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Basal ganglia and cerebellum receive different somatosensory information in rats

(barrel field/pontine nuclei/cortical lamination/vibrissae)

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ABSTRACT **There are two great subcortical circuits that relay sensory information to motor structures in the mammalian brain. One pathway relays via the pontine nuclei and cerebellum, and the other relays by way of the basal ganglia. We studied the cells of origin of these two major pathways from the posteromedial barrel subfield of rats, a distinct region of the somatosensory cortex that contains the sensory representation of the large whiskers. We injected tracer substances into the caudate putamen or the pontine nuclei and charted the location of retrogradely filled cortical cells. In preliminary studies, we used double-labeling techniques to determine whether the cells of origin of these two pathways send axon collaterals to other subcortical targets. Lamina V of the rat posteromedial barrel subfield contains two distinct populations of subcortically projecting neurons, which are organized into distinct sublaminae. Corticopontine cells are located exclusively in sublamina Vb, the deeper of two sublaminae revealed by cytochrome oxidase staining. Corticostriate cells are located almost exclusively in the more superficial sublamina Va. Experiments using double-labeling fluorescent tracers demonstrate that about one-quarter of the corticopontine cells send a collateral branch to the superior colliculus. Other studies have shown that cells in Vb are activated at very short latency after vibrissal stimulation; hence, they would seem to be an appropriate relay for the rapid transmission of sensory information to the cerebellum for use in sensory guidance of movement.**

Sensory areas of the cerebral cortex are connected to motor areas by way of corticocortical circuits and two prominent subcortically directed pathways. One subcortical pathway goes to the basal ganglia, and the other goes to the cerebellum via the pontine nuclei (1–4). The efferent terminals from the basal ganglia and cerebellum remain largely distinct in the thalamus and their thalamic targets project to nonoverlapping regions of the motor cortex (5). Do the basal ganglia and the cerebellum receive inputs from the same or a different population of sensory cortical cells?

The great majority of the cells in the sensory cortex that project to these two motor systems are located in lamina V of the cerebral cortex (2, 6–8). Lamina V may be divided into two sublaminae: a superficial sublamina Va and a deep sublamina Vb, which differ in a number of important respects. In mice, lamina Vb receives direct input from the somatosensory relay nucleus in the thalamus; Va does not (9). Stimulation of the appropriate whisker in rats activates cells in sublamina Vb at a latency that is as short as that for activating cells in lamina IV, the principal target of incoming thalamic fibers (10). In contrast, cells in Va respond at much longer latency to such stimulation. Cells in Va stain palely when tested for the presence of the metabolic enzymes cytochrome oxidase or succinic dehydrogenase (11). Cells in sublamina Vb stain far more densely, suggesting that they are capable of higher levels of tonic activity than those in Va (12).

Previous study of the efferent pathways from the rat somatosensory cortex (2) has suggested that the cell population projecting to the basal ganglia is centered on lamina Va, while the population projecting to the cerebellum is centered on Vb. However, the methods available at the time the study was done were relatively insensitive (13) and a comparison with more recent work (14) shows that only a subset of the entire population of corticopontine cells was identified. Wise and Jones (figure 6*a* in ref. 2) indicated that ≈10% of pyramidal cells in layer Vb of the somatosensory cortex were labeled after horseradish peroxidase (HRP) was injected into the basilar pons. Moreover, those corticopontine cells that were identified were described as forming clusters in which groups of labeled cells alternated with gaps containing unlabeled cells. In a recent study (14), we found that nearly all pyramidal cells in the deeper part of lamina V of the somatosensory cortex were labeled after an injection of wheat germ agglutinin-HRP (WGA-HRP), which filled the pontine nuclei, and that the corticopontine cells were distributed without any hint of clustering.

More recent evidence indicates that the origin of the corticostriatal projection may not be restricted to lamina V and that the axons of corticostriatal cells may branch. Studies with WGA-HRP (15), free HRP (16, 17), or ^{125}I-labeled tetanus toxin (18) have reported retrogradely labeled cells in both supragranular and infragranular cortical layers after injection into the basal ganglia. Are there similarly located cells in the somatosensory cortex? Both physiological and anatomical experiments have identified corticostriatal cells that send an axon collateral to the internal capsule (19–23). What is the sublaminar location of these bifurcating cells?

Current understanding of the sensory input to subcortical motor pathways is based on evidence from the study of the connections of divergent cortical areas or on methods that reveal only a small subset of the entire cell population. The conclusions of earlier studies should be reexamined by using improved tracing methods and precise identification of laminar borders in a clearly defined cortical region. We therefore reinvestigated the degree of segregation of the cells of origin of the pontine and striatal projections by using a sensitive WGA-HRP technique combined with cytochrome oxidase or succinic dehydrogenase reactions. Our aim was to identify accurately the laminar and sublaminar boundaries within a defined region of somatosensory cortex, the posteromedial barrel subfield (PMBSF), and to establish the position and distribution of retrogradely labeled cells projecting to differ-

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Abbreviations: HRP, horseradish peroxidase; WGA-HRP, wheat-germ agglutinin conjugated with horseradish peroxidase; PMBSF, posteromedial barrel subfield.

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ent targets within each sublamina. The PMBSF has clearly defined areal and laminar boundaries and receives its afferent input from the whiskers on the contralateral side of the face. The afferent projections terminate in a characteristic array of barrel-shaped structures within lamina IV of somatosensory cortex (24).

We studied the subcortical efferent pathways from the PMBSF, especially those originating in lamina V. Since cells in lamina V also project to other subcortical targets (2, 25–30), we report here preliminary results of an extensive study of the degree of axonal branching by using double-label techniques.

METHOD

WGA-HRP was injected into the entire target area of the PMBSF in the caudate putamen or the pontine nuclei (2, 14, 31). To demonstrate the axonal branching of the corticopontine fibers, in one case we injected true blue into the pontine nuclei and diamidino yellow into the superior colliculus. In controls, we injected adjacent structures in the brainstem or basal ganglia to rule out the possibility of spurious cortical labeling due to spread of the WGA-HRP to nearby structures. Under sodium pentobarbital anesthesia the animal was placed in a small animal stereotaxic apparatus, and 0.25 μl of 4% WGA-HRP was injected over a 10-min period with a Hamilton microsyringe, which was angled into the pontine nuclei through the cerebellum, or vertically to the caudate putamen. After a 48-hr postoperative survival period, the animal was given an overdose of sodium pentobarbital and perfused via the heart. The brain was removed and frozen sections were cut at 50 μm, processed according to a modification of the tetramethyl benzidine method of Mesulam (32) and Gibson *et al.* (33), and counterstained with neutral red. The laminar organization of the PMBSF was studied in alternate sections processed to reveal the presence of the enzyme cytochrome oxidase according to the method of Wong-Riley (34). In the experiments in which the succinic dehydrogenase and WGA-HRP reactions were combined on the same section, the animals were anesthetized and perfused with 10% (vol/vol) glycerol. The brain was sectioned and assayed for HRP (32, 33) and the enzyme succinic dehydrogenase (35).

RESULTS

Fig. 1 shows the PMBSF in normal rats' brains in sections assayed for cytochrome oxidase. Note the prominent barrel-shaped structures in lamina IV of the cortex and the clear sublaminar organization of lamina V below the barrels (11). Sublamina Va stains palely, while sublamina Vb stains darkly.

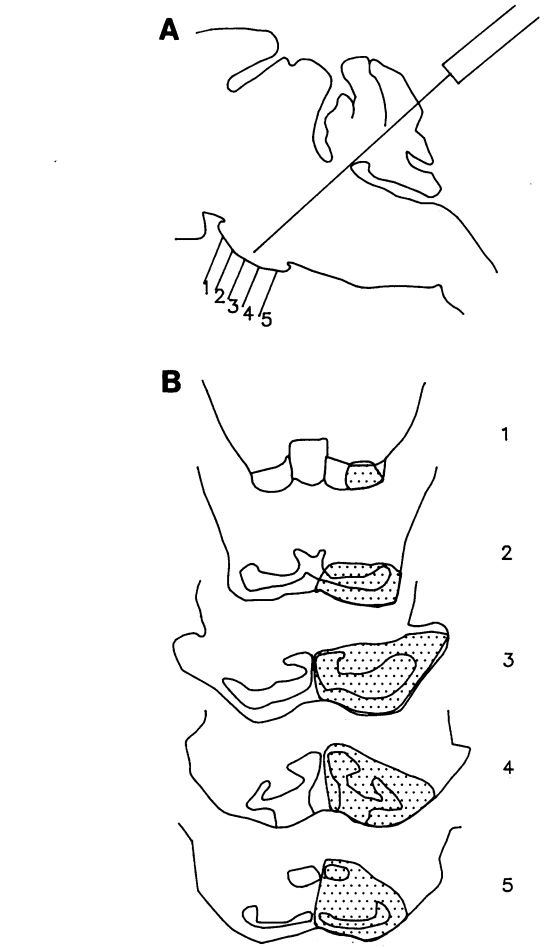
Fig. 2 shows the extent of the primary injection and the location of retrogradely labeled cells within the PMBSF after an injection of WGA-HRP had been made in the pontine nuclei. Fig. 3 shows comparable data for a case in which the injection had been made into the striatum. In both cases, we processed adjacent sections for cytochrome oxidase to calculate the laminar depth of retrogradely labeled cells in the PMBSF. In all cases in which the pontine nuclei were injected, the retrogradely labeled cells were confined entirely to sublamina Vb of the PMBSF and extended from its upper to its lower border throughout its extent. Labeled cells were distributed continuously in Vb without clusters of labeled cells or gaps. In many sections every pyramidal cell, as revealed by the neutral red counterstain, within sublamina Vb of the PMBSF was retrogradely labeled. In all cases in which the caudate putamen had been injected, the retrogradely labeled cells were largely confined to sublamina Va,

FIG. 1. A section through the caudal part of the PMBSF showing the prominent barrel-shaped structures (arrows) in lamina IV of the primary somatosensory cortex. The section was treated to reveal the enzyme cytochrome oxidase. Cytochrome oxidase and succinic dehydrogenase are present in high concentrations within each barrel and thus provide a useful means for identifying the PMBSF. (×40.)

with a narrow, ventrally located tier of cells extending into sublamina Vb.

Our procedures for WGA-HRP and cytochrome oxidase are not compatible on the same section. Therefore, to confirm the sublaminar position of the cells of origin of these two pathways, we stained the barrels and retrogradely labeled cells within the same section by a combination of techniques for demonstrating the presence of succinic dehydrogenase and WGA-HRP. Fig. 4*A* illustrates the results from a case in which the caudate putamen had been injected with WGA-HRP. The barrels are clearly revealed in the upper part of the figure by the presence of succinic dehydrogenase. The most superficially located of these retrogradely labeled cells are located just beneath the barrels and extend throughout the depth of sublamina Va with a band of labeled cells extending into the superficial part of sublamina Vb. Fig. 4*B* illustrates a case in which the pontine nuclei had been injected with WGA-HRP. The barrels are clearly visible and the retrogradely labeled cells can be seen located below a clear gap, which is completely devoid of labeled cells. The gap corresponds precisely with the extent of sublamina Va. Thus, although the combination of two histochemical techniques reduces the sensitivity of each of the methods, the results confirm the calculations made on the basis of more sensitive but less direct evidence of the differential projections from the two sublaminae.

Lamina V of the somatosensory cortex contains cells that project to other subcortical targets, including the superior colliculus, (2, 25, 28, 29), the thalamus (2, 26, 28, 30), and the trigeminal nucleus (27). Because, in many sections, every pyramidal cell is labeled in sublamina Vb after pontine injection, it seems likely that the axons of some of these Vb pyramidal cells must bifurcate. We have been studying the pattern of bifurcation by fluorescent double-labeling techniques. Fig. 5 shows a representative case in which the pontine nuclei were injected with the fluorescent tracer true blue, and the superior colliculus was injected with a different



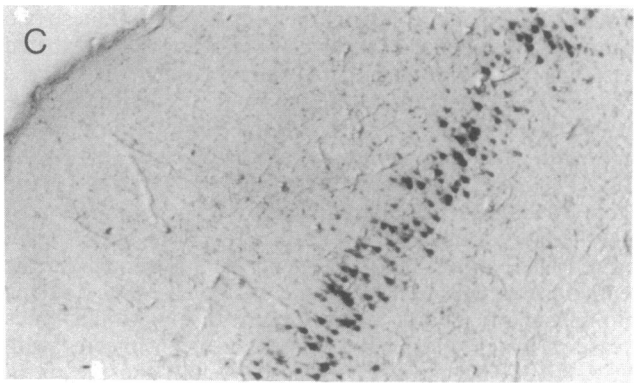
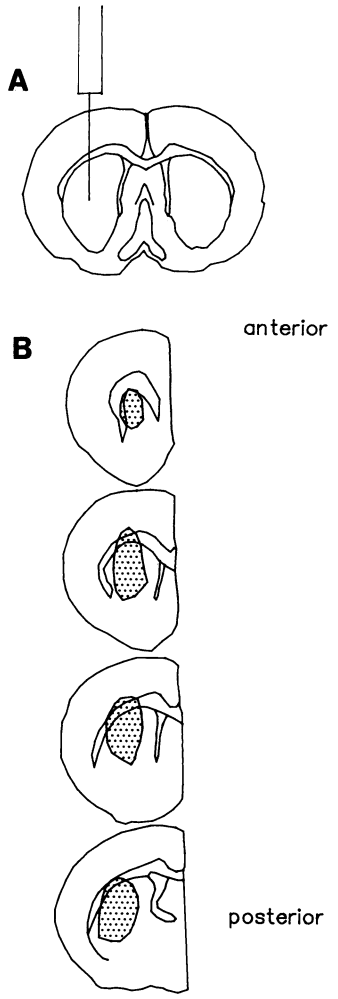


FIG. 2. A midsagittal section through the brainstem (*A*) to show the rostrocaudal level of five sections through the pontine nuclei (*B*) after an injection of WGA-HRP into the pontine nuclei. The extent of the primary injection is shown as stippling and encompasses the entire pontine projection from the PMBSF. (*C*) The resulting retrogradely labeled cells within the PMBSF. (×45.)



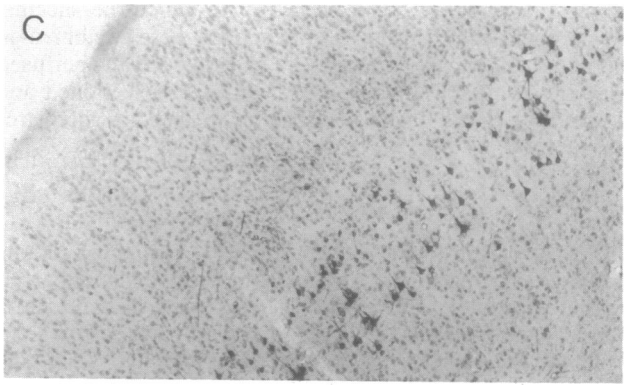


FIG. 3. The injection route (*A*) and sections through the basal ganglia (*B*) after an injection of 0.2 μl of 4% WGA-HRP into the caudate putamen. The extent of the primary injection site is shown as stippling and encompasses the striatal projections from the PMBSF. (*C*) The resulting retrogradely labeled cells within the PMBSF. (×45.)

fluorescent tracer, diamidino yellow. Confirming the observations made with WGA-HRP, the labeled pyramidal cells were evenly distributed throughout sublamina Vb of the PMBSF. About one-quarter of these cells also had a yellow-labeled nucleus. We conclude that most lamina Vb cells send an axon to the pons and that roughly one-quarter of these branch to send a collateral to the superior colliculus.

DISCUSSION

The two main targets of layer V of the PMBSF are the pontine nuclei and striatum. The cells of origin that project to these two targets are centered in distinct sublaminae, although a small number of corticopontine cells located at the upper border of lamina Vb probably send a collateral to the basal ganglia. Our evidence from double-labeling studies suggests that roughly one-quarter of Vb corticopontine cells have an axon that bifurcates and projects to the superior colliculus.

Corticopontine pyramidal cells that project to the pontine nuclei are distributed in a continuous band in sublamina Vb and are sharply restricted to that sublamina. The apparent clustering of retrogradely labeled cells seen in previous

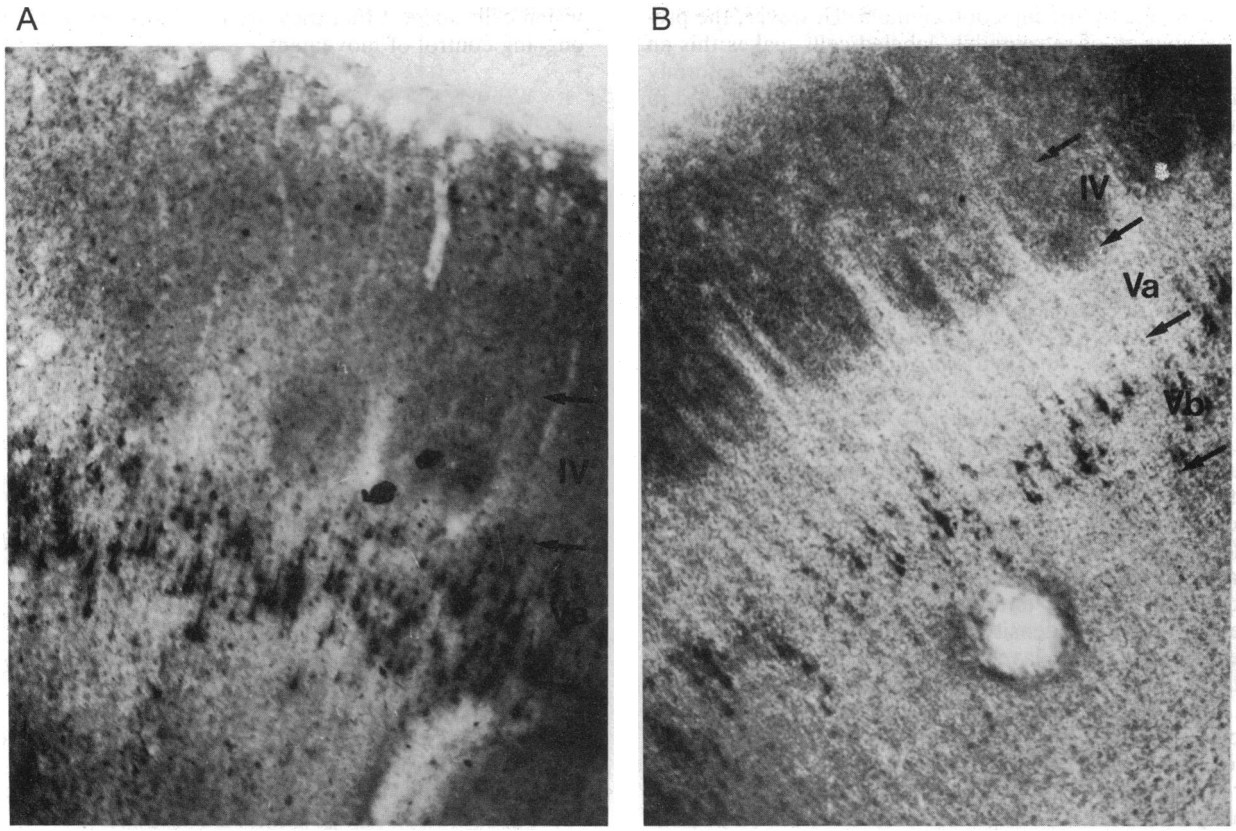


FIG. 4. The location of the barrel field and retrogradely labeled cells after an injection of WGA-HRP into the caudate putamen (*A*) and pontine nuclei (*B*). The sections were processed to reveal simultaneously the presence of the enzyme succinic dehydrogenase and WGA-HRP retrogradely labeled cells. By applying this combined technique, the sublaminar location of corticopontine and corticostriate cells was confirmed. (×75.)

studies (2, 7, 8) may have resulted from incomplete labeling of the entire set of the corticopontine fiber terminals or the low sensitivity of the available reaction techniques.

In the cases in which the WGA-HRP injection filled the striatal targets of cells in the PMBSF, the retrogradely labeled cells were confined within sublamina Va of the PMBSF with a narrow extension into the most superficial portion of layer Vb. Since the caudate putamen is penetrated by fasciculated corticopontine fibers, some or all of these Vb cells might have been retrogradely labeled as a result of

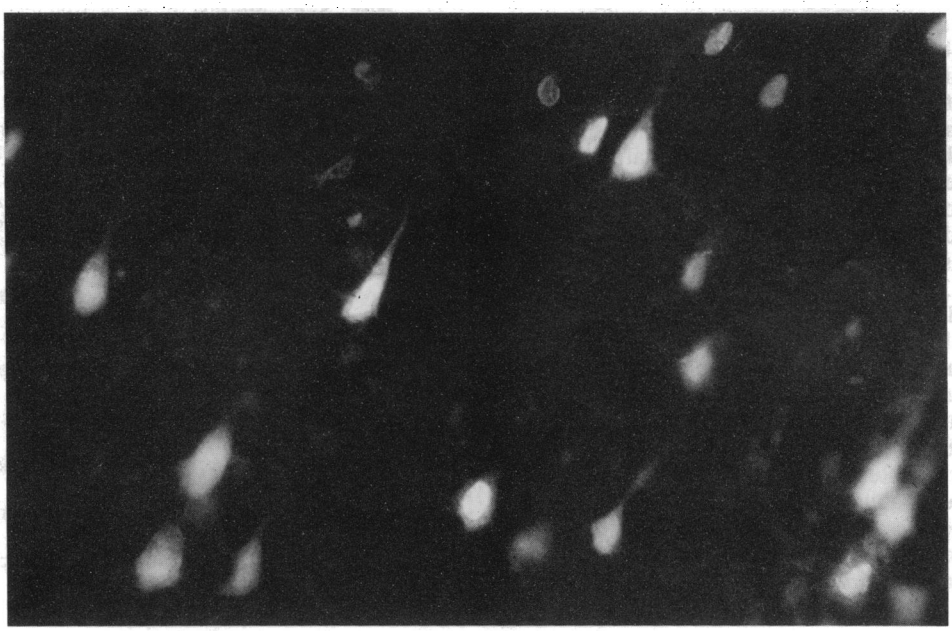


FIG. 5. Photomicrograph of a section through the PMBSF showing cells that display a blue fluorescent cytoplasm (resulting from an injection of true blue into the pontine nuclei) and in some instances yellow fluorescent nuclei (resulting from an injection of diamidino yellow into the superior colliculus). (×225.)

axonal damage by the injection cannula. However, the precise distribution of retrogradely labeled cells makes this an unlikely source of experimental artifact. Cells were uniformly distributed across the PMBSF and were always confined to the superficial part of the lamina Vb. Similarly, it is unlikely that WGA-HRP is taken up by undamaged fibers of passage (36). These superficial lamina Vb cells may be the same class of cells that were identified by Donoghue and Kitai and others (19–23) as having an axon in the internal capsule with a collateral branch to the striatum.

Other studies have been directed at establishing the cells of origin of corticostriatal projections in several mammalian species (2, 6, 15–23, 37, 38). Some studies have reported that, following injections of tracers into the striatum, retrograde labeling was not restricted to cells in cortical layer V (15–18). In the present study, we found retrogradely labeled corticostriatal cells strictly confined to layer V of the PMBSF. There are two possible sources of error in our results. (*i*) The striatal injection may have failed to encompass all of the terminals of corticostriatal axons. (*ii*) Our methods may have been biased and failed to identify all corticostriatal cells. However, the primary injection site appears to include all of the area of the striatum that receives terminals from the PMBSF (2, 31). Moreover, the great density and the range in size of retrogradely labeled cells in layer Va suggests that the WGA-HRP was transported retrogradely by axons of all caliber. It seems most likely that the reported variations in the laminar origin of corticostriatal cells must relate to the type of cortex studied. In previous work, corticostriatal cells outside of layer V were found in frontal areas of cortex; hence, the disparity in results must represent a fundamental difference in efferent corticostriatal organization of different cortical areas.

Although the distribution of corticostriatal cells extended slightly into Vb, the great majority were restricted to Va. All of the corticopontine cells were restricted to Vb. Accordingly, with the exception of cells at the border of these two sublaminae, the striatal- and cerebellar-directed pathways remain distinct at the cortical level. Recent anatomical evidence in monkeys (5) suggests that the thalamic targets and cortical projections of these two great systems also remain segregated.

Our preliminary evidence (unpublished data) using double-label techniques suggests that nearly all of the other subcortically directed fibers to the thalamus or the trigeminal nucleus arise from bifurcations of either corticopontine or corticostriate axons. For technical reasons, we have not yet attempted by double-labeling techniques to demonstrate directly a bifurcated corticopontine corticostriatal projection arising from these superficial Vb cells. While uptake by damaged or unbroken fibers of passage is minimal when WGA-HRP is used as a retrograde tracer, injection of fluorescent compounds results in a large zone of necrosis within the injection site (39). Therefore, there would be the likelihood of spurious double-labeling from damaged corticopontine fibers caused by the striatal injection.

The evidence suggests that pyramidal cells in lamina Vb relay sensory information rapidly from the whiskers to the cerebellum in contrast with slower transmission by cells in lamina Va to the basal ganglia. The rapidly activated, fast conducting sensory system relaying to the cerebellum is reminiscent of an analogous pathway in cats. Cells in lamina V of area 18 and the lateral suprasylvian area are activated at short latency and project directly to the pontine nuclei (40). The receptive fields of corticopontine somatosensory and visual cells suggest that they are both specialized for rapid, ongoing control of movement.

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