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Characteristics of synaptic connections between rodent primary somatosensory and motor cortices

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Abstract
The reciprocal connections between primary motor (M1) and primary somatosensory cortices (S1) are hypothesized to play a crucial role in the ability to update motor plans in response to changes in the sensory periphery. These interactions provide M1 with information about the sensory environment that in turn signals S1 with anticipatory knowledge of ongoing motor plans. In order to examine the synaptic basis of sensorimotor feedforward (S1–M1) and feedback (M1–S1) connections directly, we utilized whole-cell recordings in slices that preserve these reciprocal sensorimotor connections. Our findings indicate that these regions are connected via direct monosynaptic connections in both directions. Larger magnitude responses were observed in the feedforward direction (S1–M1), while the feedback (M1–S1) responses occurred at shorter latencies. The morphology as well as the intrinsic firing properties of the neurons in these pathways indicates that both excitatory and inhibitory neurons are targeted. Differences in synaptic physiology suggest that there exist specializations within the sensorimotor pathway that may allow for the rapid updating of sensory-motor processing within the cortex in response to changes in the sensory periphery.

Keywords: Barrel cortex, motor cortex, EPSPs, feedforward, feedback, cortico-cortical connections

Introduction
Both sensory and motor cortices contain topographic maps (Woolsey 1952; Li and Waters 1991). Previous anatomical studies have shown that direct, somatotopically organized reciprocal connections exist between these areas in the rodent (Porter and White 1983; Miyashita et al. 1994; Hoffer et al. 2003), and that the cell bodies of the cortico-cortical efferents from S1 to M1 are located primarily in infragranular layers (Izraeli and Porter 1995). In vivo physiological studies have revealed strong monosynaptic inputs onto M1 efferent neurons originating from S1 (Ghosh and Porter 1988). In fact, greater than half of all projection neurons in the infragranular layer of M1 receive direct inputs from S1 (Zarzecki 1989). Functionally, in primates, inactivation of these inputs can result in disruption of motor acts such as chewing (Lin et al. 1998), fine motor coordination, sustained muscle contractions, appropriate grip force, and the ability to determine the weight of an object (Johansson and Westling 1984; Hikosaka et al. 1985; Brochier et al. 1999). Changes in the sensory environment are thought to be relayed via an intracortical pathway and not via direct connections from that thalamus based on in vivo latency analyses (Blum et al. 1968; Herman et al. 1985; Farkas et al. 1999). Thus, sensorimotor interactions enable M1 and S1 to have adaptive responses to changes in the periphery. Despite this pathway’s critical role in modulating ongoing motor...
plans, little is known about the synaptic basis of these interactions or the intrinsic properties of the neurons involved.

The functional dynamics of feedforward and feedback connections have been widely studied within the visual system. These studies have shown that feedforward and feedback connections often vary in their laminar distribution and their synaptic targets (Lund et al. 1975; Weller and Kaas 1983; Johnson and Burkhalter 1996). Neurons involved in these pathways have demonstrated differences in responses to evoked stimuli in vitro (Shao and Burkhalter 1996) and to stimuli presentation in vivo (Angelucci and Bressloff 2006). Within the sensorimotor system, S1 neurons projecting to M1 have receptive field characteristics that differentiate them from other neurons (Sato and Svoboda 2010), but the manner in which their responses impact neurons in M1 are unknown. Characterizing the components of these pathways and their function is an important step towards understanding how the brain translates sensory input into motor responses and how changes in motor activity can influence sensory neurons.

Materials and methods

All experiments were performed on CD-1 mice (postnatal day 14–21) of either sex (Charles River Laboratories, Wilmington, MA, USA). All experiments were performed in accordance with the Institutional Animal Care and Use Committee guidelines of Queens College, CUNY and the National Institutes of Health.

Preparation of tissue for in vitro recording studies

Mice were anesthetized and the brain was quickly removed and sectioned at 350 μm in the sensorimotor plane as previously described (Rocco and Brumberg 2007) in chilled, oxygenated artificial cerebral spinal fluid (ACSF) solution (in mM: 124 NaCl, 2.5 KCl, 2 MgSO₄, 1.25 NaH₂PO₄, 1.2 CaCl₂, 26 NaHCO₃, 10 dextrose). Sensorimotor sections were kept in oxygenated ACSF at 34°C for at least 1 h before the start of the experiment.

Whole-cell recordings

Cells were visualized with infrared differential interference contrast optics (Olympus BX51WI). Intracellular recording electrodes were pulled on a microelectrode puller (Sutter P-87) resulting in a final resistance of approximately 3 MΩ. Electrodes were filled with (in mM) 120 KGlu, 10 NaCl, 20 KCl, 10 HEPES, 2 Mg-ATP, 0.3 Na-GTP, 0.5 EGTA, and 0.3–1% biocytin (wt/vol) for subsequent visualization of the neurons. Cells were recorded using current clamp methods as previously described at room temperature (Ramos et al. 2008). Once a stable recording was obtained (resting $V_{m} < -55$ mV, ability to generate action potentials in response to a depolarizing current pulse), cell intrinsic properties were assessed. Resting membrane potential was determined by measuring the membrane voltage in the absence of current input. Intrinsic properties were determined by quantifying responses to a series of 500 ms steps (ranging from −300 to 300 pA) applied from rest. To calculate action potential characteristics, we analyzed responses of cells to threshold currents for their generation. The input resistance ratio ($R_{in}$ ratio) was calculated based on responses to a 500 ms step current of −30 pA. The value is equal to the resistance at the maximum hyperpolarized value divided by the resistance at 100 ms prior to the stimulus offset. The slope of the firing vs. current (FI) relationship was determined by analyzing responses of cells to threshold currents for activating action potentials to responses twice the threshold value. Once intrinsic properties were assessed, synaptic response data were collected by placing a bipolar stimulating electrode (~1 MΩ, FHC Inc., Bowdoin, ME, USA, Figure 1A) in the infragranular layers of the whisker representation in one area (e.g., M1), while simultaneously recording in whole-cell configuration in the reciprocal area (e.g., S1). A response threshold was determined by stimulating with a series of 100 μs current pulses (ranging from 1 μA to 10 mA) until a consistent response was observed in response to the electrical stimulation (see Figure 1B). Typically, <5 mA pulses were used, and larger magnitudes were used to test if non-responsive cells were truly non-responsive. If a response was not observed to a single stimulus pulse, a train of three pulses was then applied at various frequencies (10–100 Hz). Final parameters were noted and the responses to stimulation at 110% of the minimal intensity necessary to evoke a postsynaptic response were used for analyses. Analyses were performed on averages of 10 traces to ensure reliability. For each identified connection a coefficient of variation analyses was performed (Berry and Pentreath 1976; Faber and Korn 1991), wherein $CV$ (coefficient of variation) = $\sigma/\mu$, where $\sigma$ is the standard deviation of response latency (in ms) and $\mu$ is the mean response latency (in ms). Connections that had values of $CV \leq 10\%$ were assumed to be reflective of a monosynaptic connection. Direct comparisons between cells were only conducted between those with identical stimulus parameters.

Four synaptic response categories were identified based on individual cell responses to stimulation in the reciprocal area: (1) short latency, presumed monosynaptic connections that had little jitter...
(values of CV ≤ 10%) and occurred ≤25 ms following the stimulus (Figure 1B), (2) long latency, presumed polysynaptic connections without a short-latency component, which occurred >25 ms following the stimulus, (3) both, includes short- and long-latency responses, and (4) no response. Long-latency responses were unlikely due to antidromic stimulation since deactivation of the stimulation site with muscimol, which would not impact antidromic activation, abolished all responses in the target area (Rocco and Brumberg 2007), but are most likely due to either polysynaptic activation and/or activation of synapses mediated by metabotropic receptors (e.g., Covic and Sherman 2011).

Neuronal reconstruction

Following recording sessions, slices were transferred to a 4% paraformaldehyde solution in 0.1 M Phosphate Buffer for subsequent biocytin histochemistry (Ramos et al. 2008). Biocytin-filled cells from whole-cell recordings were reconstructed for morphological analysis using the Neurolucida software program (version 8.0, MicroBrightField Inc., Williston, VT, USA). Tracing of soma and dendrites was done with an oil immersion objective (60×, 1.4 N.A.) and analyzed using NeuroExplorer (a component of Neurolucida). Specifically, soma shape and perimeter were quantified, as well as dendritic parameters such as quantity, length, and branching patterns.

Data analysis

Off-line analyses were performed using Clampfit 9.2 (Molecular Devices, Sunnyvale, CA, USA). Pairwise comparisons were made post hoc using Fisher’s Least Significant Difference tests. Cells were categorized based on their response to the stimulus. T-tests were used for comparisons between groups. A power analysis was conducted to determine necessary sample sizes; we assumed β = 0.8, α = 0.05, σ = 0.1. The resultant analyses suggested group sizes based on estimated means from pilot data (Donovan et al. 2008) were 17. The Mann–Whitney test was used to compare synaptic response results due to unequal
variance and $\chi^2$-tests were used to compare population distributions between different groups. Statistical analyses were done with Statistica (StatSoft, Tulsa, OK, USA). Data are represented as population means $\pm 1$ SEM.

Results

Direct connections are observed between S1 and M1

Once stable recordings were obtained and the intrinsic membrane properties were assessed for a given cell (see below), electrical stimulation was applied to the reciprocal area via a bipolar electrode (Figure 1) and any resulting post-synaptic response was recorded. Despite the distance ($\sim 3$ mm) between the two regions, short-latency responses were observed in both regions following stimulation in the reciprocal area confirming that this slice preparation retains functional connections between S1 and M1.

Overall, M1 cells (18 of 20) were more responsive than S1 cells (18 of 33) to stimulation in the reciprocal area ($p < 0.01$, Figure 2A). In addition, cells in M1 (16 of 20) were more likely to have short-latency responses to stimulation in the reciprocal area than S1 cells (10 of 33). Short-latency responses were characterized by little-to-no jitter in their latencies or amplitudes (see Figure 1B). Cells in S1 had a higher proportion (8 of 33) of cells showing purely long-latency responses than those in M1 (2 of 20), suggesting that it may be more difficult to target the subpopulation of S1 neurons that receive short-latency M1 inputs or that inputs in the feedback direction are less numerous than those in the feedforward direction. Interestingly, there was little evidence of IPSPs (inhibitory post-synaptic potentials) in our recordings, suggesting that our stimulation resulted in only subthreshold responses preventing the initiation of disynaptic inhibition. Furthermore, fibers of passage are likely not underlying these results given that muscimol application at the stimulation site prevents activation in the target region (Rocco and Brumberg 2007). These results indicate that cells receiving feedforward inputs (M1 cells) have a higher synaptic response probability and suggest that M1 signals may be easily modifiable by changes in the sensory environment conveyed by these S1 inputs.

Synaptic properties of the S1–M1 pathway

Differential responsiveness of S1 and M1 cells is also evidenced by comparing both the number of cells in

Figure 2. Short-latency inputs are more prevalent in M1. Two types of responses were observed following stimulation: short- and long-latency responses which were seen in the average trace of 10 stimuli applied to S1 with recordings in M1 (inset in A, scale bar: 2.5 mV, 100 ms). Neurons were characterized as receiving short-, long-latency, or both types of responses (see text). Neurons in M1 were more likely to receive inputs from S1 than the reciprocal relationship. Numbers in bars indicate the number of cells in each category indicated out of S1 = 33 and M1 = 20 total recordings (A). Arrows point to typical direct and indirect responses. S1 inputs to M1 were of larger magnitude (B) each direct, short-latency, response is plotted on an arbitrary axis (inset is the average of 10 stimuli presentations). The distribution of EPSP latencies (C) and population average (inset, bars represent population means $\pm 1$ SEM) demonstrate that M1 feedback to S1 arrives sooner, but is of smaller magnitude (D, bars represent population means $\pm 1$ SEM). $*p < 0.05$, $**p < 0.01$. 
each area that directly responded to low intensity stimulation (single pulse $<5.0$ mA), and the average magnitude of the short-latency responses of these cells (Figure 2B). Of the 33 sampled S1 cells, only 4 cells showed a reliable short-latency response to low intensity stimulation in M1 with an average magnitude of $2.62 \pm 0.85$ mV. Conversely, of 20 M1 cells sampled, 9 cells directly responded to low intensity stimuli with a significantly higher average amplitude response of $33.29 \pm 8.64$ mV ($p < 0.05$; Figure 2D). The variance in response may be reflective of recordings done at room temperature that can lead to small amplitude responses and increases in failure rates (Kidd and Isaac 2001; Volgushev et al. 2004; Ali et al. 2007). Coefficient of variation analysis on these short-latency connections revealed that all those cells in M1 that received short-latency responses had a CV $\leq 10\%$; similarly, the S1 cells had a CV $\leq 8\%$ that strongly suggests that this class of connections is indeed monosynaptic.

Although their distributions overlapped, differences were observed in the average time to peak of short-latency synaptic responses onto S1 and M1 cells (Figure 2C). When M1 was stimulated with a single pulse of less than 5.0 mA of current, cells in S1 had an average latency (time to peak) of $19 \pm 0.50$ ms (range 10–24 ms), which was faster than the response latency observed in M1 cells when similar stimulation was applied to S1 (106 $\pm 31$ ms; $p < 0.01$; range 50–215 ms). Assuming a 3 mm separation between the stimulation and recording sites, the axons projecting from S1 to M1 have an average transmittal time (including the synaptic delay) of $\sim 0.03$ m/s compared to a speed of $\sim 0.16$ m/s in the reverse direction. The relatively long latencies observed in both directions may be reflective of the age of our animals but extracellular recordings from sensorimotor slices from adult animals (Rocco and Brumberg 2007) demonstrated similar latencies. Moreover, our study was conducted at room temperature in contrast to many in vivo studies (e.g., Farkas et al. 1999), which slows down conduction velocities. Similar to our results, Farkas et al. (1999) reported a small magnitude short-latency response (likely due to direct thalamic input) followed by a much larger response that peaked at 175 ms post stimulus that is consistent with our in vitro findings. These findings indicate that the feedforward input to M1 results in a larger magnitude and slower response than the feedback input to S1. These results are in agreement with our previous study that found smaller axon diameters in the feedforward pathway that may provide the explanation as to why this pathway has longer response latencies (Rocco and Brumberg 2007).

**Intrinsic properties of responsive and non-responsive cells**

Intrinsic membrane and firing properties of both M1 and S1 neurons were assessed identically by quantifying responses to a series of step currents of 500 ms duration (Figure 3). Firing properties as well as synaptic stimulation data were obtained from the

![Figure 3](image-url). M1 inputs into S1 target multiple neuronal phenotypes. Reconstructions (left) of biocytin-filled fast spiking (middle, response to a $\pm 160$ pA, 500 ms in duration current pulse) non-pyramidal (A) and regular spiking (response to a $\pm 100$ pA, 500 ms in duration current pulse) pyramidal neurons (B) from S1 that responded to M1 stimulation (right, traces represent average of 10 stimulation presentations, arrows represent stimulation onset). Scale bars are the same for both top and bottom traces.
majority of cells recorded from S1 (31 of 33) and all cells recorded from M1 (20 of 20). All cells were categorized by their spike pattern according to previously published results (McCormick et al. 1985; Kawaguchi 1993). The majority of cells recorded in S1 were found to be regular spiking (RS, 28 of 31). A few fast spiking (FS) cells were observed (2 of 31) and one cell in this area was intrinsically bursting. A large portion of the cells recorded in M1 were also found to be RS (12 of 20). Additionally, low threshold spiking (LTS, 6 of 20) and fast spiking (2 of 20) cells were also observed in M1. While we were not able to systematically sample from different populations of cells, our sample does contain a variety of cell types that would be expected to be encountered in these two areas based on the literature (McCormick et al. 1985; Kawaguchi 1993).

The majority of RS cells recorded in S1 (17 of 28) were found to be responsive to stimuli in the reciprocal area. Approximately half of these responsive RS cells (9 of 17) showed a short-latency response. Of the three non-RS S1 cells, only one was responsive to M1 stimulation; this cell showed a short-latency response. Nearly all of the RS cells found in M1 responded to stimuli in S1 (11 of 12), almost all of which were short-latency responses (10 of 11). The response probabilities of the LTS cells (5 of 6) and the FS cells (2 of 2) in M1 were also very high. All of the FS cells (2 of 2) and all but one of the LTS cells (4 of 5) showed a short-latency response. These results indicate that in both S1 and M1, many cell types are responsive to stimulation in the reciprocal area.

The cell intrinsic properties of RS cells that fell into the both response category in S1 (n = 17) and M1 (n = 12) are compared in Figure 4. Responsive cells in S1 had significantly greater spike amplitudes (Figure 4B, p < 0.001) and input resistance ratios (Figure 4C, p < 0.009). M1 responsive cells had significantly larger magnitude after-hyperpolarization (AHP) than S1 responsive cells (Figure 4D, p < 0.001). S1 and M1 responsive cells did not differ significantly on measures of resting membrane potential, spike threshold, AHP duration, spike rise-time, or slope of the FI curve (values of p > 0.05). These results suggest that within our sample, responsive cells in S1 and M1 were comparable on most intrinsic membrane measures, but differ physiologically in some aspects of their firing properties such as action potential and AHP amplitudes.

Laminar location of recovered cells

Given the limited area of recording sites within each sensorimotor slice and the physical limitations of the
cells (3 of 14) were in the middle third. For both S1 and M1, the probability of finding a post-synaptic response following stimulation in the reciprocal area was highest in the infragranular layers.

Morphological categorization of recovered cells

A large proportion of the S1 cells were recovered (21 of 33), most of which (16 of 21) were also able to be reconstructed (see Figure 3). The majority of cells recorded from M1 (14 of 20) were recovered, of which 11 of 14 were able to be morphologically reconstructed. Of the 16 fully reconstructed cells in S1, 15 had pyramidal cell bodies. Of the 11 cells recovered from M1, 4 were pyramidal in shape and 7 were non-pyramidal. Interestingly, unlike the visual system, responsive cells did not appear to be restricted to a particular morphological cell type (Shao and Burkhalter 1996). Observing the dendritic architectures of the responsive neurons revealed a variety of phenotypes (see Figure 3). Quantitative analysis of somatic (area, aspect ratio, etc.) and dendritic parameters (quantity, length, branching patterns, etc.) did not reveal significant differences between the two areas or within response groups in either area. These results are consistent with in vivo findings that demonstrate that multiple classes of M1 efferent neurons receive inputs from S1 (Zarzecki 1989) and vice versa.

Discussion

Many studies have successfully used in vitro preparations to investigate connectivity between distant brain locations (Agmon and Connors 1991; Shao and Burkhalter 1996; Cruikshank et al. 2002). This is the first in vitro study using whole-cell recording techniques to describe and quantify the synaptic
connections between S1 and M1. Despite the ~3 mm separating stimulation and recording sites in our preparation, we can conclude that short-latency presumably monosynaptic connections exist in both the feedforward and feedback pathways of the sensorimotor system in the mouse. A limitation of our preparation is the lack of thalamic inputs that project to both areas and may play an important role in modulating S1–M1 interactions, but our findings that there are intrinsic differences between neurons in the two areas is independent of any thalamic inputs. Previous research has shown that following chemical inactivation (Chakrabarti et al. 2008) or ablation (Farkas et al. 1999) of whisker S1 there is an almost complete removal of whisker-evoked responses in S1. These results support the hypothesis that the feedforward inputs to M1 from S1 are driving the majority of the whisker-evoked activity observed in M1. Furthermore, our results do show that there are specific differences in the feedforward vs. feedback pathways that potentially can be modulated by extrinsic or local connections. Whole-cell recordings revealed that multiple cell types in S1 and M1 are responsive to stimuli from the reciprocal area. For both pathways, the probability of finding a post-synaptic response following stimulation in the reciprocal area was highest in the infragranular layers, which is consistent with the observed termination patterns of the axonal projections. In comparing synaptic responses under identical stimulation conditions, feedforward input from S1 to M1 resulted in significantly larger responses, but with significantly longer latencies than feedback responses. The faster feedback connection may be important in providing updated information about whisker position to S1 neurons.

Our results are consistent with previous findings indicating that feedforward connections appear to be stronger than feedback connections. In an in vitro preparation containing V1 and the rodent homolog of V2, activation of feedforward connections to V2 resulted in significantly larger amplitude EPSPs (excitatory post-synaptic potentials) than those resulting from feedback connections (Shao and Burkhalter 1996). One explanation for these results may be differences in the density of the projections between the two areas. Feedback inputs to V1 of the rodent are about 50% less numerous than feedforward inputs to V2 (Shao and Burkhalter 1996). Similarly, significantly fewer projections were observed in the feedback from area MT to V1 in the primate when compared to the feedforward direction (Maunsell and Van Essen 1983). Within the auditory system, the connections between primary and secondary cortex have been investigated using electrical stimulation and glutamate uncaging, and direct inputs have been found between the two areas that were mediated by either ionotropic or metabotropic glutamate receptors, suggesting that there may be two time scales of modulation between cortical areas (Covic and Sherman 2011) as seen in the present study. The long-latency responses observed in the present recordings were consistent over 10 stimulus presentations and thus even if due to polysynaptic circuits they must be wired such that they are continually activated or a result of activation of metabotropic receptors.

It is not known if a specific topography exists for the reciprocal S1–M1 connection, but S1 neurons that project to M1 preferentially lie beneath the septal areas (Chakrabarti and Alloway 2006) and within M1, neurons that receive input from different origins appear to be located within intermingled patches (Zarzecki 1991; Zarzecki et al. 1993) and perhaps neurons in these areas are more likely to receive direct feedback connections. It appears that, in general, M1 responses to sensory stimuli are dependent on initial S1 processing. In vivo calcium imaging has shown that a single, brief whisker deflection results initially in a strong depolarization in S1 followed by subsequent M1 activation (Ferezou et al. 2007). Significant reductions have also been observed in M1 stimulus-evoked responses following inactivation or ablation of S1 (Asanuma et al. 1980; Farkas et al. 1999). Additionally, in rats, trimming their whiskers from birth or during adulthood alters the somatotopic organization and responses within M1 (Huntley 1997). Behavioral data from primates has shown that following either M1 or S1 inactivation with muscimol (Brockier et al. 1999) there are characteristic deficits on a motor control task. In sum, these findings highlight the necessity of an intact cortical sensorimotor loop in order to process and complete sensorimotor tasks.

While neither M1 (Gao et al. 2003) or S1 (Harvey et al. 2001) is necessary to produce whisking, the feedforward projections sent from S1 to M1 may be important in regulating whisking dynamics (Kleinfeld et al. 2002). The functions of feedback projections from M1 to S1 in general are not well understood. In the whisker pathway, they may serve to synchronize firing of cells that respond to similar stimuli. In general, this process would serve to focus the activation such that only a specific subset of cells would be excited. Consistent with this is the finding that M1 activation specifically excites corticothalamic neurons in S1 that are responsive to the homotopic whiskers (Lee et al. 2008). In the feedforward direction, distinct activation could result in an alteration of whisker movements that would allow for better discrimination on subsequent whisk cycles.
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References


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