Substrate for Cross-Talk Inhibition between Thalamic Barreloids

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A double-labeling protocol was used to determine whether thalamocortical and reticular thalamic cells with overlapping receptive fields form open or closed loop connections in the vibrissal system of the rat. Results show that individual reticular cells exclusively project to the barreloid representing the principal whisker of their receptive field. Furthermore, solid retrograde labeling of relay cells reveals that a large number extend dendrites outside their home barreloid. This feature, together with previous demonstrations that reticular thalamic axons principally contact the dendrites of relay cells, provide a morphological substrate for cross-talk inhibition between thalamic barreloids.

Key words: barrels; barreloids; whisker; vibrissa; reticular thalamic nucleus; thalamic relay cells

The nucleus reticularis thalami (nRT) occupies a key position in the thalamocortical circuitry. It receives excitatory inputs from thalamocortical and corticothalamic axons, and it returns inhibitory projections to the thalamic relay cells. This inhibitory action is potent and can significantly modify the rate and pattern of relay cell discharges (Steriade et al., 1985; Lee et al., 1994; Hartings and Simons, 2000). A central issue about the nRT network is whether the constituent cells inhibit the relay cells from which they receive input or whether they inhibit other pools of relay neurons. However, these two possibilities are not mutually exclusive, and there exists anatomical and electrophysiological evidence supporting both (Shosaku, 1986; Bal et al., 1990; Brumberg et al., 1996; Crabtree et al., 1998; Pinault and Deschênes, 1998a). In the present study we directly addressed this question by taking advantage of the highly segregated organization of the rodent vibrissal system.

From periphery to cortex this system is made of discrete cellular aggregates that replicate the arrangement of the vibrissae on the mystacial pad. In the ventral posterior medial (VPM) nucleus of the thalamus, whisker-related modules are called barreloids, and each barreloid projects onto a corresponding module, called barrel, in the primary somatosensory cortex. Thus, on the one hand, the architecture of a single barreloid can be outlined by the injection of a retrograde tracer in its related barrel (Hoogland et al., 1987; Land et al., 1995; Pierret et al., 2000). On the other hand, it is well established that nRT and VPM cells have receptive fields composed of one principal and several surrounding whiskers (Simons and Carvell, 1989; Armstrong-James and Callahan, 1991; Diamond et al., 1992). Responses to surrounding whiskers are strongly depressed by anesthetics, which reduce the receptive field size of VPM and nRT cells to the principal whisker (Freidberg et al., 1999). Thus, under deep anesthesia the response of nRT cells to principal whisker deflection ought to be relayed by the barreloids cells responding to the very same vibrissa. On the basis of these data, a double-labeling protocol was used to determine whether nRT cells form closed or open loop connections with their thalamic targets. A thalamic barreloid was retrogradely labeled by injecting Fluoro-Gold (FG) in an identified barrel column, and the axon of single nRT cells responding to the same whisker, or to an adjacent whisker located on the same arc, was anterogradely labeled by juxtacellular application of biotinylated dextran amine (BDA).

MATERIALS AND METHODS

Experiments were performed in 30 adult rats (Sprague Dawley , 250–300 gm) in accordance with federally prescribed animal care and use guidelines. The University Committee for Animal Use in Research approved all experimental protocols. First, rats were anesthetized with a mixture of ketamine (75 mg/kg) plus xylazine (5 mg/kg), and a barrel column, usually C2 or D2, was located by recording unit responses to manual whisker deflection. Then, a micropipette (tip diameter, ~6 μm) containing FG (2% in 0.1 M cacodylate buffer, pH 7.0; Fluorochrome, Inc., Denver, CO) was lowered in layer 4 (depth, 740 μm) of the identified barrel column. The tracer was ejected with positive current pulses of 100 nA for 10 min. After completing this protocol in both hemispheres, the skin was sutured, rats were given analgesics (Anafen; 5 mg/kg), and they were returned to the animal facilities. Twenty-four to 48 hr later animals were reanesthetized with ketamine–xylazine, and we searched for nRT cells that responded to the whisker whose barreloid had been retrogradely labeled with FG. Extracellular recordings were made with fine micropipettes (diameter, 0.5–1 μm) filled with K-acetate (0.5 M) and low molecular weight BDA (2% BDA, 3 kDa; Molecular Probes, Eugene, OR). Throughout the experiments a deep level of anesthesia was maintained so that nRT, as well as VPM cells, responded to only one whisker. Once a responsive nRT unit had been isolated, it was labeled juxtacellularly by the application of positive current pulses (2–8 nA; 200 msec duration; 50% duty cycle) for ~10 min (Pinault, 1996). In most cases, only one cell was stained in each thalamus, and animals survived 2–3 hr

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after the last injection. They were perfused under deep anesthesia with saline followed by a fixative containing 4% paraformaldehyde and 0.5% glutaraldehyde in phosphate buffer (PB; 0.1 M, pH 7.4). Brains were removed, post-fixed overnight in the same fixative, and cut coronally at 70 μm with a vibratome. After three washes in PBS (0.01 M, pH 7.4) sections were treated for 30 min with a solution of 50% ethanol plus 1% hydrogen peroxide. They were rinsed several times in PBS, and preincubated for 1 hr in PBS with 3% normal goat serum and 0.2% Triton X-100. They were then incubated overnight in the same medium containing an anti-Fluoro-Gold antiserum (1:8000; Chemicon, Temecula, CA). The antibody was then revealed using a peroxidase-labeled secondary antibody (goat IgG; Chemicon) and 3,3′diaminobenzidine tetrahydrochloride (DAB) as a substrate (brown reaction product). Next, sections were processed for BDA histochemistry using the ABC kit (Vector Laboratories, Burlingame, CA) and nickel-DAB (black reaction product). Finally sections were mounted on gelatin-coated slides, dehydrated in alcohols, cleared in toluene, and coverslipped without counterstaining. Labeled material was drawn with a camera lucida using a 40× objective.

In five rats we attempted to get solid retrograde labeling of barreloid cells by injecting FG with larger currents (up to 300 nA), by allowing a longer survival period (4 days), and using a more sensitive immunohistochemical method. In these experiments FG was revealed with a biotinylated secondary antibody (Vector Laboratories), the ABC reaction, and Ni-DAB as a chromogen. Two of 10 injection sites resulted in the solid labeling of a single barreloid, whereas the labeling of multiple barreloids or cellular degeneration was observed in the other cases.

RESULTS

Fluoro-Gold injections of the size shown in Figure 1*a* led to the retrograde labeling of single barreloids in ~50% of the cases. When two barreloids were backfilled, one usually contained less darkly stained somata so that the border between the two arrays remained clearly discernible. Otherwise, data were discarded. Like the barreloids outlined by cytochrome oxidase staining (Land et al., 1995), retrogradely stained cells form a narrow array, ~100-μm-wide, that extends from the VPM–posterior group border toward the ventral posterior lateral nucleus.

Reticular thalamic cells that respond to whisker deflection are found in the central tier of the dorsal sector of the nucleus, 2.5–3.0 mm behind the bregma. Their distribution is somatotopically organized (Shosaku et al., 1984), the E and A row-responsive cells lying in the anterior and posterior pole, respectively. Arcs are represented dorsoventrally, so that in a typical descent D1-, D2-, D3-, etc. responsive cells are found in succession.

Our database comprises 24 nRT cells that exclusively responded to the motion of the vibrissa whose barreloid had been retrogradely labeled ($n = 16$) or to the deflection of an adjacent vibrissa situated on the same arc ($n = 8$). Axons were sufficiently stained to be traced to their termination site and to ensure that no main branches escape detection. Results leave little ambiguity; all stained nRT cells project to, and only to, the barreloid representing the principal whisker of their receptive field (Fig. 1*c*). Three projection patterns were found (Fig. 2): some nRT axons ($n = 5$) only distribute terminals in the dorsalmost segment of the barreloid that abuts on the posterior group, some ($n = 12$) innervate the rest of the barreloid with few terminations in the dorsal segment, and the others ($n = 7$) give off terminations throughout the whole barreloid. Otherwise cells giving rise to the three types of terminal fields could not be further distinguished on the basis of their location or somatodendritic morphology. Thus, these results clearly demonstrate that nRT and VPM cells that respond to the same principal whisker do form closed loop connections.

A network of lateral inhibition cannot be excluded, however, because a number of VPM cells have dendritic arbors that are wider than the size of a barreloid (Chiaia et al., 1991; Ohara and Havton, 1994; Zantua et al., 1996). Although no morphometric study has yet quantified the relative importance of these "extrabarreloid dendrites", the solid retrograde labeling of barreloid cells highlights their large number (Fig. 3). Because electron-microscopic studies have clearly established that nRT axons distribute contacts throughout the dendritic arbor of thalamic relay cells (Peschanski et al., 1983; De Biasi et al., 1988; Cucchiaro et al., 1991; Liu et al., 1995), extrabarreloid dendrites ought to receive synaptic contacts from the nRT cells that project to the barreloid they invade. Thus from a strict anatomical viewpoint, an nRT cell should exert both recurrent inhibition on cells that relay input from its principal whisker and simultaneously impose remote lateral inhibition onto the relay cells principally excited by an adjacent whisker.

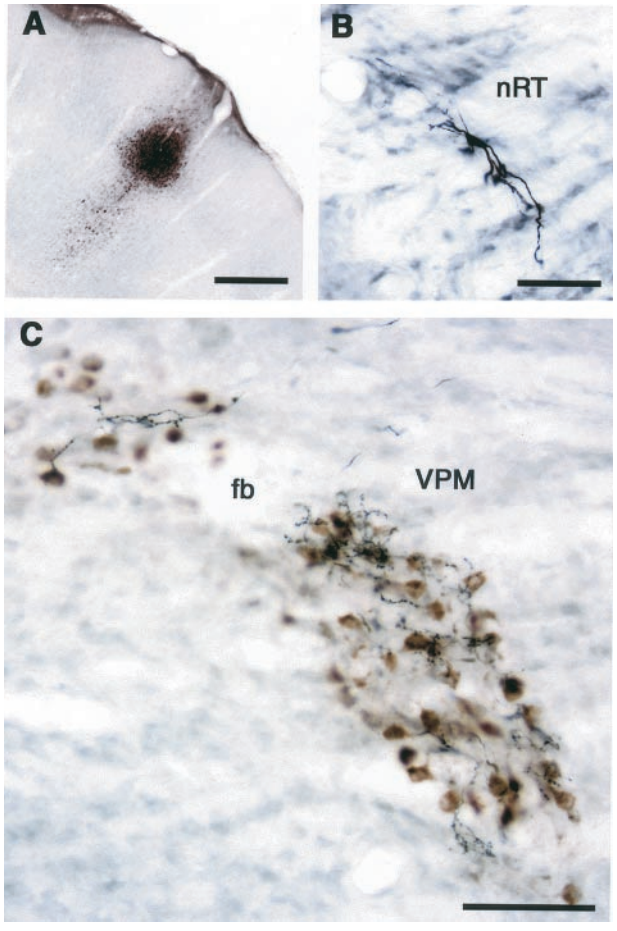

Figure 1. Terminal field of an nRT cell into a thalamic barreloid. Relay cells forming the D2 barreloid were backfilled after an FG injection into the D2 barrel column (*a*), and an nRT cell that responded to deflection of the same vibrissa was juxtacellularly stained with BDA (*b*). Note the precise overlap of terminations with the array of labeled somata (*c*). Scale bars: *a*, 500 μm; *b*, *c*, 100 μm. *fb*, Fiber bundle.

DISCUSSION

The present study revealed a point to point relationship between nRT and barreloids cells with receptive fields dominated by the same principal whisker. Reticular cells either form small compact terminal fields in the dorsalmost part of the barreloids or more extensive fields filling a large expanse of the barreloid. We were unable, however, to label any nRT cell with diffuse axonal arbors in the VPM. By using the whole-cell recording and labeling

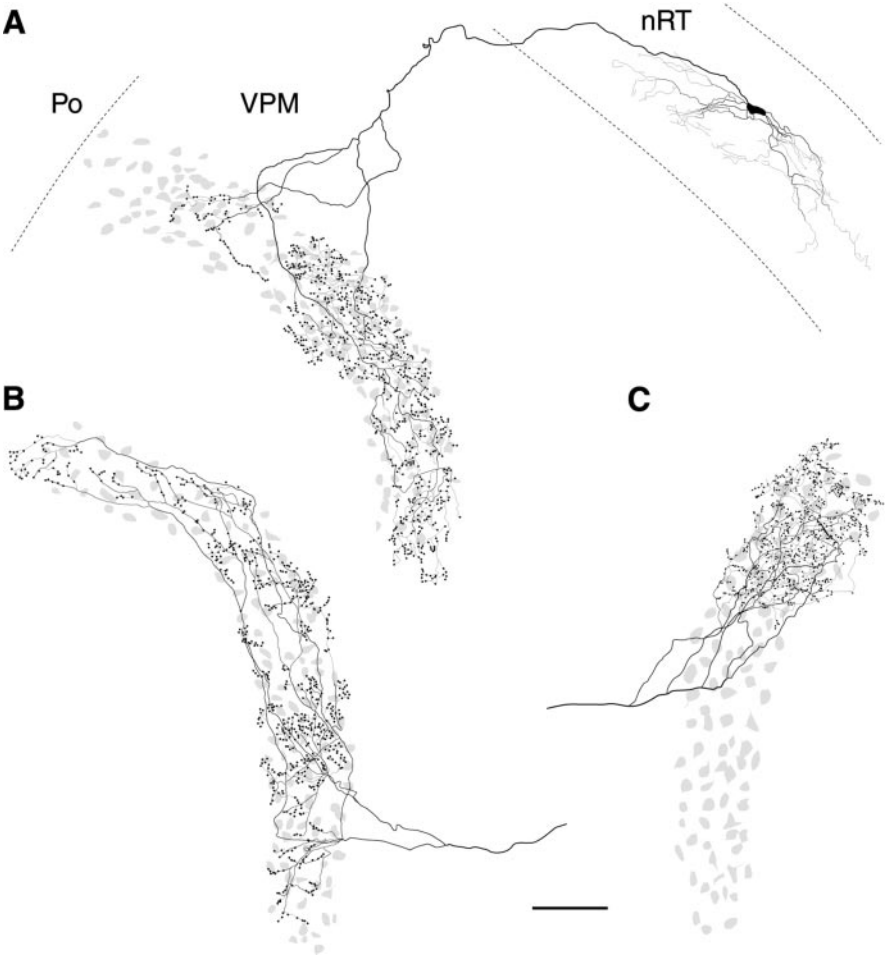

Figure 2. Projection patterns of individual reticular axons in thalamic barreloids. Arrays of retrogradely labeled cell bodies that correspond to the barreloids D2 (*A*), B3 (*B*), and C2 (*C*) are mapped in *gray tone* in the background. Terminal fields of nRT cells either distribute in different segments (*A, C*) or across the whole extent of a barreloid (*B*). Scale bar, 100 μm.

technique in thalamic slices of young rats (10- to 15-d-old), Cox et al. (1996) identified three types of nRT arbors in the VPM, ranging from diffuse to compact. Their "cluster and intermediate types" likely correspond to the patterns we found, but the "diffuse type", which contained a low density of axonal swellings, might be representative of an immature stage of development because similar diffuse and extensive nRT terminal fields are commonly observed in Golgi material obtained from young rodents (1- to 20-d-old; Scheibel and Scheibel, 1966). This is keeping with the delayed maturation of relay cells that acquire their adult form only by P21 in rodents (Zantua et al., 1996; Warren and Jones, 1997). Still, the possibility remains that we missed the diffuse type because these nRT cells do not respond to whisker motion in anesthetized animals. The same qualification applies to the possibility that some nRT cells may project to both, the barreloids and the posterior group, although the labeling of over 127 individual nRT cells in adult rats never revealed such projection pattern (Pinault and Deschênes, 1998b). Together, these results suggest that the nRT-mediated internuclear inhibition that has been disclosed between cells of the VPM and posterior group in slices (Crabtree et al., 1998) likely relies on patterns of axonal convergence within the nRT itself. Given the highly segregated organization of the vibrissal system, it would be premature to consider the present results as a model for the synaptic organization of other thalamic nuclei in rat or in other species. Different thalamic subsystems evolved to process different types of sensory inputs, and may therefore differ in the details of their internal circuitry.

Physiological significance

The innervation of the dorsalmost part of barreloids by a distinct group of nRT axons points to a functional specialization of this region. This region also stains more densely for cytochrome oxidase (Land et al., 1994; Pierret et al., 2000), receives trigeminal input from large-sized principalis cells having mutiwhisker-receptive fields (Veinante and Deschênes, 1999), and contains a larger density of labeled cells after retrograde tracer injection in layer 6 of the barrel field (Land et al., 1994). No physiological study has yet reported distinct response properties for cells located dorsally in barreloids, but the specificity of connections suggests a parallel stream of vibrissal information processing.

In agreement with the present results, cross-correlation analysis of activities simultaneously recorded from vibrissa-responsive neurons in the nRT and VPM in urethane-anesthetized rats revealed that both excitatory and inhibitory interactions are restricted to neurons with receptive field on the same vibrissa (Shosaku, 1986). Adjacent whisker-evoked inhibition of VPM cells in fentanyl-sedated rats was also reported to be weak or absent (Brumberg et al., 1996). Our results provide morphological substrate for these observations, but they also raise the possibility of subtle cross-whisker inhibitory interactions that may not be detectable with single-unit recordings. Indeed, the spread of VPM cells dendritic arbors in adjacent barreloids raises the possibility that surround inhibition acts distally in the dendrites. Remote cross-whisker inhibition might only modestly depress the

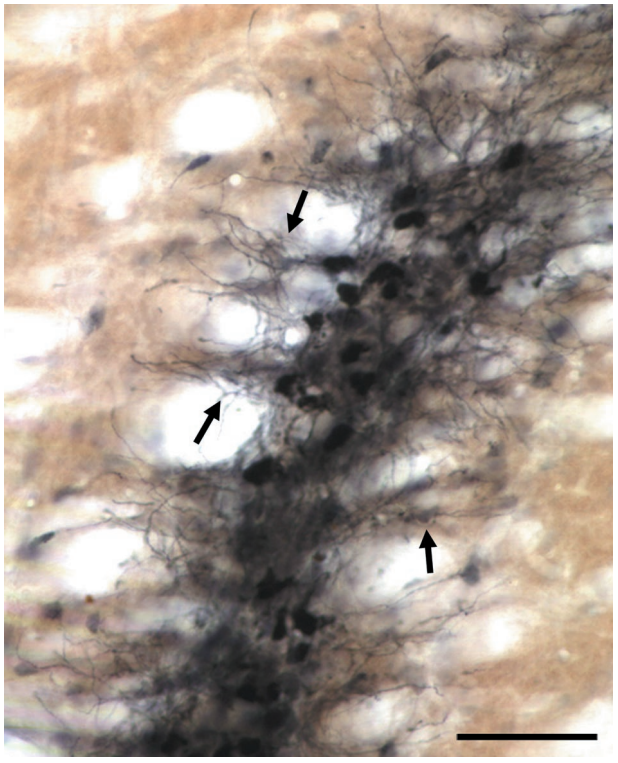

Figure 3. Solid retrograde labeling of barreloid cells after an FG injection in the C2 barrel column. Note the large number of dendrites that spread into the adjacent barreloids (*arrows*). Scale bar, 100 μm.

high security trigeminal synapses located on the proximal dendrites (Williams et al., 1994), but it may have a significant effect on corticothalamic inputs that share the same dendritic segments. The interplay of corticothalamic and nRT inputs in distal dendrites might have a subtle but decisive role in the timing of relay cell discharges.

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