Layer V Neurons in Mouse Cortex Projecting to Different Targets Have Distinct Physiological Properties

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Hattox AM, Nelson SB. Layer V neurons in mouse cortex projecting to different targets have distinct physiological properties. J Neurophysiol 98: 3330–3340, 2007. First published September 26, 2007; doi:10.1152/jn.00397.2007. Layer V pyramidal neurons are anatomically and physiologically heterogeneous and project to multiple intracortical and subcortical targets. However, because most physiological studies of layer V pyramidal neurons have been carried out on unidentified cells, we know little about how anatomical and physiological properties relate to subcortical projection site. Here we combine neuroanatomical tract tracing with whole cell recordings in mouse somatosensory cortex to test whether neurons with the same projection target form discrete subpopulations and whether they have stereotyped physiological properties. Our findings indicate that corticothalamic and -trigeminal neurons are two largely nonoverlapping subpopulations, whereas callosal and corticostriatal neurons overlap extensively. The morphology as well as the intrinsic membrane and firing properties of corticothalamic and corticotrigeminal neurons differ from those of callosal and corticostriatal neurons. In addition, we find that each class of projection neuron exhibits a unique compliment of hyperpolarizing and depolarizing afterpotentials that further suggests that cortical neurons with different subcortical targets are distinct from one another.

INTRODUCTION

Cortical pyramidal neurons in the infragranular layers provide the major output of the cortex and are therefore likely to be important modulators of sensory and motor processes. Many attempts have been made over the last 100 years to classify different subtypes of cortical neurons based on similarities in morphological and physiological properties. However, unlike in the vertebrate retina and in some invertebrate preparations, such as the stomatogastric ganglion of decapod crustaceae where there are defined neuronal cell types with distinct properties (Masland 2001; Selverston et al. 1998), the boundaries between distinct subtypes of cortical neurons are unclear. It is also unclear whether subtypes defined by morphological or physiological properties correlate to subpopulations of cortical neurons with the same set of axonal projections.

Physiologically, two major classes of pyramidal cells have been described in layer V based on differences in their intrinsic firing properties: intrinsically bursting cells, which fire bursts of action potentials in response to depolarizing current injection, and regular spiking cells, which fire trains of single action potentials (Connors et al. 1982; McCormick et al. 1985). Efforts to relate these physiological properties to morphology revealed that intrinsically bursting cells have thick apical dendrites, whereas regular spiking cells have thinner apical dendrites (Chagnac-Amittai et al. 1990). However, analysis of additional parameters, including axonal and dendritic branching patterns, spike frequency adaptation, and afterpolarizations (Agmon and Connors 1992; Gottlieb and Keller 1997; Kang and Kayano 1994; Tsiola et al. 2003), reveals a rich diversity of layer V pyramidal cells. With so many subtypes now described, it is not clear how neurons with similar properties are related to one another or how they are organized in the cortex.

One possibility is that cortical pyramidal neurons with different cortical and subcortical projection targets have distinct physiological properties (Molnar and Cheung 2006). Neurons within layer Va of the barrel cortex project callosally to the contralateral cortex, intracortically to regions including the motor and secondary somatosensory cortex and to the striatum (Bernardo et al. 1990; Hoeflinger et al. 1995; Koraek et al. 1990). The large pyramidal cells of layer Vb have subcortical projections to regions including the posteroomedial thalamic nucleus (POm), principle and spinal trigeminal nuclei (Sp5), superior colliculus, pontine nuclei, spinal cord, and striatum (Chmielowska et al. 1989; Deschenes et al. 1994; Killackey et al. 1989; Mercier et al. 1990; Wise and Jones 1977a). The properties of identified corticotectal, corticoconic, and callosal cells of the primary visual cortex have been well described in several species. Corticortical and -pontine cells are morphologically and physiologically similar to each other and distinct from callosal neurons (Hallman et al. 1988; Hubener and Bolz 1988; Kasper et al. 1994; Rumberger et al. 1998; Schofield et al. 1987; Wang and McCormick 1993). Corticocortical and -pontine cells are thick tufted, intrinsically bursting cells found in lower layer V, whereas callosal neurons have smaller somata and are thin tufted, regular-spiking cells found in upper layer V. In addition to these classes of projection neurons, rat corticospinal and cat pyramidal tract neurons of the sensorimotor cortex have been studied and found to have diverse properties (Calvin and Sypert 1976; Tseng and Prince 1993). The physiological properties of other classes of projection neurons, however, have been almost entirely neglected. In this study, we combine neuroanatomical tract tracing with whole cell recordings from corticothalamic, corticotrigeminal, corticocortical, and callosal neurons to determine whether neurons with specific subcortical targets form distinct subpopulations with characteristic firing properties.
**METHODS**

**Retrograde labeling of cortical projection neurons**

C57 black 6 mice (20–34 days) were anesthetized with 70 mg/kg ketamine, 3.5 mg/kg xylazine, and 0.7 mg/kg acepromazine. Surgery was performed using sterile technique, and body temperature was maintained using a thermostatically regulated heating pad. The mice were placed in a stereotaxic device, and a small craniotomy was made to expose the area above the targeted region. A glass micropipette (35 μm tip) containing rhodamine- or fluorescein-labeled fluorescent latex microspheres, LumaFluor Retrobeads (Naples, FL) (Katz et al. 1984), was lowered using stereotaxic coordinates. Retrobeads (0.5 μl) were pressure injected using a Picospiritzer (General Valve; 40 psi, 5-ms pulse duration). For injections in the contralateral cortex, the pipette was advanced perpendicularly to the pial surface to three sites along the rostrocaudal axis of the somatosensory and motor cortex to a depth of 0.8 mm. Neighboring sites were separated by 0.5–1.0 mm. Injections in the POm, striatum, and Sp5 were made using an oblique approach to minimize inadvertent labeling in the barrel cortex, midbrain, and brain stem. For injections in the POm and striatum, a craniotomy was made to expose the area above the caudal region of the visual cortex and the micropipette was advanced with a 40° angle from the vertical. The Sp5 was accessed by advancing the micropipette between the posterior portion of the cerebellum and rostral portion of the brain stem at a 40° angle from the vertical.

**Electrophysiology**

At least 48 h postinjection, mice (23–28 days) were anesthetized with isoflurane and used to prepare 300-μm-thick coronal slices containing primary somatosensory cortex. The slices recovered in a holding chamber for 1 h at 37°C in oxygenated artificial cerebrospinal fluid (ACSF) with final concentrations of (in mM) 126 NaCl, 3 KCl, 2 MgSO₄, 1 NaH₂PO₄, 25 NaHCO₃, 2 CaCl₂, and 10 dextrose. Slices were transferred to a thermo-regulated recording chamber and continuously perfused with oxygenated ACSF containing the following synaptic blockers (in continuously perfused with oxygenated ACSF containing the following synaptic blockers (in μM): 50 2-amino-5-phosphonovaleric acid (APV), 20 6,7-dinitroquinoxaline-2,3-dione (DNQX), and either 20 bicuculline methobromide or 50 picrotoxin. Cortical projection neurons were identified by their content of fluorescent RetroBeads and laminar position. Visually guided whole cell patch-clamp recordings were made using near-infrared differential interference contrast microscopy. Recording pipettes of 3–5 MΩ resistance contained internal solution with final concentrations of (in mM) 20 KCl, 100 K-glucuronate, 10 HEPES, 4 Mg-ATP, 0.3 Na-GTP, 10 Na-phosphocreatine, and 0.1% bicytin. Recordings were obtained from cells in layer V of primary somatosensory cortex using an AxoPatch 200B amplifier (Axon Instruments, Foster City, CA), filtered at 10 kHz and were not corrected for liquid junction potentials. Data were collected on a Dell computer using custom software running on Igor Pro (WaveMetrics, Lake Oswego, OR). Analyses of recorded responses were performed in Igor and Matlab (The Mathworks, Natick, MA).

**Reconstruction and morphological analysis of recorded cells**

Cells were filled with biocytin through the recording pipette and fixed overnight in a buffered solution containing 4% paraformaldehyde. To visualize labeled cells, sections were rinsed in PBS, incubated for 20 min in PBS with 0.5% Triton-X, and incubated for 1 h in PBS with 0.5% Triton-X and Texas Red Avidin D (1:500; Vector Laboratories, Burlingame, CA). Apical dendrite length was the distance between the shaft at the base of the dendrite to its most superficial point directly above the soma. An apical dendrite was counted as a primary apical dendrite if it bifurcated from the main apical dendrite, and extended to and formed a dendritic tuft near the pia. Apical tuft width was the horizontal width of the full dendritic tuft, which included tufts of all primary apical dendrites. Apical dendrite shaft width was measured as the width of a horizontal ellipse with axes including the maximal length and width of the cell body.

**Physiology analysis**

We plotted the relationship between the amount of injected current and the firing frequency. To calculate the initial slope of the firing-current (FI) relationship, we analyzed responses of cells to threshold currents for generating action potentials and responses to two times the threshold current. The difference in the number of action potentials in the two traces was divided by the difference in current levels. To capture the slower phase of spike frequency adaptation, which may represent a biologically separate process from fast adaptation, we defined the adaptation ratio as the ratio of the third interspike interval to the last interspike interval. This adaptation ratio was analyzed using responses recorded at two times the threshold current. To calculate action potential characteristics, including firing threshold and after-potentials, we analyzed responses of cells to threshold currents for generating action potentials. Firing threshold is calculated as the internodal membrane potential at which dV/dt equals 20 V/s (Bekkers and Delaney 2001). To measure post spike potentials, we searched for minimum values within 50 ms of the spike and maximum values within 70 ms of this minimum. If the minimum value is more hyperpolarized than both the action potential threshold and the measured maximum voltage, the fast afterhyperpolarization (fAHP) amplitude is defined as the difference between the firing threshold and the average membrane potential in a small time window about the minimum value. The depolarizing afterpolarization (DAP) amplitude is the difference between the fAHP minimum and the average membrane potential in a small time window about the maximum value. Spike height is defined relative to firing threshold. Half-width is the width of the action potential at its half-height. Quantitative data are expressed as the means ± SE.

**Histology**

In separate anatomical experiments, Lumafluor Retrobeads were injected into the POm, Sp5, striatum, or contralateral cortex (2 animals for each injection site). Forty-eight hours after injections, mice were anesthetized, perfused with 4% paraformaldehyde and sectioned at 40 μm. For NeuN immunofluorescent labeling, sections from these animals were incubated in PBS, incubated in PBS with 5% normal goat serum and 0.3% H₂O₂ for 30 min, and incubated in PBS with the neuronal nuclei monoclonal antisera (NeuN, 1:2000; Chemicon International, Temecula, CA) for 48 h at 4°C. Sections were then rinsed in PBS and incubated in PBS containing Alexa Fluor 488 or 594 goat anti-mouse IgG (1:400; Invitrogen, Carlsbad, CA). In some mice, double injections of red and green Retrobeads were made in the POm and Sp5 (n = 2) or in the striatum and contralateral cortex (n = 2). Sections from these animals were triple labeled by incubating them in PBS with 0.3% Triton-X and NeuroTrace blue fluorescent Nissl stain (1:500; Molecular Probes, Eugene, OR) for 18 h.

**Quantification of labeled cells**

We used unbiased stereological methods (Howard and Reed 1998) to quantify retrogradely labeled projection neurons and NeuN immunoreactive neurons in layer V of the barrel cortex. The optical dissector method was used with a forbidden plane analysis. Acquired images were digitized using a Leica spectral scanning confocal microscope and Leica confocal software (Leica, Wetzlar, Germany). To estimate the thickness of layer 5 and establish a counting frame height, we distinguished boundaries between cortical layers based on differences in cell size and density after NeuN immunohistochemical staining. Whereas the border between layers 4 and 5 is clear, the border between layers 5 and 6 is a bit more ambiguous. We therefore
used NeuN-labeled sections that also contained labeled corticotrigeminal neurons found exclusively in deep layer 5. In every case, the boundaries we previously distinguished based on differences in cell morphology were located just beneath the deepest corticotrigeminal neurons. In these sections, we calculated the average thickness of layer 5 to be 298.2 ± 4.0 (SE) μm. We therefore used a 300-μm thick counting frame height and aligned at its top with the border of layers 4 and 5. Injections of retrograde tracers are likely to cover only a fraction of a projection’s target area and are therefore unlikely to label all neurons projecting to that area. We therefore analyzed sections with dense retrograde labeling in the barrel cortex (3–4 sections per animal) and centered the width of the counting frame about the maximum density of retrogradely labeled neurons. For sections that contained a single retrograde tracer and were double labeled with NeuN, as well as sections that contained tracers injected in the striatum and contralateral cortex, we used a counting frame of 400 × 300 × 18 μm. In sections that contained labeled corticocortical and -trigeminal neurons, there were few double-labeled neurons. To obtain a more accurate percentage of double-labeled neurons in these sections, we used a large counting frame (1,200 × 300 × 18 μm). Within these counting frames, retrogradely labeled and NeuN immunoreactive neurons were examined along the z axis of the tissue at 2-μm increments. Approximately 160–220 NeuN-labeled neurons were counted in each section. We calculated the numerical density (Nv) of labeled neurons (per mm³) as follows: Nv = Q/ΣVdis where ΣQ is the total number of cells counted by all optical dissectors and ΣVdis is the total volume of the counting frames. Quantitative data are expressed as the means ± SE.

RESULTS

Subpopulations of cortical projection neurons

Corticothalamic projections to the POm arise from pyramidal neurons in layers V and VI (Chmielowska et al. 1989). The thalamic projections from layer V have been shown to be collaterals of long-range corticofugal axons that project more caudally (Deschenes et al. 1994). To test whether layer V corticothalamic projections are collaterals of corticotrigeminal axons, we injected animals with fluorescent retrograde tracers in the POm and the spinal trigeminal nucleus, interpolar part (Sp5). Retrogradely labeled corticothalamic neurons were found in lower layer V and layer VI, whereas corticotrigeminal neurons were found exclusively in lower layer V (Fig. 1A). A higher-magnification image of the labeling in layer V is shown in Fig. 1B. Figure 1C shows photomicrographs of Lumafluor red and green Retrobead injections restricted to the POm and Sp5. Using stereological techniques, we counted the total number of neurons in layer V that were labeled with red, green, or both red and green Retrobeads. Few neurons were double labeled compared with the number of single-labeled neurons (Fig. 1, B and D). In all sections analyzed, double-labeled neurons represented only 1.9 ± 0.8 and 3.1 ± 1.2% of the total number of corticotrigeminal (n = 279) and corticothalamic (n = 189) neurons, respectively. Corticotrigeminal neurons
have also been reported to have little overlap with corticotectal (3.1%) and pyramidal tract (7.4%) neurons (Killackey et al. 1989). Although these percentages are likely to underestimate the true overlap, our findings suggest that the main axon of cortical neurons with collaterals to the thalamus is not the trigeminal nucleus. Instead, these projections may terminate on other known caudal targets of barrel cortex neurons including the spinal cord and medullary reticular formation (Landry et al. 1984; Wise and Jones 1977a). Further, our findings suggest that corticothalamic and -trigeminal neurons are two largely distinct subpopulations of cortical projection neurons.

Callosal neurons are primarily located in layers III and upper layer V (Ivy and Killackey 1981). Callosal neurons in the primary visual cortex do not send collaterals to the superior colliculus (Hallman et al. 1988), suggesting that callosal neurons may also be largely nonoverlapping with other lower layer V corticofugal neurons including corticothalamic and -trigeminal neurons. Intratelencephalic-type (IT) corticostriatal neurons, found mainly in upper layer V, send ipsilateral projections to the striatum and cortex as well as contralaterally to either the striatum or cortex (Wilson 1987). To determine the degree of overlap between callosal and corticostriatal neurons, we injected red Retrobeads in the striatum and green Retrobeads in multiple sites of the contralateral somatosensory and motor cortex and looked for the proportion of neurons labeled with both retrograde tracers. Retrogradely labeled corticostriatal and callosal neurons were located mainly in upper layer V but were also found in lower layer V (Fig. 1E and F). There is a second major class of corticostriatal neurons, the pyramidal tract-type (PT) corticostriatal neurons, which are found mainly in lower layer V. Their striatal projection is a collateral of the main axon which passes through the pyramidal tract on its way to the brain stem and spinal cord (Landry et al. 1984; Reiner et al. 2003). This suggests that striatal injections retrogradely labeled both IT and PT corticostriatal neurons, although it is possible that we inadvertently labeled corticothalamic axons when lowering the pipette to the striatum. Analysis of physiological properties described in the following text, however, revealed differences in firing properties between corticothalamic and most corticostriatal neurons found in lower layer V. Across all sections analyzed, double labeled neurons represented 15.1 ± 0.8 and 31.5 ± 3.1% of the total number of corticostriatal (n = 359) and callosal (n = 171) neurons, respectively (Fig. 1G). Although a considerable proportion of corticostriatal neurons have callosal projections, the overlap is not complete. This is consistent with the prior observation of subtypes of corticostriatal neurons, including PT-type neurons that project through the pyramidal tract, and intratelencephalic or IT-type neurons that have ipsilateral projections to the striatum and cortex, but do not project callosally (Donoghue and Kitai 1981; Wilson 1987).

Composition of cortical layer V

Corticothalamic, corticotrigeminal, and callosal neurons are largely nonoverlapping subpopulations of layer V neurons. Corticostriatal neurons, however, have extensive projections to the contralateral cortex and the pyramidal tract. We next estimated the percent of layer V neurons that made up each of these four classes of projection neurons. Mice received single injections of Retrobeads in the POM, Sp5, striatum, or contralateral cortex. We stained sections from these mice for NeuN immunohistochemistry (Fig. 2, A and B), which labels nuclei of excitatory and inhibitory neurons (Tamamaki et al. 2003). We then counted the total number of cells in layer V labeled retrogradely and immunohistochemically. As in the previous experiment, we analyzed only sections where dense retrograde labeling was observed (3–4 sections per animal) and centered the counting frame about the maximum density of

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**FIG. 2.** Composition of layer V of the barrel cortex. A: photomicrograph of a section containing retrogradely labeled corticotrigeminal neurons (red) and stained for NeuN immunohistochemistry (green), demonstrating that corticotrigeminal neurons represent only a fraction of layer V neurons. B: photomicrograph of a section containing retrogradely labeled callosal neurons (green) and stained for NeuN immunohistochemistry (red). C: pie chart depicting the representation of corticothalamic, corticotrigeminal, corticostriatal and callosal neurons, as well as the overlap in their distributions, as a percent of the total number of neurons in layer V. The estimated proportion of interneurons is from the stereological study of Ren et al. (1992). D: using stereological techniques, we quantified the number of retrogradely labeled projection neurons and plotted them as a percent (means ± SE) of the total number of neurons in layer V.
labeled neurons in the barrel cortex. The mean numerical density of NeuN labeled neurons in layer V of the barrel cortex was $90.9 \pm 5.3 \times 10^3$ neurons per mm$^3$. Our results indicate that corticothalamic and -trigeminal neurons make up a small percentage ($7.6 \pm 0.9$ and $7.2 \pm 0.8$) of total neurons in layer V, whereas callosal ($17.8 \pm 1.1\%$) and corticostriatal ($26.5 \pm 1.4\%$) neurons account for a larger fraction (Fig. 2, C and D). Inhibitory interneurons have been estimated using stereological methods to make up approximately $30\%$ of neurons in layer V of the rat barrel cortex (Ren et al. 1992). Taken together, our results reveal that the projection neurons included in our study account for $>50\%$ of layer V neurons and $>75\%$ of the estimated layer V excitatory pyramidal cells.

Morphological differences between subpopulations of cortical projection neurons

Morphological differences have been observed between callosal and corticotectal cells in the visual cortex of several species (Hallman et al. 1988; Hubener and Bolz 1988; Kasper et al. 1994). They have also been observed between subtypes of layer 5 neurons and pyramidal tract neurons in the cat and monkey motor cortex (Deschenes et al. 1979; Hamada et al. 1981; Samejima et al. 1985; Spain et al. 1991). To test whether cells included in this study also exhibited morphological differences, cells were labeled with biocytin. All corticothalamic ($n = 10$; Fig. 3A) and corticotrigeminal neurons ($n = 21$; Fig. 3B), and most corticostriatal ($n = 14$; Fig. 3, C and D) neurons extended apical dendrites that terminated in dendritic tufts near the pia. Unlike the layer 5 callosal neurons of rat visual cortex (Hallman et al. 1988; Hubener and Bolz 1988; Kasper et al. 1994), the apical dendrites of all layer 5 callosal neurons in mouse somatosensory cortex also extended their apical dendrites to layer 1 ($n = 16$; Fig. 3E). Callosal neurons had significantly shorter apical dendrites than the other three classes of projection neurons (Fig. 3F) likely because most callosal neurons are found in the upper part of layer 5. In the majority of corticotrigeminal (19 of 21) and corticothalamic (7 of 10) neurons, the apical dendrite bifurcated into two to four primary apical dendrites that each terminated in a dendritic tuft near the pia. This bifurcating branching pattern was observed in only 2 of 14 corticostriatal (Fig. 3C) and 2 of 16 callosal neurons. The remaining corticostriatal and callosal neurons (Fig. 3, D and E) had a single apical dendrite that terminated in a dendritic tuft. Corticotrigeminal neurons had significantly more primary apical dendrites than callosal and corticostriatal neurons (Fig. 3G). Further, there was a trend for the corticothalamic neurons to have fewer primary apical dendrites than corticotrigeminal neurons and more apical dendrites than callosal neurons. The laminar positions of the bifurcation points were typically observed near the border of layers 3 and 4 and were not different between subtypes of projection neurons. In every case, bifurcation points were observed $>200\mu m$ below the pia. In contrast, terminal dendritic tufts began branching more superficially in neurons with single and multiple primary dendrites that terminated in dendritic tufts near the pia.

![FIG. 3. Morphology of identified cortical projection neurons.](http://jn.physiology.org/)
apical dendrites. The widths of the apical dendritic tufts of corticothalamic and corticotrigeminal neurons were significantly wider than those of callosal neurons (Fig. 3H). The projection neurons all had first-order branches of the apical dendrites, mainly found in layers 4 and 5. However, there were significantly more dendritic branches in corticothalamic and -trigeminal neurons than in callosal neurons and corticostriatal neurons (Fig. 3J).

In rat primary visual cortex, the diameter at the base of the apical dendrites of corticocortical cells is wider than that of callosal neurons (Kasper et al. 1994). Similarly, the apical dendrite shaft of mouse corticothalamic and corticotrigeminal neurons (4.19 ± 0.28 and 5.34 ± 0.17 μm, respectively) is significantly wider than the dendritic shaft of both callosal and corticostriatal neurons (3.01 ± 0.24 and 2.73 ± 0.35 μm; P < 0.001, Sheffe post hoc comparison). Moreover, the apical dendrites of layer 5 projection neurons terminated almost entirely in layers 5 and 6 and there were similar numbers of dendrites between the classes of projections neurons all had first-order branches of the apical dendrites, mainly

Corticothalam and corticotrigeminal neurons differ physiologically from callosal and corticostriatal neurons

We next asked whether cortical projection neurons with different targets had characteristic intrinsic membrane and firing properties. We made whole cell patch-clamp recordings from identified corticothalamic (n = 18), corticotrigeminal (n = 19), corticostriatal (n = 28), and callosal (n = 16) neurons. To study the intrinsic membrane and firing properties of these cells, we conducted experiments in the presence of antagonists of ionotropic glutamate and GABA receptors (APV, DNXQ, and bicuculline). At their resting membrane potentials (Table 1), corticothalamic and corticotrigeminal neurons did not fire spontaneously. In response to depolarizing current injections, most corticostriatal (14 of 19) and corticothalamic (13 of 18) neurons including those shown in Fig. 4, A and B, fired an initial doublet with an interspike interval of <12 ms. The doublet was typically followed by a particularly long, large-amplitude AHP. After this rapid phase of adaptation, the cells fell into regular trains of action potentials that exhibited little or no spike frequency adaptation. Neurons that did not fire an initial doublet also fired trains of action potentials at nearly constant interspike intervals. In contrast, most callosal (15 of 16) and corticostriatal (25 of 28) neurons showed strong spike frequency adaptation (Fig. 4, C and D). A similar distinction in adaptation has previously been reported for RS1 and RS2 cells (Agnon and Connors 1992), although RS1 cells did not exhibit large spike afterpotentials as we will later show for corticothalamic and corticotrigeminal neurons.

We quantified the slower phase of spike frequency adaptation, which we refer to as adaptation for the remainder of the text, as well as the slope of the FI curve and the threshold for action potential generation for each group of neurons projecting to the same target. The firing frequency of corticothalamic and -trigeminal neurons remains nearly constant, demonstrated by adaptation ratios of 0.96 ± 0.12 and 0.96 ± 0.19, respectively, and interspike intervals (ISI) that increased or decreased by <10% from the third interval.
The extent of adaptation is much larger in callosal and corticostriatal (0.43 ± 0.05 and 0.33 ± 0.03, respectively) cells and significantly different from the adaptation in corticothalamic and -trigeminal cells (P < 0.001, Scheffe post hoc comparison). Nearly half of the callosal and corticostriatal cells show more than a 200% increase from the third to last interspike interval. In response to a series of depolarizing currents, corticothalamic and -trigeminal cells have relatively uniform mean firing rates. The slopes of their FI curves are significantly larger than those of callosal and corticostriatal neurons (Fig. 4, G and H; P < 0.01, Scheffe post hoc comparison). The threshold for action potential generation is also significantly more negative in corticothalamic and corticotrigeminal neurons than in callosal and corticostriatal neurons (Table 1; P < 0.05, Scheffe post hoc comparison). In addition, the firing threshold in corticothalamic neurons is significantly more negative than the threshold in corticotrigeminal neurons (P < 0.05). These results indicate that the corticothalamic and -trigeminal neurons in layer Vb have characteristic firing properties that are significantly different from the callosal and corticostriatal neurons found primarily in layer Va. Therefore as in the visual cortex, we find a major division between long-range corticofugal neurons and callosal neurons in SI. They further suggest that there may also be differences between corticofugal neurons with different subcortical targets.

The membrane potentials of corticothalamic and -trigeminal neurons are significantly more positive than those of callosal and corticostriatal neurons, which may have contributed to the differences seen in spike frequency adaptation, FI slope and firing threshold. To test this possibility, we repeated these analyses in a subset of cells that were held at a membrane potential of –65 mV, including corticothalamic (n = 9), corticotrigeminal (n = 9), corticostriatal (n = 16), and callosal (n = 7) neurons. The adaptation ratios (P < 0.001) and FI slopes (P < 0.05) of corticothalamic and -trigeminal neurons as well as the firing threshold (P < 0.001) of corticothalamic neurons remained significantly different from those of callosal and corticostriatal neurons (Scheffe post hoc comparison). Within each group of cortical projection neurons, these values measured at −65 mV were not significantly different from values calculated at resting membrane potential (Student’s t-test). This suggests that factors other than membrane potential, such as the complement of ionic conductances, are responsible for differences in intrinsic firing properties.

Halide salts of bicuculline have been shown to block SK potassium channels at low concentrations (Khawaled et al. 1999), which may have altered some of the physiological measurements. We therefore made separate recordings from corticotrigeminal (n = 15) and callosal (n = 20) neurons, using picrotoxin in place of bicuculline in the bath solution. The resting membrane potentials, FAHP and DAP ampli-
tudes, adaptation ratios and FI slopes all remained significantly different between corticotrigeminal and callosal neurons (Student’s t-test, \( P < 0.01 \)). In addition, we compared properties of neurons with the same projection target recorded in either bicuculline or picrotoxin. Callosal neurons recorded in the two conditions exhibited no significant differences in the membrane or firing properties listed above, as well as in input resistance. Likewise, almost all of the membrane and firing properties of corticotrigeminal neurons recorded in picrotoxin were not significantly different from those recorded in bicuculline. In picrotoxin, there was a small but significant shift to a more hyperpolarized membrane potential of \(-65.4 \pm 1.2 \) mV (Student’s t-test, \( P < 0.05 \)).

Subcortical projection target correlates with action potential characteristics

Corticothalamic and -trigeminal neurons exhibit no differences in their passive membrane properties or in many of their intrinsic firing properties. However, our anatomical findings revealed that these two classes of projection neurons are largely nonoverlapping and differ in the morphology of their apical dendrites. We next tested whether neurons with different projection targets exhibited distinct action potential characteristics. We analyzed properties of the action potential including fAHPs and DAPs elicited in response to near-threshold depolarizing current pulses and found that each subtype of projection neuron produced a unique complement of afterpotentials. Shown in Fig. 5, A–F, are representative responses of different classes of cortical projection neurons to depolarizing current injection. Callosal neurons displayed either no afterpotentials (13 of 16) or very small afterpotentials after only the first spike in the train (3 of 16). In contrast, most corticothalamic (17 of 18) and all corticotrigeminal neurons produced prominent fAHPs and DAPs that followed every spike. Analysis of group data (Fig. 5, G and H; Table 1) revealed that the amplitudes of fAHPs in corticothalamic (7.0 ± 1.0 mV) and corticotrigeminal (3.6 ± 0.8 mV) neurons were significantly greater than the infrequent, small fAHPs observed in callosal neurons (0.1 ± 0.1 mV; \( P < 0.05 \), Scheffe post hoc comparison). Corticothalamic (7.2 ± 0.8 mV) and corticotrigeminal (2.3 ± 0.4 mV) neurons also had significantly larger DAPs than callosal neurons in which they were largely absent. Further, the amplitudes of corticothalamic fAHPs and DAPs were significantly larger than those of corticotrigeminal neurons (\( P < 0.01 \), Scheffe post hoc comparison), indicating that neurons with different subcortical projection targets can have unique firing properties.

Axons of corticostriatal cells also terminate in the contralateral cortex, contralateral striatum and pyramidal tract. In addition, Levesque et al. (1996b) found that in the rat all layer V corticothalamic neurons in the second somatosensory cortex have collaterals to the striatum. The corticostriatal projection neurons correspondingly exhibited several types of firing patterns (Fig. 5, D–F). The majority of corticostriatal cells (26 of 28) showed strong spike frequency adaptation (Fig. 5F). Of these, 19 had no, or very small spike afterpotentials. Biocytin reconstructions of these

FIG. 5. Projection neurons with different subcortical targets have different action potential characteristics. Corticothalamic and -trigeminal neurons display prominent fast afterhyperpolarizations (fAHPs) and depolarizing afterpolarizations (DAPs, A and B). In contrast, afterpotentials are largely absent in callosal cells (C) and variable in corticostriatal cells (D–F). Analysis of group data revealed that afterpotential amplitudes of corticothalamic and corticotrigeminal cells are significantly greater than those of callosal and corticostriatal neurons (G and H). Moreover, the fAHP and DAP amplitudes of corticothalamic neurons are significantly greater than corticotrigeminal neurons indicating that projection neurons with different subcortical targets have different intrinsic firing properties. Group data are plotted as means ± SE, and statistics are reported as described in Fig. 3.
neurons revealed that these neurons had thin apical dendrites and were located in superficial layer V. Corticostriatal cells that adapt and have no afterpotentials physiologically and morphologically resemble callosal neurons, as well as corticostriatal neurons that project bilaterally to the striatum (Morishima and Kawaguchi 2006). They are therefore likely to be IT-type corticostriatal neurons. The remaining seven adapting neurons exhibited large-amplitude fAHPs (4.1 ± 1.6 mV) and DAPs (2.3 ± 0.6 mV), had thin apical dendrites and were found in layer V (Fig. 5E). Corticospinal cells have heterogeneous properties that include adapting neurons with fAHPs and smaller somatic areas (Tseng and Prince 1993). Therefore these may be PT-type corticostriatal neurons the main axon of which projects through the pyramidal tract. The remaining 2 of 28 corticostriatal neurons showed little spike frequency adaptation as well as large amplitude fAHPs and DAPs (Fig. 5D). They were located in lower layer V and were the only two corticostriatal neurons with thick apical dendrites. The properties of these corticostriatal neurons closely resembled the morphological and physiological properties of corticothalamic neurons and thus may be representative of corticostriatal neurons that send a collateral to the thalamus.

DISCUSSION

Subpopulations of mouse cortical projection neurons are morphologically and physiologically distinct

As for pyramidal neurons that do and do not project corticofugally in the visual cortex of rats and other mammals, we find morphological and physiological differences between corticofugal and callosal neurons in primary somatosensory cortex of the mouse. These include significant differences in apical dendrite length, shaft and tuft widths, the number of primary apical dendrites, and the number of first-order dendritic branches. In addition, there were significant differences in resting membrane potential, action potential (AP) threshold, half-width, spike frequency adaptation, and fAHP and DAP amplitudes. Corticofugal neurons with different targets, including corticotectal, -pretectal, and -pontine neurons, have been found to have similar physiological properties (Rumberger et al. 1998; Wang and McCormick 1993), whereas corticospinal neurons have been found to have diverse properties (Tseng and Prince 1993). Unlike these corticofugal neurons, the cells included in our study with different subcortical targets have uniform properties that are different from one another.

Previous findings have shown that neurons that project to different subcortical targets occupy different laminar positions within layer Vb (Killackey et al. 1989; Wise and Jones 1977a). Further, although some corticofugal neurons project to multiple targets (Levesque et al. 1996a; Veinante et al. 2000), few retrogradely labeled corticotrigeminal neurons have collaterals to the thalamus, or to the superior colliculus or spinal cord (Killackey et al. 1989). Instead we find that corticotrigeminal and -thalamic neurons comprise largely discrete subpopulations of cortical neurons. Interestingly, Gao and Zheng (2004) showed that layer V corticothalamic neurons of primary motor cortex have distinct morphological features from another class of long range projection neurons: compared with the spiny apical dendrites of corticospinal neurons, corticothalamic neurons were largely spine free. Similarly we find significant morphological differences in the width of the apical dendrite shaft between corticothalamic and -trigeminal neurons. Further, these two classes of projection neurons exhibit uniform firing properties that are distinct from one another. In response to depolarizing current, corticothalamic and -trigeminal cells fired an initial doublet followed by repetitive firing without adaptation and prominent spike afterpotentials. However, the fAHPs and DAPs of corticothalamic neurons were significantly larger and their firing thresholds were significantly more negative than corticotrigeminal neurons. These findings suggest that corticothalamic and -trigeminal neurons are largely non-overlapping subpopulations of neurons with distinct morphological and electrophysiological properties.

Spike afterpotentials have also been observed in pyramidal tract and corticospinal neurons (Calvin and Sypert 1976; Koike et al. 1968, 1970; Stafstrom et al. 1984; Takahashi 1965; Tseng and Prince 1993) as well as in cat lumbar (Schwindt and Crill 1982), phrenic (Jodkowski et al. 1988), trigeminal (Takata et al. 1982), and rat hypoglossal motoneurons (Viana et al. 1993). They have been shown to be mediated by several distinct potassium and calcium conductances (Friedman et al. 1992; Schwindt et al. 1988). Although spike afterpotentials have been observed in several types of neurons, correlating their amplitudes with projection target has only been documented for fast and slow pyramidal tract neurons (Calvin and Sypert 1976). Here we reveal that corticothalamic, -trigeminal, and -striatal neurons have significantly different fAHP and DAP amplitudes. It is likely that similar types of conductances underlie the firing properties in corticothalamic, -trigeminal, and -striatal cells and that different levels of expression of specific channel subunits account for the observed differences in fAHP and DAP amplitudes.

The responses of corticothalamic and -trigeminal neurons to depolarizing current injection, an initial doublet followed by trains of nonadapting single action potentials, differed from rhythmic burst firing that has been described in corticotectal and unlabeled cells in the visual and sersorimotor corticies (Connors et al. 1982; Kasper et al. 1994; Rumberger et al. 1998). The primary somatosensory cortex has been shown to contain both corticoticellular and corticopontine neurons (White and DeAmicis 1977; Wise and Jones 1977b). Interestingly, recordings from cat motor cortex showed that two populations of pyramidal tract neurons exhibited either rhythmic burst firing or an initial doublet followed by a train of single action potentials (Calvin and Sypert 1976). It is therefore possible in SI that these patterns of firing are distinct and only found in discrete subpopulations of projection neurons where the latter pattern of firing is observed in corticothalamic and -trigeminal neurons and, as in the visual cortex, burst firing is exhibited by corticotectal and -pontine neurons.

There are several additional possibilities, including species related differences, which explain the discrepancy of cortical firing properties. Whereas most previous studies on the firing properties of identified neurons have been conducted in rat and cat, we recorded instead from projection neurons in mouse primary somatosensory cortex. In addition, several factors have been shown to shift the firing pattern of cortical neurons. The propensity of corticotectal neurons to burst is increased after prolonged exposure to anesthesia (Christophe et al. 2005). Potassium channels underlie spike afterpotentials and can pre-
vent bursting (Friedman and Gutnick 1989). The concentration of potassium ions in the ACSF may therefore also contribute to the burstiness of a neuron. Finally, in vivo intracellular studies in behaving animals have correlated transitions in firing pattern from intrinsically bursting to regular spiking with transitions between slow-wave sleep and waking or REM sleep, demonstrating that the firing pattern of individual cortical neurons is flexible (Steriade 2004; Steriade et al. 2001). It therefore may be possible to drive corticothalamic and corticotrigeniminal neurons to fire bursts of action potentials under particular recording conditions.

Diverse properties of corticostriatal neurons

In contrast to the uniform properties of corticothalamic and -trigeminal cells, the morphological and physiological properties of corticostriatal neurons, like those of corticospinal neurons (Tseng and Prince 1993), are heterogeneous. The majority of corticostriatal neurons we recorded from, like callosal neurons, showed strong spike frequency adaptation and no afterpotentials. However, some adapting corticostriatal neurons, as well as two weakly adapting neurons, showed prominent fAHPs and DAPs. These may be corticostriatal cells in lower layer V the projections of which are collaterals of axons that terminate in the pyramidal tract and thalamus. Although Levesque et al. (1996b) reported that all layer V corticostriatal neurons send a collateral to the striatum, we find that only a small proportion of corticostriatal neurons have firing properties that resemble corticothalamic neurons. Several factors may account for this discrepancy. Whereas the majority of cells reconstructed in their study were corticothalamic cells from lower layer V of the second somatosensory cortex, we recorded from corticostriatal neurons in both upper and lower layer V of primary somatosensory cortex. In addition, the striatal projection of corticothalamic neurons terminated in a narrow band in the dorsolateral region of the striatum just below the white matter. Although the pipettes containing retrograde tracers used in our study were targeted to the dorsolateral striatum, tracer deposits in this narrow region containing collaterals of corticothalamic neurons were likely only a fraction of the total injected volume. Because the sample of neurons we studied was influenced by the placement and volume of fluorescent beads, one limitation of the retrograde tracing technique, the sample therefore may not be a true representation of the relative density of different types of corticostriatal neurons.

Functional significance

The distinct intrinsic firing properties observed in cortical projection neurons may reflect differences in the readout of cortical activity they transmit to their targets. Cortical excitatory neurons provide massive recurrent excitation onto other excitatory neurons (Kisvarday et al. 1996); this has been suggested to play a role in amplifying their feedforward signals (Douglas et al. 1995). In addition to inputs from inhibitory neurons, strong spike frequency adaptation of corticocortical excitatory neurons, including callosally projecting neurons, may be essential for stabilizing the network and preventing runaway excitation. Corticofugal neurons in lower layer V instead exhibit little or no spike frequency adaptation as well as large amplitude fAHPs and DAPs. Spike afterpotentials are also hallmark properties of many described motoneurons and corticospinal neurons and have been suggested to be important for maintaining rhythmic firing in these cells (Schwindt and Crill 1982; Schwindt et al. 1988). Long-range targets of corticofugal neurons, including the thalamus and trigeminal nucleus, may therefore require a more linear readout of cortical activity.

The present results suggest that neuronal cell types in the mammalian neocortex, though perhaps represented by larger numbers of individual neurons, may be just as “identified” as in sensory structures like the retina and small networks like those that comprise invertebrate central pattern generators. Indeed, the majority of layer V excitatory pyramidal cells in the barrel cortex have been accounted for by the identified projection neurons included in this study. Although the properties of layer V pyramidal neurons are heterogeneous, by combining neuro-anatomical tract tracing with whole cell recordings, we demonstrate that cortical projection neurons with different targets form discrete subpopulations and have stereotyped physiological properties that are distinct from one another.

References


