

Axonal conduction properties of antidromically identified neurons in rat barrel cortex

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Abstract

Physiological studies of the rodent somatosensory cortex have consistently described considerable heterogeneity in receptive field properties of neurons outside of layer IV, particularly those in layers V and VI. One such approach for distinguishing among different local circuits in these layers may be to identify the projection target of neurons whose axon collaterals contribute to the local network. *In vivo*, this can be accomplished using antidromic stimulation methods. Using this approach, the axonal conduction properties of cortical efferent neurons are described. Four projection sites were activated using electrical stimulation: (1) vibrissal motor cortex, (2) ventrobasal thalamus (VB), (3) posteromedial thalamic nucleus (POm), and (4) cerebral peduncle. Extracellular recordings were obtained from a total of 169 units in 21 animals. Results demonstrate a close correspondence between the laminar location of the antidromically identified neurons and their anatomically known layer of origin. Axonal properties were most distinct for corticofugal axons projecting through the crus cerebri. Corticothalamic axons projecting to either VB or POm were more similar to each other in terms of laminar location and conduction properties, but could be distinguished using focal electrical stimulation. It is concluded that, once stimulation parameters are adjusted for the small volume of the rat brain, the use of antidromic techniques may be an effective strategy to differentiate among projection neurons comprising different local circuits in supra- and infragranular circuits.

Key words: antidromic stimulation, barrel cortex, cortical efferents, local circuits, cerebral peduncles, thalamus, motor cortex

Introduction

Physiological and anatomical findings from a variety of species and cortical regions indicate broad similarities in the columnar organization underlying the processing and transformation of afferent inputs (Hubel and Wiesel, 1962; Mountcastle, 1979). Early microelectrode investigations showed that cells at different depths within a cortical column display characteristic differences in peripheral receptive field properties, suggesting that different layers of a cortical column contain different types of local circuits. Even within the same lamina, nearby cells may participate in different circuits. Thus, anatomical findings have shown that neurons within the same neuropil may receive quite different patterns of thalamocortical inputs, and, at least in the case of efferent neurons, these patterns vary according to their projection targets (White and Hersch, 1982; Zarzecki, 1991).

The rodent whisker/barrel system is a useful model for studying cortical mechanisms underlying sensory processing. The correspondence of an individual whisker with anatomically well-defined cell groups in layer IV has greatly facilitated analysis of local

circuits there. Such analyses are more difficult outside of layer IV because of the greater variety of inputs and outputs. A consistent finding is that non-granular neurons, particularly those in layers V and VI, display considerable heterogeneity with respect to a variety of receptive field properties (Simons, 1978, 1985; Brumberg *et al.*, 1999). Unlike layer IV, where different types of local circuits, e.g., barrel vs septum, are anatomically distinguishable, different circuits in non-granular layers are spatially intermingled, with no obvious cytoarchitectural signatures. Because different projection neurons receive different patterns of thalamocortical, and probably other, synapses, it nevertheless may be possible to distinguish different circuits in these layers by identifying the target of projection neurons whose axon collaterals contribute to the local network. *In vivo* such identification can be accomplished using antidromic stimulation methods (Swadlow, 1989, 1994; Zarzecki, 1991). Such approaches, however, have been used relatively infrequently in rodents.

As a prelude to more detailed studies of local circuitry in non-granular laminae of the barrel cortex, we have characterized the axonal properties of four classes of cortical efferent neurons using

antidromic methods. Results demonstrate a close correspondence between the laminar location of the antidromically identified efferent neurons and their known layer of origin. We conclude that, once a number of technical difficulties related to the small size of the rat brain are considered, the use of antidromic techniques can be an effective strategy to distinguish among heterogeneous local circuits outside the barrels.

Materials and methods

Experiments were conducted on 21 adult, female Sprague-Dawley rats (250–350 g, Hilltop, Scottsdale, PA). Animals were cared for in accordance with Institutional Animal Care and Use Committee policies.

Initial surgical preparation

Anesthesia was initially induced with Metofane (methoxyfluorane, Pitman-Moore, Mundelein, IL), and animals were subsequently maintained on 1–2% halothane anesthesia during the remainder of the surgical procedures. The trachea and external jugular vein (Harms and Ojeda, 1974) were cannulated. After exposing the skull, small stainless steel screws were placed over the left frontal and occipital cortices to anchor an acrylic base; one of the screws was attached to a ground wire. A steel post was embedded in the acrylic base and used to hold the head in a fixed position with unimpeded access to the whiskers. The rat's head was positioned in the approximate plane of the Paxinos & Watson Atlas (Paxinos and Watson, 1982). Stereotaxic coordinates, estimated using bregma as a landmark, and physiologic mapping procedures were used to locate the appropriate sites for both electrical stimulation and cortical recordings. A core temperature of 37°C was maintained throughout the experiment using a servo-controlled heating blanket (Harvard Apparatus) Holliston, MA.

In most experiments, animals were subsequently maintained in a deeply anesthetized state using i.v. Nembutal (pentobarbital sodium) or halothane. In a final series of seven experiments, animals were maintained during the recording sessions in a lightly narcotized state using fentanyl sedation, a synthetic opiate (Sublimize, Jansen, Titusville, NJ, pharmaceuticals: 5–10 mg/kg/h), and were immobilized using pancuronium bromide to prevent spontaneous whisker movements. Immobilized animals were maintained using positive pressure ventilation. Details of the methods and the monitoring of the animals during the experiments have been described previously (Brumberg *et al.*, 1996).

Electrophysiologic identification of antidromic stimulation sites

Our initial experiments examined thalamic and motor cortex projecting neurons. Subsequently, we conducted additional experiments to identify cortical neurons projecting specifically to the posteromedial nucleus of thalamus (POm) and those projecting to the brainstem via corticofugal axons in the crus cerebri. In all cases, we identified the stimulation sites physiologically, matching the vibrissal representations in those areas with that of the cortical recording site in the barrel cortex. Histologic verification was used to confirm our stimulation and/or recording sites.

In nine experiments, under halothane anesthesia, we identified the vibrissa representation in motor cortex using intracortical microstimulation through Teflon-coated stainless steel wires (diameter = 125 μ m) insulated to within 0.5 mm of a beveled tip. Thirty-ms trains of 0.2 ms duration pulses were delivered at 300 Hz through the stimulating electrode at depths of 1,300–1,500 μ m; this depth was selected to target the large pyramidal cells in the output layer of the motor cortex. Current intensity required to evoke movement of 1–3 whiskers under halothane anesthesia ranged from 200 to 900 μ A and was found to be highly dependent on the depth of anesthesia.

Antidromic activation of putative corticothalamic fibers was accomplished using stimulation techniques described by Swadlow (1989). The representation of the whiskers was first determined using lacquer-coated tungsten or stainless steel microelectrodes to record whisker-evoked multi-unit activity. Following this, an array

of two or three wires, as above, spaced \sim 0.5 mm apart were positioned at subcortical locations that corresponded topographically to the cortical recording site. This was verified by again recording whisker-evoked multi-unit activity from each of the wires. Later experiments employed single stainless steel microelectrodes for antidromic stimulation (see Results). In experiments employing POm stimulation, the whisker representation in VB was identified as above, and the stimulating electrodes were positioned directly medial to VB in a location where whisker-evoked responses could be elicited (Carvell and Simons, 1986; Diamond *et al.*, 1992).

For antidromic activation of corticobulbar neurons, stimulating electrodes were inserted into the white matter of the caudal diencephalon at coordinates of -4.0 A-P, 2.5 – 3.5 M-L, and 7.7 – 8.0 mm deep, according to the atlas of Paxinos and Watson. Placement in the crus cerebri was confirmed physiologically using electrical stimulation to elicit whisker movements at low current thresholds.

Criteria for antidromic identification

The method of activating and identifying antidromic responses was adopted from Swadlow's studies in rabbit somatosensory cortex (Swadlow, 1989, 1990). Monophasic pulses (0.1 ms duration) at 0.8 Hz were produced using a Grass Instruments, West Warwick, RI, (S-48) stimulator and a constant voltage stimulus isolation unit. Polarities were set to evoke the lowest threshold responses at a given stimulation site. Neurons were considered to be antidromically activated if the following conditions were met: (1) the presence of a discrete threshold; (2) an invariant latency; and (3) a refractory period < 2 ms. We also noted the presence or absence of a supernormal conduction period defined as an increase in conduction velocity in response to the second of two pulses spaced 8–10 ms apart. We calculated supernormality as the percent change in antidromic latency (Swadlow *et al.*, 1978).

The use of collision tests was limited because many antidromically activated units lacked peripherally driven receptive fields and were not spontaneously active. When employed, collision tests confirmed the antidromic nature of the response (see Fig. 1). Units were isolated using an amplitude window discriminator and acceptance pulses were used to trigger the Grass

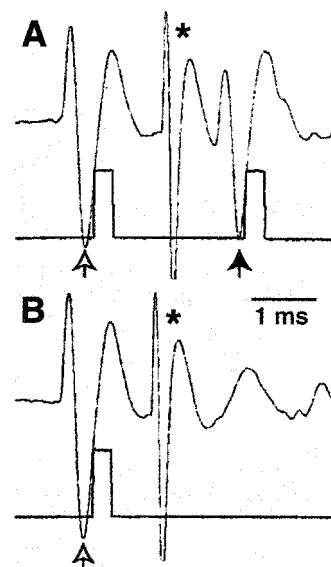


FIGURE 1. Collision test verifying antidromic activation. (A) Upper trace shows the oscilloscope recording of the spontaneous spike elicited from the barrel cortex (open arrow on left) used to trigger the electrical stimulus (denoted with the asterisk), which in turn evoked the antidromic spike (filled arrow on right). (B) As the interval is shortened (1.41 ms), the spontaneous and antidromic spikes collide, preventing the latter's arrival at the cell body. Note that there is no closed arrow on right in (B). Lower trace in panels A & B shows acceptance pulse from window discriminator used to trigger the delayed stimulus.

stimulator (delayed trigger). Frequently, synaptically evoked (orthodromic) responses were robust and nearly latency invariant. In these cases, a conditioning current was used to help discriminate such responses from antidromic ones. The conditioning stimulus was presented 30 ms prior to the test stimulus, rendering the synaptic response relatively refractory to the second pulse. Synaptic, but not antidromic, responses were abolished or greatly diminished under these conditions.

Estimating conduction velocity

The conduction distances between the cortical recording site and the stimulation sites were estimated using the atlas of Paxinos and Watson. Conduction velocity was then calculated using the obtained latency measure (in ms) divided by the estimated conduction distance (in mm) to obtain a measure in m/s. The following were used as the estimated distances from the barrel cortex: VB = 5.0 mm; Crus = 6.5 mm; Motor cortex = 7.0 mm and POm = 5.25 mm.

Single unit recordings

Extracellular single unit recordings were obtained using tungsten or glass microelectrodes (5–10 M Ω), positioned over the barrel cortex and advanced perpendicular to the pial surface using a stepping microdrive equipped with a digital counter. Antidromically identified units could be found reliably only when the stimulating and recording electrodes were topographically aligned. Data were obtained from neurons at various depths and in one or more penetrations in all animals. Electrolytic lesions (7 μ A anodal current \times 10 s) were made to confirm depth readings. Because marker lesions were not made in every track, recording location is defined by microdrive readings rather than cortical laminae. In general, layer IV corresponds to 800–1,000 μ m.

Histology

At the conclusion of the experiment, animals were given a lethal dose (100 mg/kg) of pentobarbital sodium and transcardially perfused with either formalin or 4% paraformaldehyde solution. The brain was removed, placed in the perfusion solution overnight, and then put in a 30% sucrose solution for 24–48 h. Tangential or coronal sections (60 μ m) were processed for cytochrome oxidase and/or Nissl staining. Electrode tracks from recording and stimulating electrodes, including lesion markers, were verified histologically.

Results

A summary of the identified classes of neurons and their axonal conduction properties is found in Table 1. A total of 168 units were studied in 21 animals.

Neurons antidromically activated by motor cortex stimulation

We identified 45 barrel cortex neurons that were antidromically activated by motor cortex stimulation. Corticocortical neurons were found throughout the depths of the barrel cortex. Figure 2 (panels C & D) shows the distribution of conduction velocities and supernormality of these neurons. Neurons having the slowest CVs (mean = 1.5 m/s; range = 0.51–6.1) were found at the most superficial and deep levels of the cortex, whereas neurons in the middle depths (800–1,400 μ m) conducted more rapidly (mean CV = 3.0 m/s; range = 0.4–6.0). Supernormality was least likely to be observed for these fast conducting, middle depth neurons. Many of the most superficially located neurons displayed increases in conduction velocity \geq 10%.

Neurons antidromically activated by VB stimulation

Initially we stimulated VB using an array of two or three widely spaced, large diameter wires (see Methods). Thirty-six units in six rats were identified. Antidromically activated neurons were found only when the thalamic stimulating and cortical recording electrodes were precisely aligned topographically. Also, the highest yield of antidromic units was obtained when the stimulating array was positioned such that the pair (or triplet) of wires was oriented mediolaterally so as to be in the same functionally identified barreloid or in immediately adjacent barreloids.

Figure 2 (panels A & B) shows CVs and supernormality as a function of recording depth. Antidromically identified neurons were found throughout the full extent of the infragranular cortex, and this group varied widely with respect to both conduction velocity (range: 0.58–5.95 m/s) and supernormality (0–26%) (see Table 1). There was some depth-dependence to these properties. For

TABLE 1. Summary of data

Class of neurons	<i>n</i>	Conduction velocities (m/s)	% supernormality
Corticothalamic	36	2.5 \pm 1.5 (0.58–5.95)	3.6 \pm 5.1 (0–26)
Corticofugal	36	5.9 \pm 1.6 (2.2–9.0)	0 0
Cortico-VB	15	2.8 \pm 1.4 (1.0–5.43)	5.8 \pm 4.9 (0–14)
Cortico-POm	36	1.82 \pm 1.4 (0.39–5.2)	2.4 \pm 2.7 (0–8.2)
Corticocortical	45	2.2 \pm 1.5 (0.38–6.4)	4.1 \pm 5.8 (0–26)

Values are means \pm SDs; numbers in parentheses are ranges.

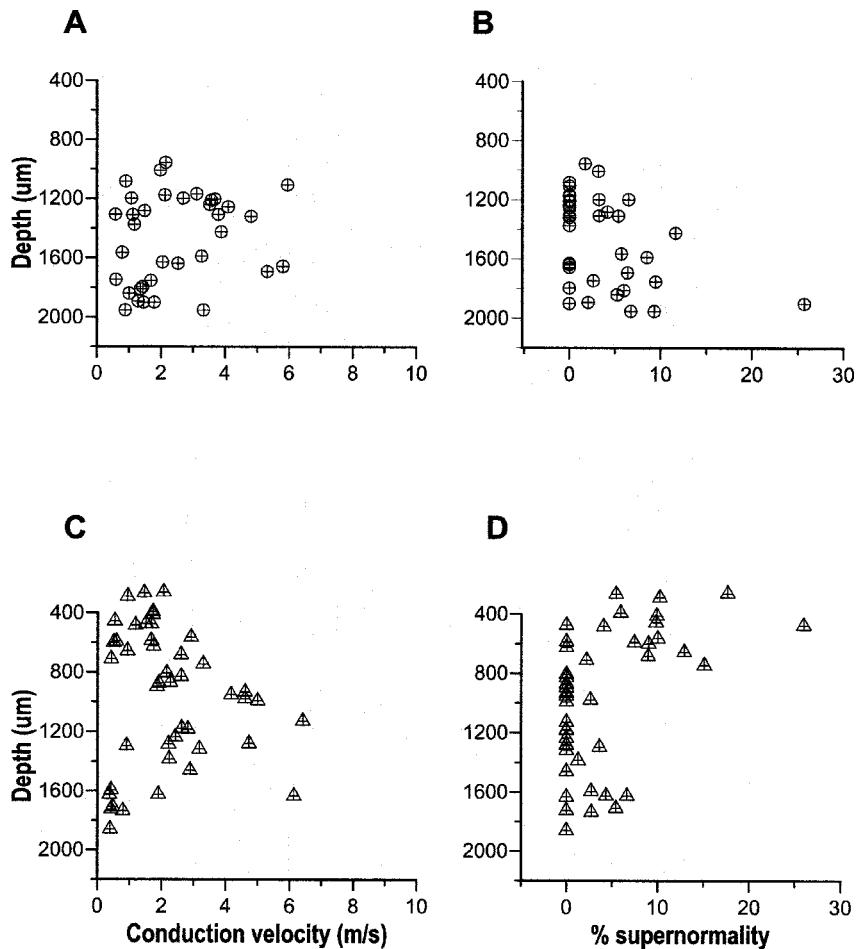


FIGURE 2. (A & B) Properties of corticofugal neurons activated using widely spaced electrodes to stimulate VB. (A) Relationship between microdrive reading (μm) and axonal conduction velocity. Symbols represent antidromic units obtained across all experiments in which the VB nucleus was stimulated. The faster conducting units tended to be located at the middle cortical depths. (B) Relationship between microdrive reading and the magnitude of supernormal period. Note the wide range of supernormal periods displayed by this group. The more superficially located units tended to have either no, or less than a 4% increase in antidromic latency. Symbols represent antidromic units obtained across all experiments. (C & D) Properties of corticofugal neurons activated by stimulation of the vibrissal representation in motor cortex. (C) Relationship between conduction velocity and microdrive readings. Note that the slowest conducting units tend to be located at the most superficial and deep aspects of the cortex. (D) Percent supernormality by microdrive reading of depth. Middle depth neurons, in addition to having faster conduction times, also displayed little or no supernormality. Symbols represent antidromic units obtained across all experiments.

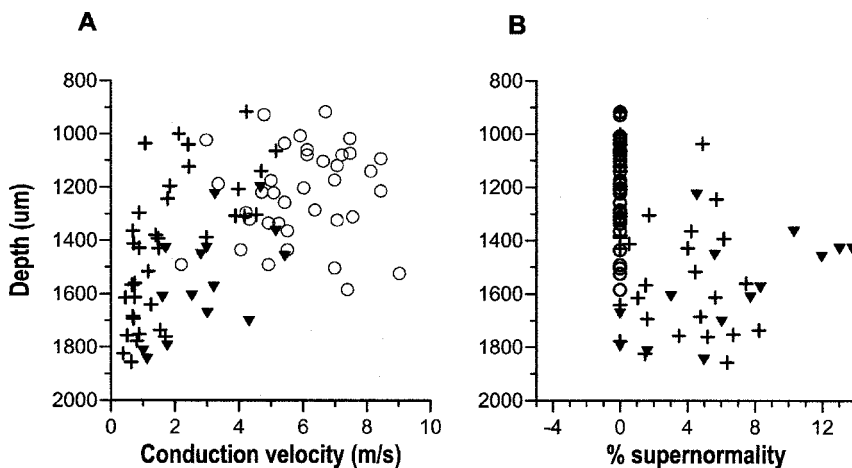


FIGURE 3. Properties of neurons activated using focal stimulation. (A) Relationship between microdrive reading (μm) and axonal conduction velocity for sites activated using focal stimulation of the crus cerebri (○), POM (+) and VB (▼). Symbols represent antidromic units obtained across all experiments. Note that POM- and VB-activated units had slower conduction times compared to the faster conducting units activated with crus cerebri stimulation. (B) Relationship between microdrive reading and the magnitude of supernormal period. Note that for units activated with crus cerebri stimulation, none displayed a supernormal period. In general, neurons activated at the deepest aspects of the cortex tended to display slow conduction times and greater supernormality compared to more superficial ones.

example, units located more superficially in the infragranular layers either lacked a supernormal period altogether or had less than a 4% increase. As well, eight of the ten fastest conducting neurons were located at depths of 1,100–1,350 μm , and only one had a supernormal period. In contrast, neurons recorded at the deepest locations had slower CVs, and most displayed supernormal periods.

Neurons identified using targeted stimulation sites

Results obtained with the relatively large VB-stimulating electrodes suggest that a heterogeneous population of corticofugal axons was activated. These were likely to include not only cortical-VB projecting neurons, but also cortical neurons projecting to nearby POM and/or corticobulbar fibers of passage in the adjacent internal capsule. In order to account for this heterogeneity, we conducted experiments in which we selectively targeted projection sites in POM and in the crus cerebri (see Methods). A final set of experiments employed single fine-tipped stainless steel electrodes ($\sim 8\text{--}10\text{ M}\Omega$) to produce focal VB stimulation.

Neurons activated by crus cerebri stimulation ($n = 36$) were relatively homogeneous (Fig. 3). They were found most often at depths of 1,100–1,400 μm and appeared to be good indicators of the recording electrode's entry into mid-layer V. These neurons had an average conduction velocity of 6.3 m/s (range = 2.2–9.0), which was the fastest of all the groups studied. Most striking, none showed a supernormal period of increased conduction.

Data obtained from neurons activated by stimulating electrodes confined to POM are shown in Figure 3. Even with this targeted stimulation, the population of antidromically activated neurons were heterogeneous with respect to cortical depth, conduction velocity and supernormality. POM-activated neurons were distributed uniformly throughout the infragranular laminae, from near the layer IV/V boundary to the white matter. The deepest neurons generally had the slowest CVs ($x = 0.86$; range 0.39–1.7) and were most likely to display supernormality. Approximately 20% of POM projecting neurons, all of which were located in upper layer V, had conduction velocities $> 3.0\text{ m/s}$ and were well within the range of the corticobulbar neurons. However, in contrast to the corticobulbar cells, 23% of the superficially located POM-activated units displayed supernormal periods, the remainder had little or no supernormality.

In the last set of experiments, we utilized single, fine-tipped metal microelectrodes in order to produce focal stimulation within VB. Except for two neurons, which were recorded at microdrive readings $< 1,250\ \mu\text{m}$, this sample of antidromically identified units was located relatively deep in the infragranular layers, corresponding to deep layer V and layer VI (Fig. 3). As observed with the neurons activated with POM and VB stimulation, axons of neurons recorded

at the deepest microdrive readings tended to display the slowest CVs. In contrast to the crus cerebri-activated and POM-activated neurons, about one-third of the VB-projecting neurons had conduction velocities $> 3\text{ m/s}$ and displayed supernormal periods $> 4\%$; these were most likely to be recorded at the layer V/VI boundary.

Peripherally driven receptive fields

Most of the antidromically identified units were recorded under conditions of general anesthesia, as the main goal of the experiment was to characterize their laminar and axonal properties. Under these conditions, most cells were unresponsive to peripheral stimulation using manual whisker deflections. Receptive field assessment was also difficult because of the burst firing characteristics of the neurons. We therefore conducted several experiments in which rats were maintained during the recording session in a lightly narcotized state using fentanyl sedation as described in detail previously (see Brumberg *et al.*, 1999). All 15 antidromically activated cells using focal VB stimulation were recorded under these conditions. Of these, only two units displayed peripherally driven receptive fields, even when controlled whisker stimuli were used to generate peristimulus histograms accumulated over repeated trials. Both of these peripherally responsive units were located relatively superficially in the infragranular layers (microdrive readings of 1,450 and 1,360 μm , respectively). Similar results were obtained for units activated by crus cerebri stimulation. Only one of nine antidromically identified units recorded under fentanyl sedation was responsive to whisker stimulation; this cell was located at a microdrive reading of 1,140 μm . None of the other VB- or crus cerebri-activated cells were responsive to whisker stimulation, and most displayed little or *no* spontaneous activity. Interestingly, in every case unresponsive antidromically identified neurons were recorded at sites where nearby neurons were spontaneously active and peripherally driven.

Discussion

Previous studies by Swadlow and co-workers have yielded a detailed body of work in rabbits describing the laminar location, thalamocortical inputs and axonal properties of different classes of somatosensory cortical neurons based on their projection targets determined using antidromic stimulation methods (Swadlow, 1989, 1994; Swadlow and Weyand, 1987). In the present study, we adopted a similar approach to investigate the axonal properties of different classes of efferent neurons in the rat whisker/barrel cortex, with the longer term goal of characterizing such neurons with respect to their receptive field properties. In most respects, the findings obtained in the rat are comparable to those

from the rabbit, suggesting common organization features across species and across cortical areas, as well (see below).

Methodological issues

During the course of this study, we encountered a number of methodological problems related to the small size of the rat brain and to the specific anatomical characteristics of the whisker/barrel system. For experiments examining corticothalamic or corticocortical projections, the stimulating electrode, in either the thalamus or motor cortex, was located only a few mm from the cortical recording electrode. Because of short axonal conduction times, typically only several ms, the evoked spikes occurred close in time to the stimulus artifact, rendering visualization of the antidromic spikes difficult. This problem is exacerbated by the relatively small caliber of the axons, and hence their high stimulation thresholds, requiring large stimulus currents. Relatedly, descending axonal pathways and/or projection sites are in close proximity to each other, so that, for example, large stimulation currents applied to VB can readily activate lower threshold axons descending in the nearby internal capsule.

These problems were particularly acute when attempting to identify cortico-VB projecting neurons. Because VB and POM are immediately adjacent to each other and at the same rostrocaudal level, VB stimulation can produce unintentional activation of axons projecting to POM, as well as low threshold corticobulbar axons in the nearby internal capsule. In order to minimize this confound, we eventually utilized fine-tipped microelectrodes to produce focal stimulation, which appears to have been more selective in activating VB-projecting corticothalamic neurons (see below). Moreover, because of the high current densities produced near the tips of these microelectrodes, antidromic spikes could be elicited at relatively low currents, producing smaller stimulus artifacts as well as less orthodromic activation of thalamocortical afferents. Under these conditions, however, stimulating and recording microelectrodes must be positioned in precise topographic alignment. In the case of cortico-VB projections, the rat whisker/barrel system presents an additional constraint. Anatomical experiments have shown that cortico-VB projecting neurons have a clustered horizontal distribution, being located preferentially deep to the center of the overlying layer IV barrel (Chmielowska *et al.*, 1989). Even when electrodes appear to be precisely aligned with respect to whisker-evoked responses, there is a reduced likelihood of finding antidromic units if the recording electrode is not in a cluster of VB-projecting neurons. Finally, there is anatomical evidence suggesting that the terminals of cortico-VB neurons may be non-uniformly distributed in the target thalamic barreloid (Land *et al.*, 1995; Keller and Carlson, 1999; Wright *et al.*, 2000).

Properties of corticothalamic projecting axons

Our initial studies employing relatively large, bipolar stimulating wires in VB yielded a heterogeneous population of antidromic units in the barrel cortex. One group of units appeared to be quite distinctive with respect to their rapid conduction velocities, their lack of supernormality and their consistent location at the middle depths of the infragranular layers. Subsequent experiments using stimulating electrodes placed explicitly in the crus cerebri confirmed that such units were indeed representative of corticobulbar projecting neurons. In addition to their being located within a relatively narrow range of depths, the antidromic units obtained using direct stimulation of the crus cerebri were homogeneous, all but one having conduction times ≥ 3 m/s and *none* demonstrating a supernormal period. These properties are highly similar to those characterized by Swadlow as belonging to layer V corticofugal (CF-5) cells, the major difference being the slower CVs in the rat compared to the rabbit (means: 6.3 vs 13.9 m/s). The relatively large diameter of these axons, evidenced by their rapid conduction times, probably rendered them susceptible to even small currents that could spread to the internal capsule when large stimulating electrodes are placed in VB. We did not investigate whether such neurons have dual projections to other sites (Levesque *et al.*, 1996).

Even after accounting for the presumed corticobulbar neurons, our initial sample of antidromic units varied considerably with respect to axonal conduction properties and especially laminar location. In particular, many units were found at depths too superficial to be cortico-VB projecting (Chmielowska *et al.*, 1989; Zhang and Deschênes, 1997) but displayed relatively slow conduction velocities and large supernormal periods that were inconsistent with their being corticobulbar neurons. A second sample of units obtained using stimulating electrodes placed in POM yielded a similarly heterogeneous population. Comparable, superficially located neurons with slow conduction velocities and large supernormal periods have not been reported in the rabbit (Swadlow, 1989). Perhaps in the rabbit, the whisker representations in VB and POM are further apart, relative to those in the rat, in both the mediolateral and anteroposterior dimensions. A particularly striking finding is that the putative POM-projecting neurons are scattered throughout the infragranular depths; many appeared to be located as superficial as upper layer V and as deep as lower layer VI, near the white matter. This is consistent with retrograde labeling patterns observed after tracer injections into POM (Veinante *et al.*, 2000). The most deeply situated POM-activated units had the slowest conduction velocities, and this general relationship between depth and speed of conduction is similar to that reported by Swadlow (1989). The

findings suggest that POM-projecting neurons may vary widely in terms of their receptive field properties and, perhaps, their influences on thalamic neurons. In this respect, the corticofugal projection to POM may parallel its afferent pathway in being less tightly coupled to specific parameters of whisker stimulation compared to its lemniscal counterpart (Ahissar *et al.*, 2000; Pierret *et al.*, 2000).

We further attempted to account for the heterogeneity of our original sample by using fine-tipped microelectrodes to produce spatially restricted stimulation currents in VB. The highly focal nature of this stimulation was evidenced by our finding antidromic units *only* when the stimulating and recording electrodes were precisely aligned topographically and by the ability to use lower stimulation currents. As noted above, the same factors that improved the resolution of the stimulation also limited the likelihood of finding cortico-VB projecting neurons. Using this approach, most antidromically activated neurons were found within a relatively restricted range of cortical depths, corresponding closely to the known laminar distribution of VB-projecting neurons, which are located largely in lower layer V and upper layer VI with fewer neurons deep within the latter (Chmielowska *et al.*, 1989). Two-thirds of the units displayed supernormal periods > 4%, with 4 of 15 having values > 10%, the largest observed with thalamic stimulation. Unlike POM-identified units, virtually all displayed supernormal conduction periods. Similarly, 91% of layer VI thalamic projecting units in the rabbit had supernormal periods > 4%. Interestingly one outlier unit was located superficially (~ 1,200 μm) and displayed no supernormality, raising the possibility that even focal VB stimulation can activate axons of POM-projecting neurons as they course through VB. Again, we did not test whether any of the antidromic units had dual projection sites.

Taken together, our findings suggest that, in the rat, at least three different populations of corticofugal neurons can be activated when large stimulating electrodes are placed in VB. Corticobulbar axons can be recognized by their location in layer V, their rapid conduction (> 4 mm/s) and the absence of supernormality. Antidromic units located at approximately the same depth but displaying slower conduction velocities (< 2 mm/s) and supernormality are likely to project to POM. Disambiguating POM- from VB-projecting neurons is highly problematic when large stimulating electrodes are used. Though the deepest and most slowly conducting neurons are likely to project to POM, antidromically activated neurons in upper layer VI and lower layer V display ranges of conduction properties that are almost completely overlapping within the two populations. The use of focal VB stimulation, using a single microelectrode, considerably reduces the likelihood of activating axons of efferent neurons other than those that

project to VB, though even here results must be interpreted with caution as axons of passage to POM might still be activated.

Cortical efferent neurons in the rat, as in the rabbit, clearly display differences in baseline conduction velocity and its modification by immediately preceding impulse activity (supernormality). Corticobulbar neurons appear capable of faithfully and rapidly transmitting the temporal sequence of action potentials discharged at the soma to distant sites where they may modulate both afferent signals in the trigeminal nuclei and efferent whisking control centers in the brainstem. Corticothalamic neurons (both VB and POM projecting) vary widely with respect to conduction velocity and supernormality. Slower conducting axons tend to display greater activity-dependent increases in conduction velocity, perhaps rendering slower arriving signals more efficacious by means of temporal summation (see Waxman and Swadlow, 1977). Together, the findings suggest that, as proposed by others (Landry and Dykes, 1985), corticothalamic systems may exert a range of effects.

Properties of corticocortical efferent projections

We also examined a class of cells that were antidromically identified as corticocortical projecting. These were activated by stimulation in the physiologically defined vibrissa motor cortex. Consistent with anatomical findings (Izraeli and Porter, 1995), such units were found throughout the cortical depths, with some concentration in layers III and V. The conduction properties of the latter group can be directly compared with those of the corticothalamic and corticobulbar projection neurons, which are located at the same laminar depths. Corticocortical and corticofugal units were similar to each other in that cells located more deeply in the infragranular layers had slower conduction velocities and were more likely to display supernormality, whereas those located more superficially were faster conducting and showed little or no supernormality. Motor cortex projecting neurons in the supragranular layers had relatively slow conduction times and displayed the largest supernormal periods compared to all other classes studied. Thus, despite their common projection target, motor cortex projecting neurons differed widely in their conduction properties, based on their laminar location.

Unresponsive neurons

In several experiments we attempted to characterize the receptive field properties of the antidromic units. We found that the vast majority of those activated with focal VB stimulation were silent and unresponsive to peripheral stimulation of the whiskers; only 2 of 15 units displayed peripherally driven

receptive fields. Similarly, only one of nine crus cerebri projecting units had a demonstrable peripheral receptive field. As noted above, in every case, nearby units were spontaneously active and had whisker-driven receptive fields. Thus, without the benefit of antidromic activation, many extracellularly studied efferent neurons would be undetectable using standard electrophysiologic techniques. Similarly, Landry and Dykes (1985) reported that 63% of antidromically activated CT neurons in SI of anesthetized cats were unresponsive to tactile stimulation, and Swadlow reported, in awake, restrained rabbits, that 45 and 49% of CT neurons in the vibrissa and forelimb cortex, respectively, were not peripherally driven (Swadlow, 1989, 1990). In a more recent study, 89% of CT neurons were found to be unresponsive (Swadlow and Hicks, 1996). In both cats and rats, large percentages of previously unresponsive cells in the somatosensory cortex had receptive fields that were unmasked using glutamate microiontophoresis (Dykes and Lamour, 1988). In the case of CT cells, these physiological findings are somewhat surprising in light of anatomical data, in mice, demonstrating that VB-projecting corticothalamic neurons receive a relatively abundant number of thalamocortical synapses (White and Hersch, 1982). A recent anatomical study in rats (Zhang and Deschênes, 1998) described a substantial projection from vibrissa motor cortex to layer VI of the barrel cortex, raising the possibility that motor commands for whisking may directly influence the excitability of CT and other corticofugal neurons, perhaps rendering them responsive to whisker deflection during active whisking.

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