Thalamic Projections From the Whisker-Sensitive Regions of the Spinal Trigeminal Complex in the Rat

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ABSTRACT

This study investigated the axonal projections of whisker-sensitive cells of the spinal trigeminal subnuclei (SP5) in rat oral, interpolar, and caudal divisions (SP5o, SP5i, and SP5c, respectively). The labeling of small groups of trigeminothalamic axons with biotinylated dextran amine disclosed the following classes of axons. 1) Few SP5o cells project to the thalamus: They innervate the caudal part of the posterior group (Po) and the region intercalated between the anterior pretectal and the medial geniculate nuclei. These fibers also branch profusely in the tectum. 2) Two types of ascending fibers arise from SP5i: Type I fibers are thick and distribute to the Po and to other regions of the midbrain, i.e., the prerubral field, the deep layers of the superior colliculus, the anterior pretectal nucleus, and the ventral part of the zona incerta. Type II fibers are thin; branch sparsely in the tectum; and form small-sized, bushy arbors in the ventral posterior medial nucleus (VPM). Accordingly, a statistical analysis of the distribution of antidromic invasion latencies of 96 SP5i cells to thalamic stimulation disclosed two populations of neurons: fast-conducting cells, which invaded at a mean latency of 1.23 ± 0.62 msec, and slow-conducting cells, which invaded at a mean latency of 2.97 ± 0.62 msec. 3) The rostral part of SP5c contains cells with thalamic projections similar to that of type II SP5i neurons, whereas the caudal part did not label thalamic fibers in this study. A comparison of SP5i projections and PR5 projections in the VPM revealed that the former are restricted to ventral-lateral tier of the nucleus, whereas the latter terminate principally in the upper two tiers of the VPM. These results suggest a functional compartmentation of thalamic barreloids that is defined by the topographic distribution of PR5 and type II SP5i afferents. J. Comp. Neurol. 420:233–243, 2000.

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Brainstem nuclei that receive vibrissal primary afferents in rats include the principal trigeminal nucleus (PR5) and all subdivisions of the spinal trigeminal complex (SP5). Each of these (sub)nuclei contributes axons to the trigeminothalamic tract, but the main stream of ascending fibers arises from PR5 and from the interpolar division of SP5 (SP5i; Smith, 1975). Bulk labeling with anterograde tracers has shown that both PR5 and SP5i axons innervate the ventral posterior medial (VPM) and the posterior group (Po) nuclei (Peschanski, 1984; Chiaia et al., 1991a; Williams et al., 1994). For the moment, the axonal distribution of whisker-sensitive cells of the oral and caudal divisions of SP5 (SP5o and SP5c, respectively) remains ill defined.

At each level of the neuroaxis, the peripheral arrangement of the vibrissae is maintained in arrays of cellular aggregates referred to as barrelettes (brainstem), barre-
loids (thalamus), and barrels (cortex). Whereas the whisker-like patterning of the terminal fields of PR5 axons in rat VPM has been well documented both at the ensemble level and the single-cell level (Williams et al., 1994; Veinante and Deschénes, 1999), the topographic distribution of SP5i projections remains unclear. Previous studies reported either a complete overlap with PR5 terminal fields (Peschanski, 1984) or a patchy, scattered distribution lacking a clear, barreloid-like arrangement (Chiaia et al., 1991a; Williams et al., 1994). Whatever the case, SP5i terminals should be relatively abundant across the field of barreloids, because electron microscopic studies consistently reported SP5i profiles presynaptic to VPM cells dendrites (Chiaia et al., 1991a; Wang and Ohara, 1993; Williams et al., 1994). In addition, large numbers of VPM neurons exhibit an overt multiwhisker responsiveness in PR5 lesioned rats (Rhoades et al., 1987) that disappears after subsequent ablation of the SP5i (see also Freidberg et al., 1999). For the moment, these ultrastructural and physiologic results are difficult to reconcile with those provided by tract-tracing studies.

A confusing issue with most studies that used massive tracer injections to map SP5 projections to the thalamus relates to their lack of specificity with regard to the different populations of orofacial afferents. This makes it hard to reach strong conclusions about the projections of whisker-sensitive trigeminothalamic cells. A second problem concerns the heterogeneity of this cellular population: Intracellular staining of cells antidromically invaded from the thalamus revealed various morphologic types of vibrissa-responsive neurons across the SP5 subnuclei (Jacquin et al., 1986a, 1988, 1989; Renehan et al., 1986; Jacquin and Rhoades, 1990). A similar diversity was highlighted by parvalbumin and calbindin immunostaining of retrogradely labeled trigeminothalamic cells (Bennett-Clarke et al., 1992). It appears likely that these different populations of neurons give rise to different patterns of axonal projections. When studied at a single-cell level, for instance, the axonal projections from the PR5 demonstrate far more complexity than what is apparent immediately in conventional tract-tracing studies (Veinante and Deschénes, 1999). In the current study, we sought to determine the single-cell make-up of SP5 projections to the thalamus by labeling small groups of axons that arise from the whisker-responsive regions of the three SP5 subnuclei.

MATERIALS AND METHODS

Anterograde labeling experiments

Experiments were carried out in 52 adult rats (Sprague-Dawley) in accordance with federally prescribed and university animal care and use guidelines (Olfert et al., 1993). Rats were anesthetized with ketamine (75 mg/kg) and xylazine (5 mg/kg), and injections of biotinylated dextran amine (BDA; molecular weight, 10,000; Molecular Probes, Eugene, OR) were made with small-sized micropipettes (5–8 μm) into the three SP5 subnuclei. The stereotaxic coordinates of the atlas of Paxinos and Watson (1986) were used to target the injections in SP5o and SP5i. The subnucleus caudalis and the caudal part of SP5i were reached through the opening of the cisterna magna by using the obex as a landmark. After recording vibrissae-evoked responses to ascertain the correct placement of the injections, BDA (2% in 0.5 M potassium acetate) was ejected by iontophoresis (positive current pulses of 300–1,000 nA, 500 msec duration, half-duty cycle for 30 minutes). After a survival period of 5–7 days, animals were perfused with saline followed by a fixative containing 4% paraformaldehyde and 0.8% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.4. Brains were postfixed in the same fixative for 2 hours, cryoprotected overnight in 30% sucrose, and cut at 75 μm on a freezing microtome along the horizontal plane. Sections were processed for cytochrome oxidase and BDA histochemistry according to previously described protocols (Wong-Riley, 1979; Horikawa and Armstrong, 1988). Finally, sections were mounted on gelatin-coated slides, dehydrated in alcohols, cleared in toluene, and coverslipped without counterstaining. Labeled trigeminothalamic fibers were drawn with a camera lucida by using ×25 or ×40 objectives. Photomicrographs were taken with a digital camera (Agfa-Gevaert NV, Montsel, Belgium) and were processed with Photoshop software (version 3.0; Adobe Systems, Mountain View, CA). Apart from brightness and contrast adjustments, they were printed without modifications.

Control experiments

Control experiments were made to address anatomic issues raised by the anterograde labeling experiments. These included calbindin immunostaining of sections containing labeled fibers to determine whether some of these fibers terminate in the anterior intralaminar nuclei. After bilateral injections of BDA into SP5i, one brain was cut at 50 μm along the frontal plane, and alternate sections were processed either for cytochrome oxidase and BDA reactions or for revealing BDA and calbindin immunoreactivity. Once the BDA reaction was terminated, sections were rinsed three times in 0.01 M phosphate-buffered saline (PBS, pH 7.4), and incubated overnight in a solution containing 5% normal horse serum, 0.2% Triton X-100, and anticalbindin D-28K antisera (Sigma; St. Louis, MO; dilution, 1:2,500). After three rinses in PBS, sections were incubated for 1 hour in the secondary antibody (biotinylated horse immunoglobulin G; Vector Laboratories, Bur-
lingame, CA), rinsed again three times in PBS, and reacted with the avidin-biotin-peroxidase complex (ABC; Vector Laboratories). Bound peroxidase was revealed by using 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma) as a substrate.

Finally, four experiments were conducted to map in frontal sections the terminal fields of PR5 and SP5i afferents. Rats were anesthetized with ketamine/xylazine, and BDA was injected iontophoretically into the whisker-sensitive division of both subnuclei by means of coarse micropipettes (tip diameter ~15 μm) and large-current pulses (3–4 μA, 7 seconds on/7 seconds off). After a survival period of 6 days, animals were perfused, and the tissue was processed as described above.

Electrophysiological experiments

Data pertaining to the conduction velocity of thalamic projecting SP5i neurons were obtained in the course of a separate series of experiments carried out previously by one of the authors (for a description of the methods used, see Jacquin et al., 1989). In these experiments, the latencies of antidromic invasion to VPM stimulation were measured for 96 SP5i neurons. Most of these cells responded to whisker stimulation, and some were injected intracellularly with horseradish peroxidase. These data were used in conjunction with the anatomic data base to assess the hypothesis that SP5i projections to the thalamus arise from two populations of neurons that have different conduction velocities.

RESULTS

Data base

In horizontal sections of the brainstem, cytochrome oxidase staining clearly delineated SP5 subnuclei. Six sites were located in SP5o, 16 were located in SP5i, and 17 were located in SP5c (see Fig. 1). The thalamic projections of 51 darkly stained trigeminothalamic fibers were reconstructed totally; then, proceeding backward, the main trunks and collateral branches were mapped to the other terminal sites in the mesencephalon. An additional group of 65 axons was reconstructed in part to verify whether their projections conformed to those of the fully reconstructed fibers.

Projections from SP5o

Previous anatomic studies reported few labeled cells in SP5o after the injection of retrograde tracers into the thalamus (Smith, 1975; Silverman and Kruger, 1985; Bruce et al., 1987; Mantle-St.-John and Tracey, 1987; Bennett-Clarke et al., 1992). Accordingly, large injections of BDA (1 μA) made with relatively coarse micropipettes (~15 μm) labeled very few SP5o trigeminothalamic fibers (typically, 0–3 axons per injection site). The reconstruction of these axons (n = 7) revealed a common pattern of innervation in the caudal-ventral part of the Po and in the crescent-shaped region adjacent to the anterior pretectal nucleus (Fig. 2). In matching horizontal sections that were stained for calbindin, this later region stained positively and displayed a sieve-like appearance. It comprised the ethmoid nucleus, the medial part of the medial geniculate...
nucleus (MGm), and the caudalmost extension of Po intercalated between the pretectal and the medial geniculate nuclei. Axons from the SP5o formed loose clusters of terminations in caudal Po and more dispersed clumps in the rest of their terminal fields. As a rule, SP5o axons also gave off a number of branches in the superior colliculus (SC), and some innervated the dorsal division of the zona incerta (ZI).

Projections from SP5i

In agreement with previous electrophysiological studies (Woolston et al., 1982; Jacquin et al., 1986a, 1989), recordings made in SP5i prior to the injections disclosed almost exclusively units that responded to the movement of multiple whiskers. On the basis of the distribution of terminations in the upper mesencephalon and thalamus and of the shape of terminal arbors, two types of axons make up the ascending projections from this subnucleus.

Type I axons (n = 23) have diameters of 2–4 μm and consistently showed up after all injections made into SP5i. Collectively, these axons project to a number of sites in the thalamus and upper brainstem. The most robust projections are to the superior colliculus, the pretectum, the ventral division of the zona incerta (ZIv), and the prerubral field, including the parvocellular division of the red nucleus. Terminal fields in these regions often are so dense that even the labeling of only two or three fibers makes their complete reconstruction hazardous. Tracing the main branches of single axons clearly shows, however, that most distribute terminals to each of these targets. In the thalamus, terminal fields are less dense and are concentrated principally in Po (Figs. 3, 5A). After giving off a series of collaterals in the mesencephalon and ZIv, type I axons enter the thalamus and divide in a number of secondary branches that form one or multiple clusters of medium-sized terminations (2–4 μm) concentrated principally in a shell-like region over the dorsal aspect of VPM and/or the caudal part of VPM lacking barreloids. Among these fibers, some also innervate the dorsal part of the ventral-lateral nucleus (VL) and/or give off branches that make a distinct cluster of terminations in a dorsomedial region of Po situated behind the anterior ventral nucleus. This later region is not an actual part of the intralaminar thalamus, as evidenced by its lack of calbindin immunoreactivity in double-stained sections. Rather, it seems to correspond to the angular nucleus in the atlas of Paxinos and Watson (1986). Finally, most axons distribute scattered terminals throughout caudal Po between the parafascicular and the lateral geniculate nuclei. Although many type I axons give off branches that ascend through VPM, none gives off terminations in the field of barreloids.

Type II axons (n = 15) are observed more frequently after injections made into the caudal, barrelette-patterned region of SP5i or the rostral part of SP5c. Injections made into the rostral SP5i label few of these axons, and, when it is present, the staining is often faint. Most type II axons are of small diameter (1–2 μm), they branch very sparsely in the tectum and MGm, and none terminates in ZI. In VPM, they make bushy terminal fields containing one to three clumps of medium-sized boutons (2–4 μm; see Figs. 4, 5B). In horizontal sections, terminal fields form thin (∼80 μm), narrow sagittal bands (∼100 × 250 μm) that most often are present in sections cut deep through the thalamus.

Projections from SP5c

Whereas recordings made in SP5o and SP5i prior to the injections disclosed almost exclusively units that responded to the movement of multiple whiskers, the recordings made in SP5c yielded a number of single-whisker cells; however, very few of these cells seem to project to the thalamus. Of 17 injections made into this subnucleus, only those located rostrally (n = 6 injections), within ∼0.8 mm from the caudal border of SP5i, produced anterograde labeling in the thalamus. Other injection sites resulted in heavy staining of intersubnuclear axons that formed a
thin, sagittal band across the full length of the trigeminal complex; however, the thalamus and upper brainstem were completely free of labeling in these cases. Sites that give rise to thalamic projections are characterized by the presence of multiwhisker cells. Trigeminothalamic axons arising from these sites (six fibers were reconstructed) are
of small diameter (1–2 μm) and form bushy terminal fields similar to those made by type II SP5i fibers. Likewise, terminal fields are distributed principally in the ventral part of VPM (Fig. 4).

Small numbers of thin-diameter axons labeled from the rostral part of SP5c projected to both VPM and Po. They arborized in the caudal part of VPM that lacks barreloids and in the neighboring region of Po. Although none of these fibers was drawn completely, partial reconstruction revealed that they also give off terminal branches in MGm and tectum.

**Comparative distribution of PR5 and SP5i projections in VPM**

A remarkable feature of SP5i axons that terminated in VPM was their preferential distribution in horizontal sec-

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**Fig. 4.** A–D: Terminal fields of type II SP5i axons in the VPM. Note the small sizes and elongated aspect of terminal fields, which are ~80 μm thick. Drawings were made from horizontal sections. For abbreviations, see list. Scale bar = 200 μm.
tions cut deep through the thalamus. Therefore, we cut two brains along the frontal plane to visualize better the location of terminal fields. After a large BDA injection into the whisker-responsive division of SP5i (two cases), the anterograde labeling in VPM was found exclusively in the ventral-lateral tier of the nucleus that is contiguous to the ventral posterior lateral nucleus (VPL). Figure 6A shows the distribution of darkly labeled terminal fields in a representative frontal section passing through VPM. After a similar injection made into PR5 (two cases), labeled terminal fields were found in a more restricted zone of VPM corresponding to the size of one or two barreloids. This zone contains dense clusters of terminations, principally in the upper two tiers of the nucleus, with a sparser distribution of boutons in the lower tier adjacent to VPL (Fig. 6B,C). Thus, these results show that the terminal fields of PR5 and SP5i axons form complementary projection patterns in VPM, except in the lower tier of the nucleus, where both projections seem to overlap.

Conduction velocities of thalamic projecting SP5i cells

The anatomic results described above show that SP5i projections to the thalamus arise from two classes of fibers that have different diameters. One would expect this feature to be related to cellular populations that have different axonal conduction velocities. The histogram of Figure 7 shows the distribution of the antidromic invasion latencies of 96 SP5i units that were backfired from the contralateral VPM. At first glance, this distribution looks neither clearly unimodal nor bimodal. Thus, a test of bimodality was carried out to determine whether the distribution contains one population or a mixture of two populations of neurons (McLachlan and Basford, 1988). This test compared the likelihood of two hypothesis: The observed distribution of latencies arises either from a single population of normally distributed latencies (hypothesis $P_1$) or from a mixture of two populations of normally distributed latencies (hypothesis $P_2$). In the latter case, it was assumed that the variance within the two populations was the same, but the mean latency was allowed to differ. The likelihood ratio of the two hypothesis ($L_2/L_1$) indicates which of hypothesis is more likely and twice its logarithmic value follows a $\chi^2$ distribution that can be used to test the null hypothesis of a single population (hypothesis $P_1$) against the alternative hypothesis ($P_2$). This test clearly favors the $P_2$ hypothesis, with a likelihood ratio $= 518$ ($\chi^2 = 12.5$; 2 degrees of freedom; $P = 0.002$). Accordingly, the histogram would contain two populations of thalamic projecting cells consisting of 74% of fast-conducting cells invaded at a mean latency of 1.23 ± 0.62 msec ($P_1$) and
26% of slow-conducting cells invaded at a mean latency of 2.97 ± 0.62 msec (P2).

DISCUSSION

Two principal findings resulted from this study. By using BDA to label small groups of whisker-sensitive SP5 cells, we demonstrated the existence of two main types of ascending fibers to the thalamus: thick fibers that distribute to the Po and to other regions of the midbrain and thin fibers that arborize principally in the VPM. In addition, the SP5 projection to VPM was shown to be restricted to the lower tier of the nucleus, where PR5 projections are sparse but present.
BDA is a very efficient and stable anterograde tracer that stains cell processes in a Golgi-like manner. This tracer seemingly enters into the cells through the small-sized axonal and dendritic branches that are severed by the micropipette (Pinault, 1996; Chen and Aston-Jones, 1998). This is probably the reason why labeling appears solid and uniform instead of granular, as with tracers that can be taken up actively (e.g., lectins or cholera toxin). Thus, after extracellular deposits, the labeling of fibers of passage is unavoidable. This drawback, which is common to most tracers, can be minimized by the use of small-sized micropipettes. The fact that injections made into the different trigeminal subnuclei labeled axons with different projection patterns is a good indication that BDA uptake through axon collaterals is not a critical issue in the current study. However, when large pipettes are used to pressure inject tracers, as in most previous studies, the probability of breaking fine collaterals increases as the square of the pipette diameter. Large injections into PR5, for instance, in combination with the use of sensitive histochemical methods, are likely to yield false-positive results, because many trigeminothalamic axons from SP5i send collaterals to this nucleus (Jacquin et al., 1986a, 1990). Consequently, labeling in the VPM may appear more extensive than what we report in this study and may be more abundant in the lower tier of the nucleus, where we found only a sparse projection. This contention is supported by the spatial distribution of the terminal fields of single PR5 axons that were labeled in a previous series of experiments (Veinante and Deschênes, 1999). Reexamination of these data confirms the preferential arborization of PR5 fibers in the two dorsal-medial tiers of VPM. Tracer injections were made into the whisker-responsive regions of SP5. It remains possible, however, that some labeled fibers may have receptive fields on neighboring regions of the mystacial pad (i.e., guard hairs, intervibrissae fur). This possibility actually is impossible to dismiss; however, insofar as the projections to the barreloids are concerned, there is no doubt that they arise from the whisker-sensitive populations of the trigeminal complex. The well-documented responsiveness of Po and of collicular and intercalar cells to whisker displacements (Tiao and Blakemore, 1976; Chalupa and Rhoades, 1977; Yamasaki and Krauthamer, 1990; Chiaia et al., 1991b; Diamond et al., 1992; Nicolesis et al., 1992) also matches with the projection patterns observed in the current study. Thus, it seems unlikely that our anatomic data base was contaminated much by nonwhisker-related axons.

Comparison with previous studies

Thalamic projections from the SP5 have been described previously in studies using anterograde degeneration and a variety of neuronal tracers (Lund and Webster, 1967; Smith, 1975; Erzurumlu and Killackey, 1980; Peschanski, 1984; Chiaia et al., 1991a; Williams et al., 1994). Because most studies used massive lesions or injections that likely involved many types of second-order orofacial afferents, there exist some minor mismatches between prior results and the current set of whisker-related data. For instance, we did not observe any SP5 projection to the submedius or to the anterior intralaminar nuclei, likely because these regions receive input from afferents of other submodalities (Ma et al., 1988). Labeled terminal fields were not observed in the ventral-medial wing of VPM, which contains the upper lip and lower mandibular representations. However, the other projection sites to the thalamus and the midbrain were confirmed again by the current study.

Although it can never be said that the current sample of SP5 axons is representative of all fiber types that make up the ascending projections from this division of the trigeminal complex, it came rather as a surprise that none of the labeled fibers actually innervated both the field of barreloids and the Po. Such axons were expected from prior double-retrograde labeling studies, which estimated that they constitute ~8% of the ascending contingent from SP5i (Chiaia et al., 1991a). Our negative result hardly can be ascribed to the small size of the injections and, thus, to a sampling bias, because injections of similar size made into the PR5 showed that 4% of ascending fibers from that nucleus terminate in both VPM and Po (Veinante and Deschênes, 1999). Interpolaris cells with a dual thalamic projection, thus, may be absent or very few in number. The double-retrograde labeling observed after injections of tracers into VPM and Po likely resulted from tracer uptake by damaged branches of SP5i axons that ascend through VPM to reach Po.

All electrophysiological studies agree on the fact that whisker-sensitive SP5i cells that project to the thalamus have multiwhisker receptive fields (Woolston et al., 1982; Jacquin et al., 1986a, 1989). In one of these studies (Jacquin et al., 1986a), SP5i neurons were backfired from the thalamus and labeled intracellularly. Although the numbers of neurons studied were small, there was a clear dichotomy among this cellular population (see Table 1 in Jacquin et al., 1986a). Half of the sample consisted of cells with thick axons (2–5 μm) that were invaded antidromically at latencies shorter than 1 msec, whereas the rest of the sample consisted of cells with thinner axons (1–2 μm) that were backfired at latencies longer than 1.5 msec. These results are fully in line with the two types of axons that were labeled by our BDA injections and with the statistical analysis of the distribution of antidromic latencies. Thus, it seems that two populations of neurons relay vibrissa information from SP5i to the thalamus: fast-conducting cells that project to Po and slow-conducting cells that project to VPM. In all likelihood, these two classes of neurons correspond to the large and small tri-
geminothalamic cells that were identified previously in the SP5i by Phelan and Falls (1991).

Ultrastructural and electrophysiological studies of the trigeminal projections in the rat VPM provided evidence that both PR5 axons and SP5i axons make synapses with the same relay cells (Chiaia et al., 1991b; Wang and Ohara, 1993; Freidberg et al., 1999). In light of the current data, it is clear that both types of prethalamic afferents should contact a fair proportion of cells in a thalamic barreloid. Only cells situated dorsally in barreloids may have dendrites that are out of reach of type II SP5i axons. However, if their dendrites extend across the VPM/Po border, then these neurons may be the target of PR5 axons and type I afferents from the SP5i that terminate in the shell region of the VPM. For the moment, this remains an open issue, and additional labeling studies will be required to settle the question. The segregation of SP5i axons in the ventral-lateral tier of the VPM suggests a functional compartmentation within barreloids. Such a notion has been introduced and discussed previously by Land et al. (1995) on the basis of the different cytochrome oxidase reactivity of the dorsal and ventral aspects of barreloids and of their differential retrograde labeling after horseradish peroxidase injections made at different depths of a barrel column. Here again, a single-cell study may reveal an as yet unsuspected specificity of connections between different regions of a thalamic barreloid and its corresponding cortical barrel.

**Projections from SP5i to barreloids**

In sections cut in an oblique sagittal plane through the VPM, cytochrome oxidase-rich barreloids form curved, tapering cylinders that extend through the thickness of the nucleus (Land et al., 1995). In the dorsal-medial part of the VPM, the long axis of barreloids lies normal to the VPM/Po border but bends horizontally in the ventral-lateral part adjacent to VPL. Most PR5 trigeminothalamic fibers that project to VPM have single-whisker receptive fields and form small-sized, bushy arboros that are restricted to the dimension of a single barreloid (Williams et al., 1994; Veinante and Deschénes, 1999). Although the shape of individual barreloids could not be visualized in our material, the terminal field of single type II axons from the SP5i appears isomorphic with the size and curved structure of barreloids in the ventral lateral part of VPM. Thus, it seems reasonable to propose that single type II axons from the SP5i convey multiwhisker information to a single thalamic barreloid.

**Receptive field of VPM cells**

The PR5 and SP5i are innervated by the same first-order vibrissal afferents that branch repeatedly throughout the trigeminal column (Hayashi, 1980, 1985; Jacquin et al., 1986b). The PR5 is situated next to the entry of the trigeminal nerve and at ~7 mm from the VPM, whereas the barrelette region of the SP5i lies ~2.5 mm more caudally. In addition, single-whisker PR5 neurons are much smaller than the multiwhisker units of the SP5i (Jacquin et al., 1988, 1989; Veinante and Deschénes, 1999), indicating that they likely possess a higher input resistance that would reduce the rise time of synaptic potentials. Assuming a factor of proportionality of 6 m/second per micrometer of axon diameter (Hursh, 1939; Rushton, 1951), the conduction velocity of PR5 axons would be about twice that of type II SP5i fibers (PR5 axons diameter, 2–3 μm, as measured from a previous data base; Veinante and Deschénes, 1999). Together, all of these factors add up to favor a faster transmission through the PR5 than the SP5i channel. Thus, these crude anatomic considerations give full support to the conclusions reached by Lee et al. (1994b), who provided clear physiological evidence that a late-arriving, multiwhisker input to the VPM arises from the SP5i.

The above-described anatomic organization is consistent with and, indeed, clarifies previous functional studies of vibrissal-sensitive cells in rat VPM. On striking a single vibrissa, relay cells in the corresponding barreloid first will be activated by the firing of fast-conducting PR5 afferents. Activation will be followed by inhibition arising from reticular thalamic cells that will prevent the late-arriving, multiwhisker input from the SP5i to reach threshold. The critical point here is how effective is the inhibition. Strong inhibition will confer on thalamic cells a single-whisker responsiveness, whereas inhibition that is not so strong will allow these cells to manifest a multi-whisker sensitivity. Because the inhibition induced by reticular thalamic cells is strongly state-dependent (Steirae and Deschénes, 1984), it becomes obvious that the weight of SP5i inputs will increase as the level of anesthesia diminishes (Freidberg et al., 1999). After lesion of the reticular nucleus, most relay cells will respond to multiple whiskers (Lee et al., 1994a). Lesion of the PR5, however, will prevent the early induction of reticular inhibition and confer on relay cells the multiwhisker responsiveness that characterizes SP5i afferents. In contrast, SP5i lesion will reduce the receptive field to one or two whiskers (e.g., see Rhoades et al., 1987; Freidberg et al., 1999).

**Projections from SP5i to Po**

The SP5 projection to Po is extensive, with some clusters, and it is particularly dense in the shell region over the VPM and in the caudal ventral part of the ventrobasal complex, where it is difficult to distinguish Po from the nonbarreloid region of VPM. This latter region demonstrates heavy retrograde labeling after tracer injections into the second somatosensory cortical area (Spreafico et al., 1987). Vibrissal information processed in this cortical area likely transits through this zone of the posterior thalamus, which receives input from all subnuclei of the trigeminal complex.

The distinct clusters of terminations in the angular and ventral lateral nuclei also clearly were present in the photomicrographs published by Williams et al. (1994; see Fig. 2). It remains unclear whether these thalamic regions project to the barrel field and/or to other whisker-related areas of the neocortex. Because these two regions receive a robust collateral input from layer 5 cells of the barrel field, whose axons also project to the deep layers of the superior colliculus (unpublished observations), they may be involved in the motor control of whiskers. The same comment also applies to the shell region over the VPM, which receives a layer 5 corticothalamic input and contains cells that project to both the somatic sensory and the motor cortices (Deschénes et al., 1998). Thus, the hodology of prethalamic and corticothalamic connections in Po suggests that this nucleus may be more involved in the processing of information related to active whisking than to passive displacements of the vibrissae.

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LITERATURE CITED


