Somatosensory Response Properties of Excitatory and Inhibitory Neurons in Rat Motor Cortex

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ABSTRACT

In sensory cortical networks, peripheral inputs differentially activate excitatory and inhibitory neurons. Inhibitory neurons typically have larger responses and broader receptive field tuning compared to excitatory neurons. These differences are thought to underlie the powerful feed-forward inhibition that occurs in response to sensory input. In the motor cortex, as in the somatosensory cortex, cutaneous and proprioceptive somatosensory inputs, generated before and during movement, strongly and dynamically modulate the activity of motor neurons involved in a movement, and ultimately shape cortical command. Human studies suggest that somatosensory inputs modulate motor cortical activity in a center excitation, surround inhibition manner such that input from the activated muscle excites motor cortical neurons that project to it, while somatosensory input from nearby, non-activated muscles inhibit these neurons. A key prediction of this hypothesis is that inhibitory and excitatory motor cortical neurons respond differently to somatosensory inputs. We tested this prediction with the use of multi-site extracellular recordings in anesthetized rats. We found that fast-spiking (presumably inhibitory) neurons respond to tactile and proprioceptive inputs at shorter latencies and larger response magnitudes compared to regular-spiking (presumably excitatory) neurons. In contrast, we found no differences in the receptive field size of these neuronal populations. Strikingly, all fast-spiking neuron pairs analyzed with crosscorrelation analysis displayed common excitation, which was significantly more prevalent than common excitation for regular-spiking neuron pairs. These findings suggest that somatosensory inputs preferentially evoke feed-forward inhibition in the motor cortex. We suggest that this provides a mechanism for dynamic selection of motor cortical modules during voluntary movements.
INTRODUCTION

Extensive research has focused on input processing within cortical networks of all sensory modalities. Comparisons between excitatory and inhibitory cortical networks consistently show that inhibitory interneurons are more broadly tuned and tend to response more strongly to inputs (Bruno and Simons 2002; Gibson et al. 1999; Keller 2001; Porter et al. 2001; Swadlow 2002). These increased response properties facilitate feed-forward inhibition in response to thalamocortical input (Agmon and Connors 1992; Beierlein et al. 2003; Gabernet et al. 2005; Gil and Amitai 1996; Inoue and Imoto 2006; Sun et al. 2006). This inhibition, widespread within the cortex, dominates excitatory responses and sharpens them in a center-surround arrangement whereby inputs originating from a neuron’s receptive field activate that neuron while inputs from adjacent regions inhibit it. The importance of this center-surround arrangement has been demonstrated clearly for processing of tactile [for principle versus adjacent whiskers see Brumberg et al. (1996); for whisker direction tuning see Wilent and Contreras (2005); for review see Miller et al. (2001)] and visual (Benevento et al. 1972; Blakemore and Tobin 1972; but see Ozeki et al. 2009) sensory input. In the auditory cortex, the same intracortical feed-forward inhibition underlies the ability to perceptually separate two temporally adjacent stimuli (Wehr and Zador 2005). This inhibition-mediated sculpting of excitatory cortical input is thought to be a key feature of perceptual processing.

Evidence points to a similar arrangement in motor cortex such that afferents originating from the muscle field represented by a motor cortical neuron are able to excite that neuron while, at the same time, inhibit motor cortical neurons projecting to other muscle fields (Rosenkranz and Rothwell 2003). In this study we address the question of how the properties of motor cortical neurons mediate this feed-forward arrangement. Somatosensory afferents—both
thalamocortical and corticocortical—target both excitatory and inhibitory neurons in motor
cortex (reviewed in Keller 1999). We hypothesize that, as in the somatosensory cortex, the
inhibitory neurons are more sensitive to these inputs, and will therefore fire readily in response
to somatosensory inputs. This will explain the widespread suppression that occurs in motor
cortex in response to somatosensory inputs (Rosenkranz and Rothwell 2003). However, when a
cortical neuron’s muscle field is stimulated (its excitatory center), somatosensory afferents will
transiently converge and summate to drive that cortical neuron to spike threshold. This will
explain the excitation of cortical neurons in response to peripheral stimulation of their muscle
field.
Consistent with our hypothesis, we found that, compared with excitatory neurons, inhibitory
neurons in motor cortex respond more readily to tactile stimulation. In addition, inhibitory
neurons are activated synchronously by widespread divergent input from the periphery. Taken
together these data are consistent with powerful feed-forward inhibition underlying
center-excitation, surround-inhibition evoked by afferent input during motor control.

MATERIALS AND METHODS

All procedures were approved by the University of Maryland School of Medicine Animal Care
and Use Committee. Experiments were conducted according to institutional guidelines and
federal regulations.

Surgical procedures
Six female Sprague-Dawley rats, weighing 250–300 g, were used in this study. Rats were
anesthetized with halothane (1–2%) and placed on a thermoregulated heating pad to maintain
body temperature at 37°C. Lidocaine was applied locally and a craniotomy was performed over
M1.

Extracellular recordings
Rats remained anesthetized under halothane, administered through a face mask, while head-fixed in a stereotaxic apparatus and depth of anesthesia was monitored every 15 min by testing reflexes to pinching of the skin and cornea stimulation. Recordings of local field potential were used continually to confirm that remained in Guedel’s anesthesia stage III-3 for the duration of the recordings. Extracellular recordings of single units were obtained with a 16-channel, multielectrode array (4 shafts separated by 125 µm, each having 4 recording sites separated by 100 µm; NeuroNexus Technologies, Inc., Ann Arbor, Michigan). The array was advanced through the hindlimb representation in M1 (0.5 to 1.5 mm anterior and 1 to 2 mm lateral to bregma; Sapienza et al. 1981). The depth of individual units was calculated based on an electrode map provided by NeuroNexus, and on micrometer readings. Spike waveforms were digitized through a Plexon (Dallas, TX) data acquisition system, sampled at 40 kHz and sorted off-line with Plexon’s Offline Sorter. Detected waveforms with a signal-to-noise ratio of 3:1 were recorded, all others were ignored. Autocorrelograms generated with NeuroExplorer software (Nex Technologies, Littleton, MA) were used to confirm that recordings were obtained from single units.

**Evoked Responses**

The tip of the array was advanced in steps of 50 µm and a handheld tactile probe (wooden, 2-mm diameter) was applied to the dorsal and plantar surfaces of the hindpaw while all 16 channels were simultaneously monitored. Receptive fields were defined as all locations on the surface of the skin which responded to brushing and tapping with the tactile probe with a clearly discernible increase in firing rate above spontaneous firing rate. Once a well-isolated unit was identified, its receptive field and the receptive fields of any other units detected through other channels were mapped with the handheld probe. Calibrated tactile stimuli (50 stimuli, 1 Hz, 5 msec duration; pipette tip attached to a linear DC-servomotor served as probe; V101
electro-dynamic mechanical stimulator, LDS Group, Royston, UK), delivered to the receptive field area evoking the greatest response, were recorded. Responses were recorded for all isolated units. Following tactile stimuli, vibratory stimuli (50 trains of 10 at 80 Hz, 6.25 msec duration) were applied. Spontaneous activity was then recorded for 90 seconds.

Data Analysis

Statistical analyses were performed with Intercooled Stata (Stata, College Station, TX). Between-group statistical comparisons were assessed with the nonparametric Wilcoxon Rank-Sum Test. K-means cluster analysis was performed to partition the waveform scatter plot into 2 clusters.

Classification of Units. Excitatory and inhibitory motor cortical neurons were identified based on their extracellular waveform patterns. The initial wave duration was measured from the onset to its recrossing of baseline, and the duration of the second phase was measured from the end of the initial wave to its recrossing of baseline (Bruno 2002).

Responses to Calibrated Stimuli. Time stamps of well-isolated units and of stimulus triggers were exported to Matlab software (MathWorks, Natick, MA) for analyses using custom written algorithms. To quantify neuronal responses to stimulation, peristimulus time histograms (PSTHs; 50 stimuli, 1 msec bin width) were constructed, and significant stimulus-evoked responses were defined as PSTH bins with response magnitudes that significantly exceeded (99% confidence interval) spontaneous activity levels, computed from a 100 msec period preceding the stimuli. Response onset was defined as the first of two consecutive bins (post-stimulus) displaying significant responses, and response offset as three consecutive bins in which response magnitude falls below the 99% confidence interval. Response magnitude was defined as the total number of spikes per stimulus occurring between response onset and offset. During vibratory stimulation the first stimulus was used as the
trigger. Responses to each stimulus in the vibratory stimuli train was also computed as the total number of significant spikes within the 12.5 msec period between each stimulation.

CROSS-CORRELATIONS. Spontaneous activity of isolated units (see above) was recorded for 15 min and used for cross-correlation analysis. The maximum distance between simultaneously recorded units was 250 µm. For each pair of units, crosscorrelograms (± 50 msec, 1 msec bin size) were computed to determine the probability of a spike from one unit (target) in relation to the spike from another unit (reference) as a function of time. Excitation of the target unit was indicated by a peak exceeding the upper 99% confidence limit, inhibition by a trough that dropped below the lower 99% confidence limit.

RESULTS

We hypothesized that excitatory and inhibitory neurons in the motor cortex respond differently to somatosensory input. Specifically, we hypothesized that inhibitory neurons would respond more readily and have larger receptive fields.

We used an established method to identify putative excitatory and inhibitory neurons from their extracellularly recorded waveforms (Mountcastle et al. 1969; Simons 1978). Units classified as fast-spiking units (FSUs) are widely accepted to represent parvalbumin-containing, GABAergic interneurons (DeFelipe et al. 2002; Kawaguchi 1995; Kawaguchi and Kubota 1996). The vast majority of regular-spiking units (RSUs) are excitatory, pyramidal projection neurons. However, some classes of cortical inhibitory neurons have action potential waveforms that meet the RSU criteria (Gibson et al. 1999; Kawaguchi and Kubota 1993). It is therefore likely that some of our RSU population represent inhibitory interneurons.

Classification of RSUs and FSUs

As has been shown in other studies, excitatory and inhibitory cortical neurons can be
determined based on their extracellular action potential waveform patterns (Bruno and Simons 2002; McCormick et al. 1985; Simons 1978). We measured the duration of the initial wave, from onset to its recrossing of baseline, and the duration of the second phase, measured from the end of the initial wave to its recrossing of baseline (inset, Fig. 1A). A scatterplot was created and K-means cluster analysis performed to identify two distinct groups (Figure 1A), corresponding to regular-spiking units (RSUs) and fast-spiking units (FSUs). Seventy-eight units were characterized: 48 were classified as RSUs and 30 were classified as FSUs. In agreement with Bruno and Simons (2002), the RSU cluster had longer initial (mean 306±48 μsec, median 300, range 220 to 400) and secondary (741 ± 54 μsec, median 753, range 600 to 800; see METHODS) phases than FSU initial (mean 246 ± 51 μsec, median 250, range 145 to 350) and secondary (mean 448 ± 71 μsec, median 450, range 300 to 585) phases. Our values are slightly larger than the values obtained in the rat somatosensory cortex by Bruno and Simons (2002), who also reported more discrete clustering of RSUs and FSUs, based on action potential indices. This may reflect differences between somatosensory and motor cortices, as recently observed in primate motor cortex (Levy et al., 2010). Nevertheless, as we show below, our main conclusions are not affected by the method used to classify these neuronal groups.

Receptive fields

In sensory cortices RSUs and FSUs can differ in their receptive field properties. Studies in somatosensory, visual and auditory cortices show that FSUs respond more readily and have larger (somatosensory) or more broadly tuned (visual and auditory) receptive fields (Bruno and Simons 2002; Atencio and Schreiner 2008; Simons and Carvell 1989; Swadlow and Weyand 1987). We compared receptive fields between RSUs and FSUs in rat motor cortex by measuring the body surface area from which responses could be evoked in these neurons (see METHODS). Statistical comparison showed no difference between RSUs and FSUs (normalized surface area,
Wilcoxon rank-sum test, \(p = 0.2\); RSUs N = 16 cells; FSUs N = 11 cells; Figure 1B). As the vast majority of our recorded units were from the hindpaw representation, receptive fields showed a high degree of overlap in hindpaw surface area. However, there was one exception in which receptive fields did not overlap. Shown in Figure 1B are two example RSU-FSU pairs which responded to tactile stimulation of the hindpaw and had overlapping (left) or non-overlapping (right) receptive fields.

**Responses to tactile stimuli**

Having distinguished RSUs from FSUs, we sought to compare response properties of each cell type. To do this, we applied calibrated tactile stimuli to the skin (see METHODS). Following receptive field characterization with a handheld probe, the calibrated probe was positioned to evoke the greatest response. Compared with RSUs, FSUs responded with a shorter onset latency [mean 11 ± 3 msec, median 10, range 6 to 21, \(n = 21\) cells; RSUs: mean 15 ± 6 msec; median 14, range 4 to 29; \(n = 27\); Wilcoxon rank-sum test, \(p = 0.004\); Figure 2B]. Response magnitude was greater for FSUs compared to RSUs (mean 0.77 ± 0.74 spikes per stimulus; median 0.56, range 0.06 to 3.3; RSUs: 0.30 ± 0.40; median 0.09, range 0 to 1.3; \(p < 0.001\); Figure 2C), and response duration was longer for FSUs compared to RSUs (mean 9.9 ± 6.5 msec, median 10, range 2 to 33; RSUs: mean 6.3 ± 4.6, median 5.0, range 2 to 18; \(p = 0.01\); Figure 2D). To determine if the response properties within each cell types varied according to cortical layer we compared superficial (layer II/III) and deep (layer V) neurons. None of the response parameters examined above were significantly different between superficial and deep RSUs (onset latency: \(p = 0.4\); response magnitude: \(p = 0.9\); response duration: \(p = 0.3\); \(n = 4\) deep, 21 superficial cells; Wilcoxon rank-sum test) or between superficial and deep FSUs (onset latency: \(p = 0.9\); response magnitude: \(p = 0.5\); response duration: \(p = 0.07\); \(n = 14\) deep, 7 superficial).

**Responses to vibratory stimuli**
Vibratory stimuli preferentially activate Ia proprioceptive muscle afferents (Burke et al. 1976; Roll et al. 1989; Woolsey et al. 1952). As was the case with tactile stimuli, responses to vibratory stimuli evoked shorter latency responses in FSUs compared to RSUs (mean 12 ± 3 msec, median 10, range 9 to 17, n = 11 cells; RSUs: mean 15 ± 5, median 15, range 8 to 26, n = 31; Wilcoxon rank-sum test, p = 0.047; Figure 3A). In addition, response magnitude was greater for FSUs (FSUs: mean 0.98 ± 1.1 spikes per stimulus, median 0.58, range 0.12 to 3.2; RSUs: mean 0.41 ± 0.45, median 0.25, range 0 to 1.7; p = 0.02; Figure 3B) as well as response duration (FSUs: mean 14 ± 8 msec, median 13, range 2 to 28; RSUs: mean 7 ± 5, median 6, range 2 to 20; p = 0.01; Figure 3C). Comparing responses from each cell type in the superficial cortical layers to those of the deep layers revealed no differences in response magnitude or duration (RSUs layer II/III vs. layers IV and V: response magnitude: p = 0.3; response duration: p = 0.3; FSUs layer II/III vs. layer V: response magnitude: p = 0.4). Layer II/III RSUs, however, showed a shorter onset latency than RSUs in layers IV and V (mean 11 ± 2.1, median 11, range 8 to 13, n = 7 cells; layers IV and V: mean 16 ± 4.9, median 16, range 9 to 26, n = 24; p = 0.006). In contrast FSUs from superficial and deep layers showed no difference in onset latencies (p = 0.6). The high frequency protocol allowed us to compare stimulus-evoked adaptation between RSUs and FSUs. Studies in the rat barrel cortex using depolarizing current injection (Beierlein et al. 2003; Gibson et al. 1999; Porter et al. 2001) and high frequency stimulation (whisker deflections; Simons 1978) show that adaptation is more pronounced for RSUs than for FSUs. Consistent with these reports, the response to vibratory stimuli was significantly higher for FSUs following the third stimulation in the stimuli train (mean 0.32 ± 0.31 spikes/stim, median 0.29, range 0 to 1.0, n = 14 cells; RSU: 0.13 ± 0.25, median 0.01, range 0 to 1.2, n = 33 cells; Wilcoxon rank-sum test, p = 0.006; Figure 3D; see Methods). Responses to stimuli 4 thru 10 are not significantly different between RSUs and FSUs. This may reflect comparable degrees of adaptation in both
cell types at these later stimuli. Given the decreased level of stimuli entrainment of FSU responses at 40 Hz (Khatri et al. 2004; Simons 1978), comparable responses might be expected at the 80 Hz used here. Moreover, adaptation may explain the lack of significant differences in overall response magnitude and response duration to vibratory stimulation.

Spontaneous firing rate

Spontaneous activity was recorded from 50 isolated motor cortical units. We found no difference in spontaneous firing rates of RSUs and FSUs (RSU: mean 2.7 ± 3.2 Hz, median 1.1, range 0 to 12, n = 33 cells; FSU: mean 1.6 ± 1.4, median 1.4, range 0 to 4.9, n = 17; Wilcoxon rank-sum test, $p = 0.8$). A comparison of spontaneous activity showed that deep layer RSUs fire at a higher rate than superficial RSUs (layer II/III: mean 0.62 ± 0.76 Hz, median 0.18, range 0 to 1.7, n = 5 cells; layer IV/V: 3.2 ± 3.4, median 1.9, range 1 to 12, n = 26; $p = 0.04$). Spontaneous firing rate between superficial and deep FSU neurons was not significantly different.

That RSU and FSU have similar spontaneous firing rates raised concerns that the statistical method by which we distinguished these groups may not have led to accurate classifications. To address this concern, we reanalyzed spontaneous firing rate as well as all of the response data after removal of one-third of the units (27) with intermediate waveform durations (phase 1 = 0.25 to 0.3 msec). The results of this analysis were indistinguishable from those performed with all the neurons included: spontaneous firing rates of FSUs and RSUs remained indistinguishable, and the differences noted above for response latency and magnitude remained unchanged.

Crosscorrelations

One hundred eighty-two crosscorrelograms between unit pairs were compiled for identified RSUs and FSUs. Of these, 79 crosscorrelograms resulted in significant features. All pairs were
recorded from different electrodes located on the same or immediately adjacent electrode shaft, and their calculated separation ranged from 100 µm to 250 µm.

The most common feature between pairs of RSUs (Figure 4A) and pairs of FSUs (Figure 4B) was a central peak, indicative of excitation by a common input source (for review see Fetz 1991). Common excitation occurred in nearly half of RSU pairs (12 out of 25 pairs, 48%). Strikingly, all FSU pairs displayed common excitation (15 out of 15 pairs), significantly more prevalent than common excitation between RSU pairs ($p < 0.001$, χ² test). Common excitation also occurred among mixed RSU-FSU unit pairs (17 out of 39 pairs, 44%), which is also statistically less prevalent than commonly-excited FSU unit pairs ($p < 0.001$).

The second most common feature between pairs of RSUs was a peak appearing after time 0, corresponding to increased firing probability of the target cell after a reference cell spike at time 0 (11 out of 25 unit pairs, 44%; see METHODS). These were interpreted as serial excitation of the target cell by the reference cell (Figure 4C; Fetz 1991; Fetz and Gustafsson 1983). Not surprisingly, the most common output from an RSU to an FSU was serial excitation (19 out of 23 pairs, 83%; Figure 4D). Some RSU-FSU pairs within this group (4 out of 23 unit pairs, 17%) displayed both common and serial excitation, indicated by a central peak and a peak appearing after time 0.

In 6 out of 16 FSU-RSU pairs (38%; FSU is reference, RSU is target) crosscorrelograms showed a trough that failed to reach below the lower 99% confidence limit. An example of this activity is seen in Figure 4F. In this example, inhibition was preceded by excitation from another unit, indicative of both excitatory and inhibitory inputs upon the target RSU. However, serial inhibition, in which the trough does reach below the lower 99% confidence limit was observed (1 out of 16 pairs, 6%; Figure 4E). Additionally, one FSU-RSU pair displayed common excitation followed by a non-significant trend towards inhibition (out of 16 unit pairs, 6%).
RSU-FSU interactions

Crosscorrelograms from 8 RSU-FSU pairs showed evidence for reciprocal connectivity. In 6 of these pairs, FSUs showed either serial inhibition or a non-significant trend towards inhibition upon a target RSU. Three of these pairs displayed common excitation. When unit pairs were analyzed with the FSUs as targets, all pairs showed either serial excitation upon the FSU (3 out of 8 pairs, 38%), a combination serial and common excitation (4 out of 8 pairs, 50%) or serial excitation preceded by a trend towards inhibition (1 out of 8 pairs, 13%). Example cross-correlograms from a reciprocal pair is shown in Figure 4: an RSU causes serial excitation of the FSU (4D) and the FSU imparts inhibition of the RSU (4E).

DISCUSSION

The motor cortex contains multiple, noncontiguous, highly overlapping representations of muscles and movements (Penfield and Rasmussen 1950; Woolsey et al. 1952; for review see Schieber 2001). This arrangement might impart computational advantages during movement execution by virtue of the close apposition of neurons involved in the same movement, such as the neurons controlling shoulder and wrist muscles during movement (Aflalo and Graziano 2006; Sanes and Donoghue 2000; Schieber 2001). A distributed, redundant organization also offers advantages for regulating the rapid plasticity in motor cortex that results from motor training or disease states (Keller 1999; Nudo 2006; Sanes and Donoghue 2000). From this it follows that during the execution of voluntary movements, this distributed network must organize dynamically to generate multi-jointed motor actions. The key question, and the one we aim to address, therefore, is: What are the parameters encoded by the activity of motor cortical neurons that govern the synthesis of movement commands?

FSUs Respond to Tactile Stimuli More Robustly Than RSUs
Excitatory and inhibitory neurons contribute in unique ways to cortical information processing. In this study we compared the response properties of putative excitatory (regular-spiking units) and inhibitory (fast-spiking units) in the motor cortex to tactile and vibratory stimuli. We found that FSUs respond to stimuli with greater responsivity, at a shorter latency, and with longer durations compared to RSUs. Studies in the somatosensory cortex showed that stimulation of thalamocortical afferents (Cruikshank et al. 2007; Porter et al. 2001) as well as stimulation by whisker deflection (Bruno and Simons 2002; Simons 1978; Zhu and Connors 1999) evokes more robust activation of inhibitory interneurons, and at shorter latencies, compared with excitatory neurons. Similarly, inhibitory interneurons in the awake rabbit have more broadly-tuned receptive fields and lower whisker deflection thresholds for activation (Swadlow 2003). A recent study in which thalamocortical afferents were stimulated with light following infection with the light-activated protein channelrhodopsin confirms these results (Cruikshank et al. 2010).

Studies in other sensory cortices indicate that increased responsivity and more broadly-tuned receptive fields are common properties of inhibitory interneurons across modalities. In the visual cortex, interneurons with action potentials of short duration similar to FSUs respond at short latency and have receptive fields that are not orientation selective (Swadlow and Weyand 1987). FSUs in the auditory cortex likewise responded to stimuli at short latencies and with broader spectral tuning compared to RSUs (Atencio and Schreiner 2008). Taken together, these and our data suggest that in both sensory and motor cortical afferent inputs preferentially activate inhibitory neurons.

**FSUs Share Greater Common Excitation**

Besides their greater tactile responses, FSU-FSU pairs showed greater incidence of common activation, compared to RSU-RSU pairs (Figure 4). This marks another similarity to the somatosensory cortex where the sharp synchrony of presumptive inhibitory neurons within the
same barrel is mediated by highly convergent/divergent, monosynaptic thalamocortical input (Swadlow 1995; Swadlow and Gusev 2002; Swadlow et al. 1998; for reviews see Swadlow 2002, 2003). A recent study showed that connectivity between topographically aligned, thalamus-barrel cortex pairs, was twice as likely for FSUs than for RSUs (Bruno and Simons 2002). Likewise, activity of FSU pairs in the dorsolateral prefrontal cortex exhibit greater correlation than RSU-RSU or RSU-FSU pairs (Constantinidis and Goldman-Rakic 2002). Moreover, synaptic efficacy is significantly greater for thalamocortical afferents projecting onto FSUs than onto RSUs (Cruikshank et al. 2007; Porter et al. 2001). Greater common input to FSUs alone could explain the increased common excitation seen in our study. In addition, gap junction coupling between cortical inhibitory neurons (Beierlein et al. 2000; Galarreta and Hestrin 1999; Gibson et al. 1999) would further contribute to synchronous activity.

A network of highly responsive, synchronized inhibitory interneurons would be effective in suppressing excitatory drive within a network. Taken together with the latency data, activation of this network that precedes that of the excitatory network further supports the notion that simultaneous excitation of projection neurons may be required to overcome feed-forward inhibition.

\textbf{RSU and FSU Receptive Fields Are Similar}

Contrary to other cortical areas studied to date, we did not find a difference in receptive field size between excitatory and inhibitory neurons (Armstrong-James et al. 1993; Bruno and Simons 2002; Simons and Carvell 1989). It is important to note that the majority of our recordings were from the hindpaw representation of the motor cortex (Neafsey et al. 1986) and, as such, nearly all the mapped receptive fields overlapped extensively. This sampling bias is likely to have affected our comparisons of receptive field sizes. Further studies in this area will be needed to definitively address this question.
Considering the significant overlap of hindpaw representations in the rat somatosensory and motor cortices (Chapin 1986; Sapienza et al. 1981), a subgroup of cells analyzed in this study are likely somatosensory in origin. We attempted to maximize recordings from motor cortical neurons by targeting the rostral portion of the MI hindpaw representation where the SI representation is scarcer (see METHODS). The fact that our data include some of somatosensory neurons, however, does not affect our conclusion that greater responsivity of FSUs contributes to the feedforward inhibition of a center-surround arrangement as SI afferents converge on motor cortical loci (Aizawa and Tanji 1994; Godschalk et al. 1984; Porter 1992; Swadlow 1994; Zarzecki 1991) and thus SI and MI inputs are likely to affect MI neurons in a similar manner.

Recent evidence from the sensory cortex suggest that the increased responsivity of inhibitory compared to excitatory neurons is not due to differences in intrinsic cellular properties, but to the differences in the strength of thalamocortical inputs targeting each cell type (Cruikshank et al. 2007; Porter et al. 2001). Considered together, the current findings of increased interneuronal responsivity and their high tendency to be coactivated are consistent with a feed-forward inhibitory motor cortical network powerfully activated by divergent thalamocortical input. This arrangement which, in S1, serves to sharpen sensory-evoked responses that lead to perception, may increase output contrast in motor cortex to functionally link areas involved in movement.
FIGURE LEGENDS

Figure 1
Motor cortical neurons types can be differentiated from extracellular waveform patterns. (A) Scatter plot of initial and secondary action potential phase durations for RSUs (n = 48; filled circles) and FSUs (n = 30; unfilled circles). Inset, example waveforms of each cell type with different action potential phases labeled (dashed line = baseline). (B) Representative receptive fields for an RSU-FSU pair with overlapping receptive fields (left) and an RSU-FSU pair with non-overlapping receptive fields (FSU responds to stimulation of dorsal surface, RSU to plantar surface; right). Darker grey indicates increased region evoking most spikes.

Figure 2
RSU and FSU responses to tactile stimuli. (A) Peristimulus time histograms (PSTH, 1 msec bin) of example sensory-evoked responses demonstrate that FSU responses are markedly higher than RSU responses. Dashed lines represent 99% confidence interval. (B, C, D) Grouped response data. Boxes represent the 25th and 75th percentile of distribution, solid horizontal lines depict the means, and dashed lines represent mean values; whiskers show the 10th and 90th percentiles. (B) FSUs (n = 21 cells) have significantly shorter response onset latency than RSUs (n = 27 cells) to tactile stimuli. (C) Tactile stimuli evoked significantly more spikes per stimulus in FSUs compared to RSUs. (D) Tactile response duration was significantly longer in FSUs compared to RSUs.

Figure 3
RSU and FSU responses to vibratory stimuli. Compared to RSUs (n = 31 cells), FSUs (n = 11 cells) responded to vibratory stimuli with shorter onset latencies (A), and with greater response magnitudes (B) and response durations (C). Boxes represent the 25th and 75th percentile of distribution, dashed lines represent mean values; whiskers show the 10 and 90 percentiles. (D)
Example responses of an RSU (gray) and an FSU (black) to stimuli 1 thru 3 of the vibratory train. Triangles represent stimuli beginning at time = 0; dashed lines represent 99% confidence intervals. Note the rapid attenuation of the RSU response compared to the FSU response.

**Figure 4**

Crosscorrelation histograms from motor cortical RSUs and FSUs. Firing probability histograms were constructed with 1 msec bins. Dashed horizontal lines indicate 99% confidence limits. For each panel, first unit type in label indicates reference unit, second indicates target. Central peaks above the 99% confidence limit indicate common excitatory input to a pair of RSUs (A) and a pair of FSUs (B). (C) A peak to the right of $t = 0$ msec indicates serial excitation between an RSU-RSU pair. (D, E) Crosscorrelograms of a reciprocally-innervated RSU-FSU pair. (D) Example serial excitation of target FSU by a reference RSU. (E) Reversing the target and reference roles of units in D shows inhibition of the target RSU (histogram drops below lower 99% confidence limit) by reference FSU. (F) FSU-RSU pair, with RSU showing a trend towards inhibition that does not pass the lower 99% confidence limit. The RSU also displays excitation just prior to time 0.
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