The present study bears on afferents that terminate in layer VI of the posteromedial barrel field in the rat. Their origin was determined by the retrograde transport of cholera toxin, and their axonal arborizations were revealed by targeting injections of biotinylated dextran amine in regions that contained retrogradely labeled neurons. Afferents to lamina VI arise from the thalamus (the ventral posteromedial, the posterior group and the intralaminar nuclei), the claustrum and the infragranular layers of other somatomotor regions of the neocortex (the motor, second somatosensory and perihinal cortices). Among these afferent systems, corticocortical axons, particularly those issuing from the motor cortex, give rise to the most profuse projections in layer VI, whereas thalamic and claustral afferents form sparse terminal fields. Because corticothalamic cells represent ~50% of the neuronal population in lamina VI and 73% of their dendritic processes are deployed locally, it seems likely that afferents arising from the infragranular layers of the motor cortex may directly influence the firing of these neurons. These anatomical data suggest that the role of corticothalamic pathways should be studied from the viewpoint that sensory perception is an active process which operates under the guidance of motor activities.

Introduction
Throughout the neocortex, lamina VI is the principal source of corticothalamic (CT) projections. In sensory systems, CT pathways are usually considered as feedback loops that modulate the responses of thalamic relay cells to peripheral stimuli. This concept, however, remains largely descriptive as no one knows what is actually being fed back to the thalamus. The hypothesis proceeds from the viewpoint that perception is a multileveled synthetic processing of elementary sensory signals that are ultimately bound into a percept by the coherent firing of ensembles of neurons. Accordingly, electrophysiological studies of CT pathways investigated how the sensory responses of thalamic neurons were modified after removal or inactivation of the primary sensory cortical areas. Most of these studies, including those carried out in non-anesthetized animals, however, failed to demonstrate any significant change. In the best cases, it was reported that CT cells could increase the excitability of thalamic neurons or modulate the timing of their discharges (Widen and Ajmone-Marsan, 1960; Baker and Malpeli, 1977; Tsumoto et al., 1978; Geisert et al., 1981; Yuan et al., 1985, 1986; Ghosh et al., 1994; Sillito et al., 1994), which may represent a small fraction of what those neurons actually do in free moving animals.

In the present study we sought to establish which afferent systems terminate in layer VI of the posteromedial barrel field in the rat. Most cells within this zone of the primary somatosensory area (SI) respond to the deflection of one or multiple vibrissae, but it is noteworthy that lamina VI cells are not very responsive to such passive stimulations (Chapin, 1986; Armstrong-James and Fox, 1987; Armstrong-James and George, 1988a,b; Armstrong-James et al., 1992). Though thalamic relay cells from the ventral posteromedial nucleus (VPM) give off terminals in lamina V1 (Jensen and Killackey, 1987), it seems that the activation of other afferent systems is required to drive CT cells. Axonal transport studies have already shown that the cortical barrel field, as a whole, receives afferents from other thalamic and cortical regions (Tracey and Waite, 1995), but information concerning those afferents that terminate specifically in layer VI is still partial. We addressed this issue by the retrograde labeling of cells that project to lamina VI of the posteromedial barrel field, and by targeting injections of an anterograde tracer in regions that contained retrogradely labeled neurons.

Materials and Methods
Experiments were made in 38 adult rats (Sprague–Dawley) under ketamine (75 mg/kg) and xylazine (5 mg/kg) anesthesia. Housing and treatment conditions adhered to federally prescribed and university animal care and use guidelines.

Injection of Tracers
Three rats received unilateral micro-iontophoretic injections of cholera toxin β subunits (CTβ; List Biological Laboratories, Campbell, CA) in lamina VI of the posteromedial barrel field. The map of vibrissae representation published by Neafsey et al. (1986) was used to guide the injections. Using an angular approach of ~40°, micropipettes penetrated the cortical surface 2–4 mm behind the bregma and 5.5 mm lateral to the midline. In this region, cells responded to vibrissa deflection but we did not attempt to further define their receptive field. Injections were made at a depth of 1650 μm with micropipettes (tip diameter ~10 μm) filled with a CTβ solution (0.5 mg of lyophilized low salt CTβ in 1 ml of H2O). The tracer was ejected by passing positive current pulses (7 s on/7 s off, 1.5 μA for 20 min).

In other rats (n = 35), unilateral or bilateral injections of biotinylated dextran amine (BDA; mol. wt 10 000, Molecular Probes, Eugene, OR) were made in regions where cells had been found to be retrogradely labeled with CTβ. Micropipettes (tip diameter ~10 μm) were filled with a solution of potassium acetate (0.5 M) plus 2% BDA, and the tracer was ejected with positive current pulses of 400–800 nA (1 s on/1 s off) for 50 min. In other experiments, single VPm cells (n = 4) were labeled by juxtacellular applications of BDA (see Pinault, 1996).

Histochemistry and Immunohistochemistry
Seven days after CTβ injections, or 4 days after BDA injections, animals were perfused with 0.9% NaCl followed by a fixative containing 4% paraformaldehyde and 0.5% glutaraldehyde in phosphate buffer saline (PBS, 0.1 M, pH 7.4). Brains were cryoprotected in 30% sucrose overnight and cut coronally at 75 μm on a freezing microtome.

Alternate sections of CTβ-injected brains were processed for cytochrome oxidase histochemistry or CTβ immunohistochemistry. Cytochrome oxidase activity was revealed following the protocol described by Wong-Riley (1979). The presence of CTβ was revealed according to the procedure of Luppi et al. (1988). Sections were first rinsed three times in PBS, preincubated in a solution containing 2% normal rabbit serum (NRS) and 0.2% Triton X-100 (Sigma, St Louis, MO) for 1 h, and then incubated for 24 h in a solution containing goat anti-CTβ (List; dilution 1:10 000), 1% NRS and 0.1% Triton X-100 diluted in PBS.
Sections were then rinsed three times in PBS and incubated for 90 min in a solution containing biotinylated rabbit anti-goat IgG (Vector Labs, Burlingame, CA; dilution 1:400), 1% NRS and 0.1% Triton X-100 diluted in PBS. After three rinses in PBS, sections were incubated in avidin–biotin peroxidase complex (ABC, Vector Labs, dilution 1:400 in PBS) for 10 h. They were rinsed once in PBS and twice in 0.05 M Tris–HCl buffer solution (TBS, pH 7.6) and then processed following the nickel ammonium sulfate intensification method. Sections were immersed in a solution containing 0.025% 3,3′-diaminobenzidine (DAB; Sigma), 0.3% nickel ammonium sulfate and 0.003% H2O2 in TBS for 5–15 min. The reaction was terminated by several rinses in TBS. It results in black punctate granules in the retrogradely labeled cells. All steps were carried out at room temperature (∼22°C).

All sections of BDA-injected brains were processed first for cytochrome oxidase histochemistry to identify cortical and subcortical structures, and then for BDA histochemistry. BDA was revealed using nickel-DAB as the enzymatic substrate, whereas cytochrome oxidase was revealed using DAB alone. To reveal BDA, sections were incubated for 24 h in the Vectastain® ABC reagent (Vector Labs) diluted in a PBS solution containing Triton X-100 (0.25%). After two rinses in PBS and two rinses in TBS, sections were reacted with a TBS solution containing 0.05% DAB, H2O2 (0.003%) and nickel ammonium sulfate (0.3%). Finally, sections were mounted on gelatin-coated slides, dehydrated in alcohols, cleared in toluene and coverslipped without counterstaining.

Data Analysis
The use of fine micropipettes and low-intensity iontophoretic currents resulted in the solid labeling of small pools of neurons. Yet the number of labeled axons that terminated in S1 was quite variable: it ranged from one (in the claustrum for instance) to several tens (in VPm) depending upon the local scattering of projection neurons. When the yield per injection site was small, multiple injections were made to obtain a representative picture. Single fibers or small groups of axons were drawn with a camera lucida using 25× or 40× objectives. Injection sites and labeled axons were also mapped at low magnification to determine their location on corresponding plates of the atlas of Paxinos and Watson (1986).

The material used to quantify the laminar distribution of the dendrites of CT cells was obtained from a previous series of experiments in which 39 CT neurons from the posteromedial barrel field have been injected juxtacellularly with biocytin (Zhang and Deschénes, 1997). CT neurons were identified individually as cells that have an axon projecting to the thalamus. Fourteen of these cells were chosen according to the quality of their labeling. The entire dendritic tree of these cells were reconstructed using a computer-based reconstruction system (Neurulucida, Microbrightfield, Colchester, VT). The length of dendritic segments was measured and the total length per lamina was computed. Boundaries between laminae were determined by counterstaining sections with thionin after completion of the drawings.

Results
Laminar Distribution of CT Cells Dendrites
Corticothalamic neurons belong to the category of short pyramidal cells. In the rat barrel cortex, these neurons are characterized by a skirt of basal dendrites that radiate out from the cell body in lamina VI and by an apical dendrite which, after giving off side branches in the infragranular layers, divides and terminates in midcortical layers (Fig. 1A). Quantitative analysis of the laminar distribution of the dendrites of 14 CT cells (see Materials and Methods) revealed that the cumulated length of dendritic segments in lamina VI represents 73 ± 12% of the entire dendritic tree (Fig. 1B). This figure suggests thatafferent that terminate at this level may represent major synaptic inputs for CT cells.

Retrograde Labeling after CTb Injections in Layer VI
Cholera toxin injection sites (n = 3) formed dark blue halos of ∼400 μm in diameter in the middle of lamina VI. The tracer probably diffused to the lower part of layer V but no sign of CTb deposit was observed along the tract of the micropipette in the upper cortical layers. The contralateral barrel field also contained <20 labeled cells per rat, indicating that CTb deposits were mainly confined to lamina VI of the granular zone.

The distribution of labeled cells was similar for the three cases (Fig. 2). Retrograde labeling was observed in layers II–V over the injection sites and in the infragranular layers of the adjacent granular and dysgranular zones of the somatosensory area (S1). Elsewhere in the cortex, clusters of CTb-positive cells were found almost exclusively in the infragranular layers of the motor, second somatosensory (S2) and perirhinal areas. Cells containing CTb were also present in the rostral part of the claustrum and in the thalamus. In the thalamus, CTb-positive cells were found in a number of structures: they were grouped in a rod-like aggregate in VPm, they were scattered throughout the rostrocaudal extent of Po, and they formed a diagonal band within the rostral intralaminar nuclei (central lateral, paracentral and central medial nuclei). A few cells were also labeled in the ventral medial nucleus.

Anterograde Labeling of Afferents to Layer VI
The laminar specificity and the density of the terminal fields generated by afferents in lamina VI of the barrel field were assessed by injecting BDA in most regions that contained CTb positive cells. Photomicrographs of Figure 3 shows representative pictures of the BDA injection sites.

Thalamic Afferents
Injections of BDA in VPm (n = 2), about the size of a barreloid (~150 μm), produce two-tiered foci of terminations in the barrel cortex: one in layer IV and the other in the upper half of layer VI. The photomicrograph Figure 4A shows the dense terminal fields that result from the labeling of tens of VPm axons. Although layer VI contains a large number of labeled profiles, many are dendrites of retrogradely labeled cells or secondary branches of thalamocortical axons that head for layer IV. The reconstruction of single fibers from juxtacellularly stained units (n = 4) shows...
that each VPm axon ramifies profusely in layer IV but much more sparsely in layer VI (Fig. 4B).

The labeling of axons issued from different parts of Po (n = 8) reveals an heterogeneous population of fibers which, collectively, project across all the somatomotor regions of the neocortex: S1, S2, insular and motor cortices. Partial reconstruction of single fibers (n = 63) at their exit from the striatum reveals that the vast majority of these axons divide in the white matter and send branches into different cortical areas where they terminate principally in layers Va and I. Among these fibers, some ramify as well, but sparsely, in the lower part of lamina VI beneath the barrels (Fig. 5A).

A similar sparsity of projections is observed in layer VI of the barrel field after injections of BDA in the intralaminar nuclei (n = 4). The bulk of intralaminar axons terminate in layers III and V of the motor areas. Before entering into the motor cortex, some intralaminar axons give off collaterals that travel caudalwards and ramify loosely in lamina VI of S1 (Fig. 5D). Few branches ascend in layer V but they never pass beyond the granular layer.

Claustral Afferents

After injections of BDA in the rostral part of the claustrum (n = 4) profuse anterograde labeling is observed in the frontal cingular areas. When present in the barrel field (2/4 cases), the number of labeled fibers is always small. Claustrofugal axons ascend through the medial part of the striatum, close to the ventricle, then they turn laterally and enter the barrel field where they form a loose plexus of branches that extends across many barrel columns in layers VI and V (Fig. 6).

Corticocortical Afferents

Injections restricted to the infragranular layers of the vibrissal motor cortex (n = 5) label numerous fibers which, after coursing deep in layer VI, arborize principally in layers I and VI of S1. In the dysgranular zone, terminal branches form a column in which terminations are distributed throughout all layers, but mainly in laminae I and VI. In granular zones, however, terminations are present almost exclusively in laminae I and VI. As shown in Figure 7, the superficial and deep projections arise from branching axons. In contrast, injections made in layer III (n = 2) or in layer VIb (n = 1) do not produce any anterograde labeling in the infragranular layers of the posteromedial barrel field.

Injections of BDA in the infragranular layers of S2 (n = 3) label fibers that form patch-like terminal fields in layers VI and V of S1 (Fig. 8A). Most of these fibers are fine and appear as collaterals of axons heading for the corpus callosum or the motor cortex. Misplaced injections restricted to lamina VIb (n = 2) result in the labeling of passing fibers in the depth of the barrel field with few axonal ramifications in the infragranular layers.

Few fibers are observed in the deep layers of S1 after BDA deposits in the infragranular layers of the perirhinal area. Most of these axons project to the motor cortex, among

Figure 2. Distribution of cells retrogradely labeled after a small injection of CTb in lamina VI of the posteromedial barrel field. Each drawing pools the distribution of labeled cells of two alternate sections (spacing 70 \(\mu\)m). The injection site is indicated by the grey spot in (C). Abbreviations: ac, anterior commissure; Cl, claustrum; CL, central lateral nucleus; CM, central medial nucleus; F, frontal plane of the sections with respect to the bregma; fmi, forceps minor corpus callosum; Fr1, frontal cortex area 1; Fr2, frontal cortex area 2; Par1, parietal cortex area 1; Par2, parietal cortex area 2; PRh, perirhinal cortex; Pc, paracentral nucleus. In this and other figures, the architectonic borders (arrows) were drawn according to Paxinos and Watson (1986).
which some give off branches in S1. Terminal fields are always concentrated in the lower half of layer VIa and in layer VIb (Fig. 8C).

**Discussion**

The main finding of the present study is that lamina VI of the posteromedial barrel field in the rat receives strong cortico-cortical projections from the infragranular layers of the motor and S2 cortices. Thalamic afferents arising from VPm form a moderately dense terminal field in layer VI. Inputs from the other thalamic nuclei or from the claustrum are either light, diffuse or involve small numbers of cells.

**Comparison with Previous Studies**

Our data show that three thalamic nuclei project to lamina VI of the posteromedial barrel field. Collectively, VPm afferents give rise to the most robust and focused projection, but this input is sparse at a single-cell level (see also Jensen and Killackey, 1987). In agreement with the autoradiographic results of Herkenham (1980), an overall sparsity of terminations also characterizes the terminal fields of Po and intralaminar axons in lamina VI of the parietal cortex. Owing to the sparsity of these projections, the Golgi-like labeling of a small number of axons revealed new features of these thalamocortical fibers. First, it was observed that most Po neurons distribute branches in at least two different areas of the neocortex. Second, it was shown that the intralaminar projection is concentrated in the infragranular layers of S1 and that it arises from branching axons whose main termination site is the frontal motor areas. This result is thus in line with a previous description of the axonal projections of

**Figure 3.** Photomicrographs of BDA injection sites. (A) Injection in the posterior group; (B) in the central medial nucleus; (C) in the claustrum; (D) in the motor cortex; (E) in the secondary somatosensory cortex; (F) in the perirhinal area.
single central lateral neurons in the rat (Deschênes et al., 1996), and further disproves the long-standing belief that the intralaminar nuclei project to layer I.

In cat and monkey the claustrum is reciprocally connected with the primary somatosensory areas (Olson and Graybiel, 1980; Mufson and Mesulam, 1982; Pearson et al., 1982). In the rat, no previous study reported similar connections, which were probably missed because retrograde tracer injections did not target lamina VI. In comparison with the massive projection of claustral cells in the rostral cingular areas, the one issued to the infragranular layers of the barrel field appears sparse. For the moment, the possibility that the claustral projection to S1 arises from neurons that also project to the cingulum cannot be excluded.

Reciprocal connections between the posteromedial barrel field and the motor, S2 and perirhinal cortices have been described previously in rats and mice (White and DeAmicis, 1977; Akers and Killackey, 1978; Chapin and Woodward, 1982; Donoghue and Parham, 1983; Carvell and Simons, 1987; Chapin et al., 1987; Koralek et al., 1990; Fabri and Burton, 1991; Miyashita et al., 1994; Izraeli and Porter, 1995). The vast majority of these connections were seen to arise from layers III and V with a variable but minimal contribution from lamina VI. In most studies, however, tracer injections were also made in layers III–V with a limited spread to layer VI. The contribution of the infragranular layers of S2 and the parietal ventral area (herein referred to as the perirhinal region) to corticocortical relationships with S1 was recognized by Fabri and Burton (1991) and the existence of ‘strong’ projections from the motor cortex to the deep layers of the granular zone in S1 was briefly reported by Chapin and Woodward (1982).

Because it gives rise to the bulk of CT projections, layer VI is usually considered ‘corticothalamic’ much as layer III is considered ‘corticocortical’. However, quantitative estimates made in the cat visual cortex (Gilbert and Kelly, 1975) and in the rat S1 area (Zhang and Deschénes, 1997) revealed that CT cells account for only half of the neuronal population in layer VI. An approximately equal percentage of cells in layer VI of the rat barrel field were shown to project to the vibrissal motor cortex and/or to S2 (Koralek et al., 1990; Zhang and Deschénes, 1997). Conversely, the infragranular layers of these cortical regions are important sources of afferents to lamina VI of the barrel field. Thus the present results add to the current concept of corticocortical relationships by disclosing a parallel organization of these connections between the deep cortical layers.

The Synaptic Weight of Corticocortical Inputs
A quantitative estimation of synaptic weight of various afferents on CT cells seems premature at the moment. The fact that a given set of afferents terminate in layer VI does not imply that these fibers make synapses with CT neurons, and one cannot exclude the possibility that afferents with sparse ramifications in layer VI may strongly influence the firing of CT neurons by controlling the activity of local circuit cells. Nonetheless, considering that CT cells represent ~50% of the neuronal population in lamina VI and that 73% of their dendritic processes are deployed locally, they stand as potential targets of cortico-cortical axons. It is worthwhile mentioning that there are two types of CT cell in lamina VI: one projects to VPm alone, and the other projects to both VPm and Po (Bourassa et al., 1995; Zhang and Deschénes, 1997). Cells that project to VPm are located in the upper half of layer VI and their apical dendrites extend into layers III–IV. These cells may be under strong thalamic influence since thalamocortical fibers make a large number of synapses onto their apical dendrites in layer IV (White and Hersch, 1982). On the other hand, cells that project to both VPm and Po are located in the lower half of lamina VI and their apical dendrites terminate in layer V. Because thalamocortical axons arborize mostly in layers III–IV and to a lesser extent in the upper half of layer VI, cells that project to VPm and Po would receive few thalamocortical inputs, and the main synaptic drive for these CT neurons may be of corticocortical origin. For the moment, no electrophysiological study has investigated the synaptic responses of lamina VI cells to stimulation of deep corticocortical pathways. In layer III cells, corticocortical postsynaptic potentials exhibit frequency facilitation and

Figure 4. Terminal fields of VPm axons in S1. The photomicrograph in (A) shows the bilaminar distribution of terminal fields observed after the anterograde labeling of tens of VPm axons. The axonal arbor of a single VPm axon is shown in (B); note the relative sparsity of terminal branches in layer VI. Scale bar in A = 500 µm.
Figure 5. Terminal fields of Po and intralaminar axons in layer VI of S1. Drawing in (A) shows the laminar distribution of three Po axons; note the branching of axons in S1 (Par1) and S2 (Par2) and the sparsity of terminal branches in the deep part of layer VI. Inserts (B) and (C) show respectively the location of the projection and injection sites on corresponding frontal sections. Drawing in (D) shows the arborizations of three axons that were labeled by a small injection of BDA made in the central medial (CM) nucleus. Note that the terminal branches in S1 arise from parent axons that project to the frontal motor areas. Insert (E) shows the location of the projection and injection sites. In this and following figures, the dashed line in the inserts indicates the upper limit of layer V. Abbreviations: CPu, caudate putamen; Fr1, frontal cortex area 1; Fr2, frontal cortex area 2; Par1, parietal cortex area 1; Par 2, parietal cortex area 2.

Figure 6. Terminal field of a claustral axon in the deep layers of the posteromedial barrel field. Inserts (B) and (C) show respectively the location of the injection and projection sites on corresponding frontal sections. Abbreviations: Cl, claustrum; Par1, parietal cortex area 1.
long-term potentiation (Iriki et al., 1989; Kirkwood and Bear, 1994; Castro-Alamancos et al. 1995). The presence of similar phenomena in lamina VI neurons, and particularly in CT cells, would be strong indication that different cortical regions can influence, in a use-dependent manner, thalamocortical cells via corticocortical–CT pathways.
Functional Implications

Most studies that investigated the responses of cortical cells to whisker deflection reported that lamina VI cells reacted poorly to sensory stimuli. Other studies aimed at determining the function of CT pathways failed to demonstrate clear-cut effects of cortical inactivation on the way VPM relay cells respond to stimulation of the vibrissae. Together, these results suggest that CT pathways exert little influence on thalamic neurons when vibrissae are passively stimulated.

It is a commonplace experience in humans that without movements of the skin relative to the surface of an object, none but the most coarsely patterned surfaces can be recognized. Behavioral studies showed that rats rely on movements of their facial whiskers, much as humans rely on the coordinated movements of their fingertips to discriminate object shape and texture (Carvell and Simons, 1990). In monkeys, many cells of the primary somatosensory areas respond more vigorously to tactile scanning of object surface than to the passive displacement of the patterned surface against the fingertips (Darian-Smith et al., 1984). The present anatomical study disclosed strong corticocortical projections, particularly from the infragranular layers of the motor cortex, to lamina VI of the barrel field. The existence of these connections suggests that CT activities in the posteromedial barrel field might be tightly coupled to the motor commands that control head and vibrissae movements in rats. We thus propose considering the role of CT pathways from the viewpoint that sensory perception is an active process which operates under the guidance of motor activities.

Notes

We thank Dr A. Parent for the gift of cholaer toxin and antibodies. This study was supported by a grant from the Medical Research Council of Canada.

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