 = 32.1$, df = 2, $P < 0.0001$).

FOOTPRINT SIZES. We found the reticulothalamic footprints to the posterior nucleus to be larger than those to the ventral posterior lateral nucleus (Fig. 4). Figure 5 summarizes the results from 11 experiments during which we simultaneously recorded a pair of relay cells, one from each nucleus. For each pair, the area of the reticulothalamic footprints were plotted against each other, and the diagonal, dashed line indicates where the two values are equal. In 9 of the 11 cases, the footprints were larger for the posterior nucleus neurons. The difference in footprint areas was statistically significant [the ventral posterior lateral nucleus vs. the posterior nucleus, 0.013 ± 0.013 and 0.033 ± 0.024 (SD) mm^2, respectively; Wilcoxon signed-rank test, $P < 0.005$].

Comparison of the responses evoked from the inner and outer tiers of thalamic reticular nucleus

A total of 12 posterior neurons had input footprints that consisted of separable areas in the inner and outer tiers of the thalamic reticular nucleus (Fig. 3*B*). We have compared the areal extent and IPSC sizes evoked from these two regions. The maximum peak IPSCs evoked from the inner and outer tiers of the thalamic reticular nucleus were 405 ± 386 pA and 365 ± 461 pA, respectively, and the difference was not significant (Wilcoxon signed-rank test, $P = 0.239$). The footprint areas located within the inner and outer tiers were 0.0165 ± 0.0151 and 0.0092 ± 0.0084 mm^2, respectively and the differences was not significant (Wilcoxon signed-rank test, $P = 0.480$).

DISCUSSION

We used laser-scanning photostimulation in in vitro slice preparations in the mouse to compare the topography of the reticulothalamic input to the ventral posterior lateral and posterior nuclei, these being examples, respectively, of first- and higher-order somatosensory thalamic relays. In confirmation and extension of our study in rats (Lam and Sherman 2005), reticulothalamic input footprint to neurons of the ventral posterior lateral nucleus in mice consisted of small, topographically organized elliptical areas (Fig. 2). The input footprints to posterior nucleus neurons were quite different and varied considerably among neurons: they could consist of a single elliptical region located near the reticulothalamic border (Fig. 3*A*), but in most cases, responses could be evoked in additional discontinuous regions of the thalamic reticular nucleus (Fig. 3*B*) or in relatively diffuse regions throughout the thickness of the thalamic reticular nucleus (Fig. 4*B*). Overall, in additional to differences in shape and topography, the reticulothalamic input footprints to posterior nucleus neurons were also larger in size (Fig. 5).

Technical considerations

We believe that the differences in footprint topography and size are not an artifact of stimulation intensity or other variations across experiments for the following reasons. In 11 dual recording experiments in which a neuron from each of the ventral posterior lateral and posterior nuclei were recorded and stimulated simultaneously, similar differences were observed. Also we used a fixed and equal threshold value during all estimations of footprint area, and because the responses of posterior nucleus neurons were smaller (even though the difference was not significant), our measurement method was actually biased toward smaller footprints for these cells, the opposite of what we observed. It is also worth noting that one might expect more axons to be cut in the slice for the longer reticulothalamic pathway to the posterior nucleus, which should reduce the size of these footprints, and yet these were larger than those of the shorter reticulothalamic pathway to the ventral posterior lateral nucleus.

Three types of reticulothalamic footprint to the posterior nucleus were seen (Figs. 3, *A* and *B,* and 4*B*). This pattern could be explained by considerable variation in a single class of posterior nucleus neuron or by variations of experimental conditions, such as the extent of axons cut in the slices. On the other hand, this might suggest that there are multiple classes of these thalamic neurons related to different patterns of reticulothalamic input, which is consistent with the proposal that the posterior nucleus consists of distinct functional populations

(Trageser and Keller 2004). In any case, our data are insufficient to distinguish between these possibilities of multiple classes or considerable variation within a single class.

For technical reasons involving practical issues of whole cell recording, we conducted our experiments on young animals (9–12 days postnatal), and we cannot rule out the possibility that some of the differences we reported represent a developmental phenomenon. We think this unlikely for the following reasons. First, in agreement with our experience in rats (Lam and Sherman 2005), the reticulothalamic input to the ventral posterior nucleus is already highly topographic even at this age, suggesting an early development of the somatosensory system in rats and mice. Second, injection of retrograde tracers into the posterior nucleus has indicated that differences between the reticular inputs to the first- and higher-order relays are found in adults (Crabtree 1996; Crabtree et al. 1998).

Finally, it is worth noting that this study was conducted using the somatosensory thalamocortical slice preparation (which has the advantage of preserving much of the thalamocortical circuit) (Agmon and Connors 1991; Reichova and Sherman 2004). The topography of the input footprints to ventral posterior neurons in these slices (Fig. 2) were very similar to what we saw in horizontal slices of rats (Lam and Sherman 2005), and therefore we believe that the results shown here is not an artifact of the plane of sectioning.

Comparisons with previous results

Injection of retrograde tracers into the posterior nucleus label a large and diffuse area in the thalamic reticular nucleus of the cat (Crabtree 1996) and rat (Crabtree et al. 1998), whereas labeling of individual reticular neurons in the rat reveal mostly compact and topographically organized axonal arbors in both the ventral posterior and posterior nuclei (Pinault and Deschênes 1998; Pinault et al. 1995). Our results suggest that these inconsistences may be different aspects of the reticulothalamic circuitry revealed using different techniques. We found that the majority of posterior nucleus neurons received reticular inputs from a relatively large region of the thalamic reticular nucleus, which could explain the wide-spread retrograde labeling of the thalamic reticular nucleus by Crabtree and colleagues (Crabtree 1996; Crabtree et al. 1998). Furthermore, we did find clear evidence of an overall topography in the reticulothalamic input to the ventral posterior lateral and posterior nuclei, consistent with the observations of Pinault et al. (1995). Also in agreement with the latter study, we found a tendency for the input to the posterior nucleus to emanate from regions closer to the reticulothalamic border (Fig. 3, *A* and *B*).

The larger input footprint to posterior nucleus neurons might result from the large axonal arbor volume of the reticular neurons targeting the posterior nucleus compared with those targeting the ventral posterior nucleus (Pinault and Deschênes 1998, Table 1). A contribution from electrical coupling between reticular neurons might also affect the footprint area, but we do not know of any data supporting different areal extent of reticular neurons that project to the ventral posterior and posterior nuclei. Moreover, experiments have indicated that electrical coupling existed only in a relatively small portion of reticular neurons (Lam and Sherman 2006; Landisman et al. 2002).

Functional significance

Figure 6 is a schematic diagram of our interpretation of the results. We propose that most or all of the reticulothalamic neurons have specific and topographically organized axonal arbors. Inputs to the ventral posterior lateral or medial nuclei derive from the regions of the thalamic reticular nucleus further from the reticulothalamic border, and reticulothalamic neurons closer to this border selectively target the posterior nucleus (Fig. 6*A*). Although we show only three neurons for these proscribed projections (Fig. 6*A, left*), there is probably a finer and continuous organization within each of these tiers, as suggested by both anatomical (Crabtree 1996) and functional results (Lam and Sherman 2005). In addition, we propose that there is a less topographically organized input from reticular neurons that are located in all parts of the thalamic reticular nucleus and that innervate both the ventral posterior and posterior nuclei; this less-topographic input results in both multiple, discrete areas or larger, continuous reticulothalamic input footprints (Fig. 6*A, right*).

The organization of the circuitry of these two types of reticular inputs could indicate different underlying functions. We propose that the topographic inputs to the ventral posterior (lateral and medial) and posterior nuclei are well-suited to mediate lateral inhibition between thalamic neurons (Lam and Sherman 2005). The less topographic inputs, on the other hand, may represent circuitry through which first and higher order somatosensory thalamic relays interact (Crabtree and Isaac 2002; Crabtree et al. 1998). We also found differences among topography of the reticular inputs of the posterior nucleus neurons, suggesting the interesting possibility of functional and anatomical variations among posterior nucleus neurons.

What is unclear at this stage is the detailed circuitry involving the reticulothalamic neurons in this less topographic pathway. Figure 6*B* illustrates several possibilities indicated by numbers to underscore the uncertainty. These neurons could

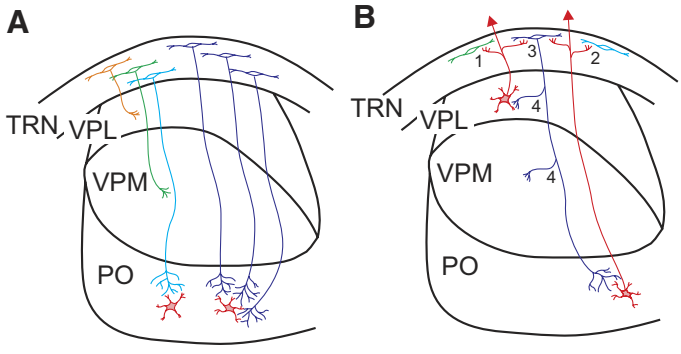

FIG. 6. Schematic diagram illustrating proposed functional organization of the reticulothalamic inputs to somatosensory thalamus. *A*: most or all of the reticulothalamic neurons have topographically organized axonal arbors. Inputs to the ventral posterior lateral or medial nuclei derive from the regions of the thalamic reticular nucleus further from the reticulothalamic border, and reticulothalamic neurons closer to this border selectively target the posterior nucleus (*left*). In addition, there is a less topographically organized input from all parts of the thalamic reticular nucleus to the posterior nucleus that results in both multiple, discrete input areas or larger, continuous footprints (right). *B*: uncertain aspects of the functional organization. The source of input to the reticular neurons involved in the less topographically organized reticulothalamic input to the posterior nucleus is unknown: these neurons could receive specific inputs from the ventral posterior (1) or posterior nucleus (2), or they could receive convergent inputs from both nuclei (3). Moreover, these neurons could also innervate multiple thalamic nuclei (4).

receive specific inputs from the ventral posterior lateral (Fig. 6*B,* 1) or posterior nucleus (Fig. 6*B,* 2), or they could receive convergent input from both of these nuclei (Fig. 6*B,* 3); also, it is possible that individual reticulothalamic axons in this pathway innervate multiple thalamic nuclei (Fig. 6*B,* 4). These circuit details are important, as they constrain the direction of information flow between first- and higher-order somatosenosory relays, but further data will be required to address this point.

Our results indicated different functional organizations of the reticular input to the first-order (ventral posterior lateral or medial nuclei) and higher-order (posterior nucleus) somatosensory relays. Another important question is whether the differences are general across different sensory systems. Injection of retrograde tracer into the visual (Conley and Diamond 1990) and auditory (Conley et al. 1991) thalamus of bushbabies revealed separated reticular regions projecting to the first- and higher-order relays, and, in agreement with our observations, the reticular inputs to the higher-order relays were more diffuse. However, we also found that some reticular neurons in the region that projects to the first-order somatosensory thalamic relay provide a second source of convergent input to the higher-order thalamic relay and act as an interface between these two thalamic nuclei. Whether this organizational principle holds true for the visual and auditory thalamus remains to be determined.

Our results can also be considered in light of the suggestion by Sherman and Guillery (2006) that first-order relays transmit subcortical information for the first time to cortex, whereas higher-order relays act as an intermediate transmission site between cortical areas. This would suggest that this cortico-thalamo-cortical route involving higher-order relays, perhaps because of its later stage in information processing, is under modulatory, inhibitory control from a larger and more diffuse zone of the thalamic reticular nucleus than are the first-order relays. Because the thalamic reticular nucleus is involved in the corticothalamic feedback, our data would also suggest information processing in the higher-order relays is under more extensive (disynaptic, inhibitory) control from the cortex. This notion is consistent with recent evidence that higher-order thalamic relays generally have a greater preponderance of modulatory inputs (Van Horn and Sherman 2007; Wang et al. 2002) and are relatively selectively innervated by GABAergic inputs from the zona incerta and anterior pretectal nucleus (Bartho et al. 2007; Bokor et al. 2005; Trageser and Keller 2004) and by dopaminergic inputs (Sanchez-Gonzalez et al. 2005).

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