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Spatiotemporal Dynamics of Cortical Sensorimotor Integration in Behaving Mice

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SUMMARY

Tactile information is actively acquired and processed in the brain through concerted interactions between movement and sensation. Somatosensory input is often the result of self-generated movement during the active touch of objects, and conversely, sensory information is used to refine motor control. There must therefore be important interactions between sensory and motor pathways, which we chose to investigate in the mouse whisker sensorimotor system. Voltage-sensitive dye was applied to the neocortex of mice to directly image the membrane potential dynamics of sensorimotor cortex with subcolumnar spatial resolution and millisecond temporal precision. Single brief whisker deflections evoked highly distributed depolarizing cortical sensory responses, which began in the primary somatosensory barrel cortex and subsequently excited the whisker motor cortex. The spread of sensory information to motor cortex was dynamically regulated by behavior and correlated with the generation of sensory-evoked whisker movement. Sensory processing in motor cortex may therefore contribute significantly to active tactile sensory perception.

INTRODUCTION

Sensory information is actively acquired by animals. This is particularly evident for the sense of touch, where self-generated movements often drive tactile input. To understand somatosensory perception it is therefore important to consider the interactions between sensory and motor systems. The rodent whisker system provides a relatively simple mammalian sensorimotor pathway (recently reviewed by Kleinfeld et al., 2006, and Petersen, 2007). During active sensation, the mystacial vibrissae predominantly move in a forward and backward motion termed "whisking." Such one-dimensional movement has the advantage that it can be easily and accurately quantified. Whisker movements can be evoked by the direct stimulation of neurons in the primary whisker motor cortex (Brecht et al., 2004; Haiss and Schwarz, 2005; Berg et al., 2005). Motor cortex activity can phase lock to whisker movements in rats trained to whisk for rewards (Ahrens and Kleinfeld, 2004) and during epileptiform activity (Castro-Alamancos, 2006). However, other data suggest that action potential firing in motor cortex is not normally phase-locked to the whisking cycle (Carvell et al., 1996). Equally, the frequency of whisking evoked by intracortical microstimulation is different from the frequency of the stimuli delivered to the motor cortex (Haiss and Schwarz, 2005; Cramer and Keller, 2006). From a behavioral perspective, it is clear that whisking occurs even in rodents with lesioned motor cortex (Gao et al., 2003), lesioned neocortex (Welker, 1964; Semba and Komisaruk, 1984), and in decerebrate rats (Lovick, 1972). Finally, a serotonergic input onto the facial nucleus motor neurons is sufficient to evoke whisking (Hattox et al., 2003; Cramer et al., 2007). The major role of the whisker motor cortex is therefore unlikely to be the simple rhythmic control of each whisking cycle.

Anatomical studies have provided evidence for sensory input to motor cortex originating from somatosensory cortex and thalamus (White and DeAmicis, 1977; Porter and White, 1983; Miyashita et al., 1994; Izraeli and Porter, 1995; Deschenes et al., 1998; Hoffer et al., 2003; Alloway et al., 2004). The whisker motor cortex could therefore serve to integrate sensory input with motor commands. In support of such a hypothesis, previous electrophysiological studies have shown that electrical stimulation of trigeminal sensory afferents (Farkas et al., 1999) or repetitive whisker deflections (Kleinfeld et al., 2002) could evoke responses in motor cortex. In this study, we applied voltage-sensitive dye to sensorimotor cortex to directly image the interactions between somatosensory and motor cortex in both anesthetized and awake head-fixed mice during behavior. We find that a single whisker deflection can evoke a highly distributed sensory response, with complex spatiotemporal dynamics, which begins in somatosensory cortex and is then relayed to motor cortex, in a manner dependent upon ongoing behavior. The

sensory-evoked activity in motor cortex in turn correlates with sensory-evoked whisker movement. Our results suggest that the whisker motor cortex is guided by powerful sensory input from the primary somatosensory barrel cortex directing active control of whisker movement during tactile sensory perception.

RESULTS

Voltage-Sensitive Dye Imaging of Sensorimotor Cortex

A large fraction of mouse somatosensory and motor cortex was exposed bilaterally and stained with voltage-sensitive dye (VSD) RH1691 (Figure 1A). Imaging the voltage-dependent fluorescence changes of neocortex stained with RH1691 allows the visualization of the ensemble membrane potential dynamics of the supragranular layers at millisecond temporal resolution and subcolumnar spatial resolution (Shoham et al., 1999; Seidemann et al., 2002; Slovin et al., 2002; Kenet et al., 2003; Petersen et al., 2003a, 2003b; Jancke et al., 2004; Grinvald and Hildesheim, 2004; Civillico and Contreras, 2006; Ferezou et al., 2006; Borgdorff et al., 2007; Berger et al., 2007; Lippert et al., 2007; Benucci et al., 2007; Xu et al., 2007).

A single brief passive deflection of the C2 whisker evoked a stereotypical pattern of cortical activity imaged with VSD in urethane-anesthetized mice (n = 15; Figures 1B–1F and see Movies S1 and S2 in the Supplemental Data available with this article online). The earliest sensory response occurred with a latency of 7.2 ± 0.9 ms following whisker deflection and was highly localized, specifically exciting the C2 barrel column of the contralateral primary somatosensory cortex (S1) (Ferezou et al., 2006). Over the next few milliseconds, the depolarization spread across a large part of the barrel cortex. Supragranular neurons in the surrounding barrel columns therefore become depolarized, in good agreement with electrophysiological membrane potential recordings revealing broad subthreshold receptive fields of L2/3 cortical neurons (Moore and Nelson, 1998; Zhu and Connors, 1999; Brecht et al., 2003). Approximately 8 ms after the earliest response in S1 cortex, a second localized anteromedial cortical region is depolarized, located 1.4 ± 0.2 mm anterior and 1.1 ± 0.2 mm lateral relative to Bregma (n = 15 experiments). This is within the previously identified location of the mouse motor cortex (M1) (Caviness, 1975; Franklin and Paxinos, 1996). The motor cortex depolarization occurred with a latency of 15.3 ± 1.3 ms following whisker deflection and also spread over the following milliseconds. Finally, after ~30 ms following whisker deflection, the sensory-evoked activity propagates to the other hemisphere, although depolarization in the hemisphere ipsilateral to the stimulated whisker is relatively weak (Figure 1C). Deflection of the C2 whisker therefore initiates cortical activity in two clearly separate focal regions, from which propagating waves of depolarization can spread to a large part of the sensorimotor cortex.

VSD Fluorescence Changes Reflect Cortical Synaptic Activity

In previous studies, we found that RH1691 VSD fluorescence changes in somatosensory cortex in vivo were correlated with the local ensemble subthreshold membrane potential changes of layer 2/3 pyramidal neurons (Petersen et al., 2003a, 2003b; Ferezou et al., 2006; Berger et al., 2007). These previous measurements were made in small craniotomies encompassing only part of the barrel cortex. In order to test whether local synaptic drive underlies the VSD signals in the larger craniotomies studied here, we combined local field potential (LFP) recordings with simultaneous VSD imaging (n = 4 mice, an example experiment is shown in Figures 2A–2C). The LFP signal correlated closely with the VSD signal, as reported by Lippert et al. (2007). A single brief deflection of the C2 whisker evoked VSD and LFP signals first in S1 and ~8 ms later in M1 (Figure 2A). The LFP signal, however, has a shorter duration because it primarily reflects the extracellular synaptic currents, whereas the VSD signal reflects membrane potential changes and is therefore prolonged due to the neuronal membrane time constants.

To address concerns that the large craniotomies might damage the cortex, we carried out control experiments applying VSD on two small craniotomies (one centered on S1 and the other in M1) and found a similar sequential activation of S1 and M1 following single C2 whisker deflection (n = 8 experiments, including 5 experiments in anesthetized mice and 3 experiments in awake mice; Figures S1A–S1C). We also observed a similar sensory-evoked LFP response in M1 with a small craniotomy centered on motor cortex leaving the somatosensory cortex untouched (n = 5 experiments, data not shown). Finally, in whole-cell recordings from small M1 craniotomies, we found neurons responding with depolarizing membrane potentials to single brief C2 whisker deflections at similar latencies (21 ± 6 ms, n = 7) to those recorded with VSD and LFP (an example experiment is shown in Figure S1D).

VSD Imaging of Spontaneous Activity in Sensorimotor Cortex

The spatiotemporal dynamics of cortical spontaneous activity can also be imaged using VSD (Arieli et al., 1996; Kenet et al., 2003; Petersen et al., 2003b; Ferezou et al., 2006; Berger et al., 2007). The ability to resolve spontaneous activity is of importance when considering the real-time imaging of cortical function during behavior (see Figures 7 and 8). We found close correlations between spontaneous VSD fluorescence changes and LFP signals in both S1 (Figure 2B) and M1 (Figure 2C) in urethane-anesthetized mice. The images and movie (Movie S3) reveal complex patterns of spontaneous activity across the sensorimotor cortex. Typically activity propagated as waves of depolarization reflecting UP states (Steriade et al., 1993; Cowan and Wilson, 1994; Petersen et al., 2003b; Cossart et al., 2003; Shu et al., 2003; Volgushev et al., 2006) and often very large fractions of sensorimotor cortex were synchronously depolarized. The amplitude of

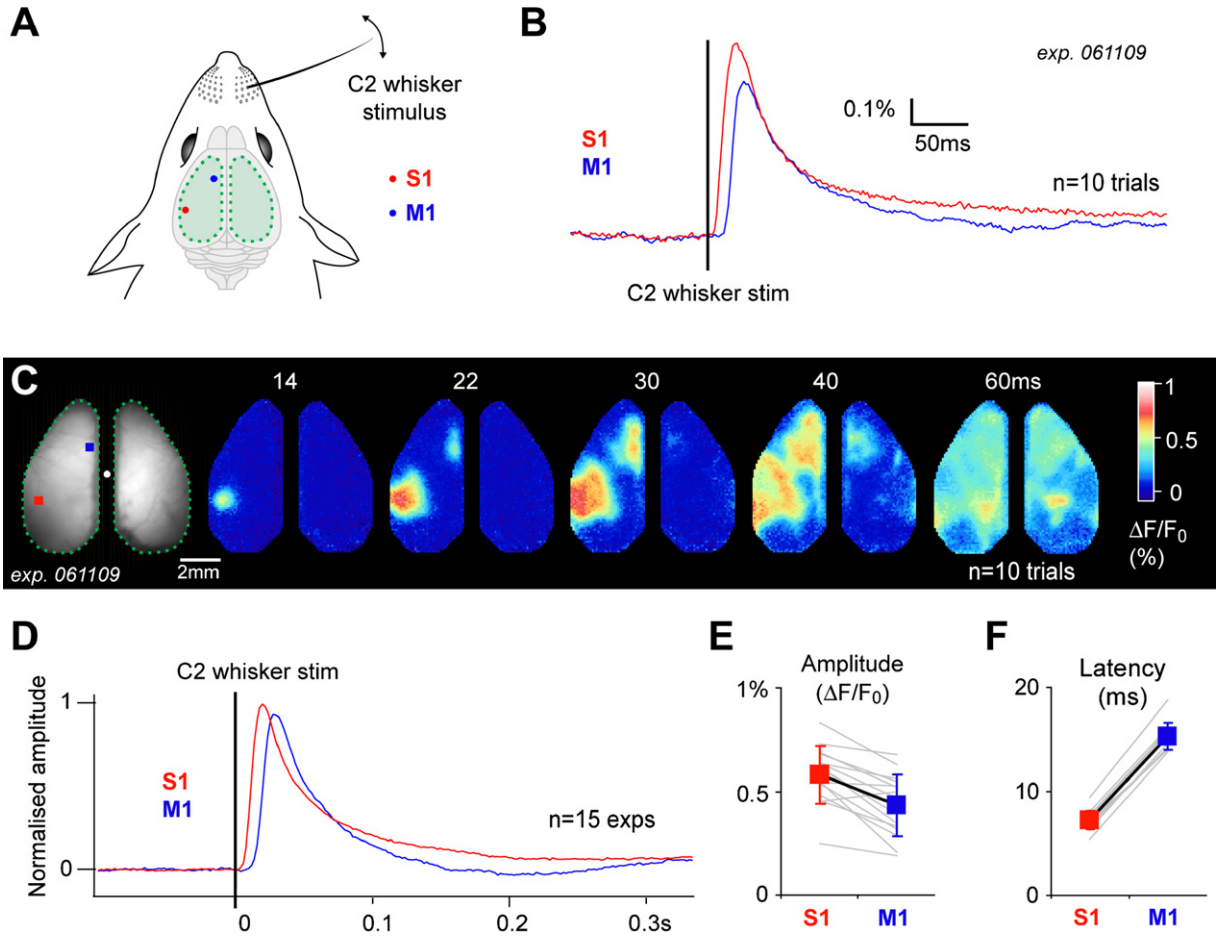


Figure 1. A Brief Deflection of a Single Whisker Evokes a Depolarizing Response in Both Somatosensory and Motor Cortex

(A) The green-shaded region schematically indicates locations of bilateral craniotomies. The craniotomies include a large fraction of somatosensory and motor cortex. The red dot indicates the position of the C2 barrel column of primary somatosensory cortex, and the blue dot is located in motor cortex. A small part of the primary visual cortex may be included in the posterior part of the craniotomies in some experiments.

(B) The craniotomies were stained with VSD RH1691 in a mouse under urethane anesthesia. A single brief deflection of the right C2 whisker evoked a transient increase in VSD fluorescence, first in primary somatosensory barrel cortex (S1, red trace) and then approximately 8 ms later in motor cortex (M1, blue trace). These traces were averaged across ten trials. Images from the same experiment showing the regions of interest from which the traces were computed are shown in (C).

(C) The left image shows resting VSD fluorescence with a white dot indicating the location of Bregma. The red square corresponds to the location of the C2 whisker representation in S1, and the blue square is located in M1. The single brief deflection of the right C2 whisker evoked an early localized response in the contralateral barrel cortex, followed by depolarization of the motor cortex. At later times, the sensory response is highly distributed, even spreading to parts of the ipsilateral cortex.

(D) Similar sequence of cortical activation, first in the somatosensory cortex and followed by motor cortex, was imaged with VSD in the 15 mice tested. Changes of fluorescence induced by a single C2 whisker deflection were quantified from S1 (red) and M1 (blue), normalized to the S1 peak response amplitude and averaged.

(E) Comparison of the peak amplitudes of the sensory responses measured in S1 and M1, for each individual experiment (grey lines) and mean ± SD (red for S1, blue for M1, linked by a black line).

(F) The latency of the sensory-evoked cortical activation was quantified in S1 and M1 by extrapolation of a linear 20%–80% fit of the rising phase of the signal. Data from individual experiments are shown in grey lines. Mean ± SD is indicated in red for S1, blue for M1, and linked by a black line.

spontaneous activity was similar, although usually smaller, to that of evoked activity (Figure S2).

We also imaged spontaneous activity in awake head-fixed mice, finding similarly diverse patterns of dynamic depolarizations propagating across sensorimotor cortex (Figure 2D). The example images show a correlated wave of depolarization spreading synchronously across somatosensory and motor cortex. We simultaneously filmed the whisker-related behavior of the mice at 500 Hz and matched the behavioral movie frame-by-frame to the VSD images of sensorimotor cortex function, allowing precise quantification of whisker movement. In order to quantify whisker-related behavior, the large mystacial vibrissae were trimmed immediately before the recording session,

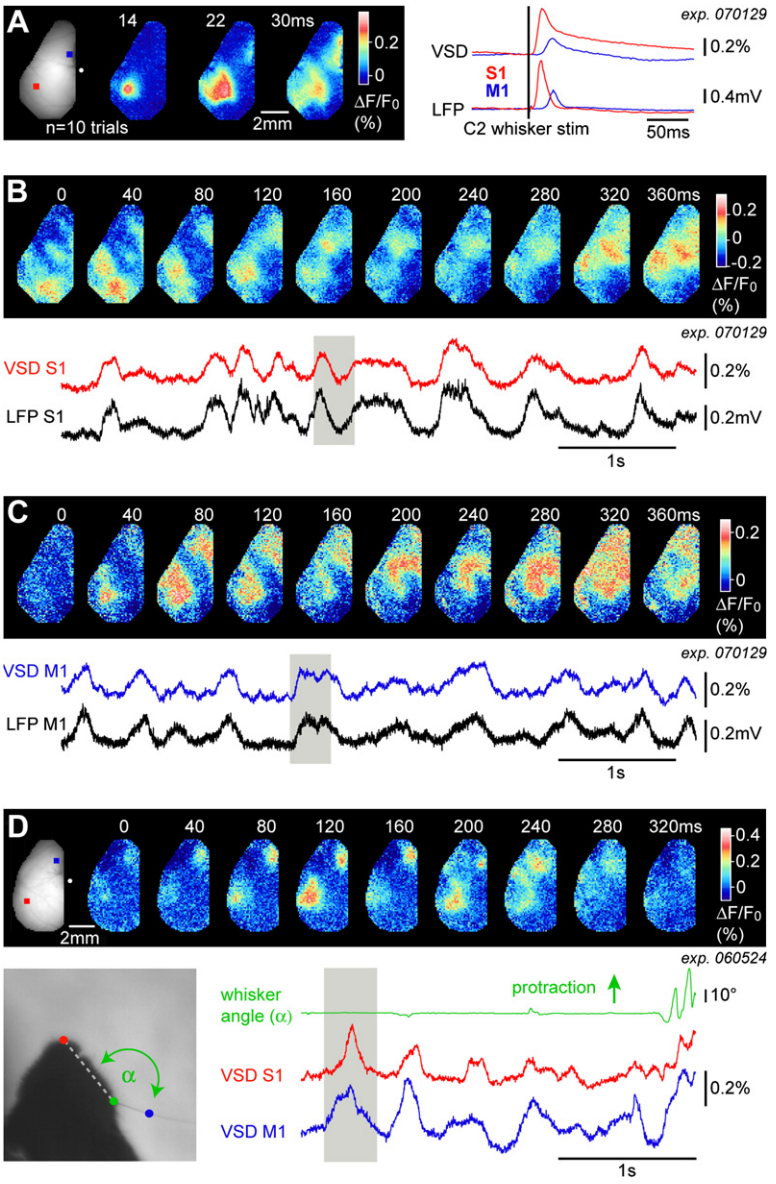


Figure 2. Voltage-Sensitive Dye Imaging and Local Field Potential Recording of Evoked and Spontaneous Activity in Sensorimotor Cortex

(A) The local field potential (LFP) in a urethane-anesthetized mouse was sequentially recorded in S1 and M1 locations corresponding to the regions of interest used to quantify the VSD fluorescence. Recordings of responses to C2 whisker deflection (n = 10 trials for each LFP location) revealed a close correlation between the simultaneously recorded optical and electrophysiological signals. Since the LFP signal relates to extracellular synaptic currents, the LFP response has a shorter duration than the VSD signal, which relates to membrane potential and is therefore slower due to the membrane time constants. The sign of the LFP signal is inverted for ease of comparison with the VSD signal.

(B) The LFP and VSD signals in the same urethane-anesthetized mouse were also correlated in the absence of sensory input, during spontaneous cortical activity. Lower traces show the quantification of the VSD fluorescence and the corresponding LFP recording in S1. The VSD images above illustrate the spontaneous events highlighted in gray.

(C) A different period of spontaneous activity in the same experiment, now with the LFP recording and VSD quantification in M1.

(D) VSD imaging of spontaneous activity in the sensorimotor cortex of an awake head-fixed mouse. The VSD images in this example show a correlated wave of spontaneous activity in motor (blue square) and somatosensory (red square) cortex. The whisker-related behavior was filmed at 500 Hz, and images were matched frame-by-frame to the VSD images (lower left image shows an example frame). The whisker angle was quantified for each frame (green trace) and plotted together with the changes in fluorescence in S1 (red trace) and M1 (blue trace). The period of spontaneous activity shown in the images above is highlighted by gray shading. At the end of the trial, the mouse begins to whisk, which is accompanied by depolarization of both somatosensory and motor cortex.

leaving only the right-hand C2 whisker intact. Under these conditions, we could precisely measure the rostrocaudal whisker movements. Toward the end of the trial, the mouse begins to whisk with concomitant depolarization of both motor and somatosensory cortex. From this trace, it is clear that the supragranular motor cortex can depolarize without whisker movement and, conversely, from other trials (data not shown), we found that whisker movement can be initiated without strong VSD signals in motor cortex. These results, highlighting the complex relationship between motor cortex activity and movement, may not be surprising in view of the fact that whisking can occur in rodents with lesioned motor cortex (Gao et al., 2003), lesioned neocortex (Welker, 1964; Semba and Komisaruk, 1984), and in decerebrate rats (Lovick, 1972). Equally, it is important to stress that the VSD imaging primarily reflects subthreshold depolarizations of layer 2/3, whereas it is the action potential activity of the layer 5/6 pyramidal neurons that is directly involved in the regulation of movement.

Somatotopic Organization of Tactile Responses in Sensorimotor Cortex

Having established the utility of imaging VSD fluorescence in these big craniotomies encompassing a large extent of somatosensory and motor cortex, we next began to functionally map the spatiotemporal dynamics of sensory responses evoked by different tactile stimuli. An example experiment is shown in Figure 3. The earliest responses

in somatosensory cortex were highly localized. The position of these early localized tactile responses changed depending upon the stimulus delivered. Deflection of the left C2 whisker evoked an early response in the right hemisphere (Figure 3A and Movie S2). Deflection of the right C2 whisker evoked an early localized response in the mirror symmetric location on the left hemisphere (Figure 3B and Movie S1). The dynamic patterns of evoked activity are very similar and allow a direct comparison of the bilateral propagation of the sensory signal. Interestingly, the response in the hemisphere ipsilateral to the stimulated whisker was not most prominent in the barrel cortex, but rather in frontal and posteriomedial parts of the dorsal cortex (likely motor cortex and parietal association cortex, respectively). That the spreading VSD signal appears to avoid exciting the ipsilateral barrel field is in good agreement with the lack of sensory responses to ipsilateral whisker deflections in whole-cell recordings of neurons in the supragranular barrel cortex (Brecht et al., 2003).

Single brief deflections of different individual neighboring whiskers evoked early localized sensory responses in accord with the somatotopic map of barrel cortex (Woolsey and Van der Loos, 1970). Responses in S1 to right C2 whisker deflections (Figures 3B and 3F) were mapped to lie between responses to whiskers B2 (Figures 3C and 3F), D2 (Figures 3D and 3F), C1 (Figure 3F), and C3 (Figure 3F). Tactile stimulation of the right forepaw (Figure 3E) evoked a sensory response anteromedial to the whisker responses. Superimposing these early localized responses provides a functional map of the somatosensory cortex (Figure 3F). However, as noted previously, the sensory responses do not remain localized to these cortical columns for more than a few milliseconds. Instead, the sensory information spreads across the supragranular cortex and even single brief whisker deflections can inform a large cortical area (Figure 3G).

The second localized region of activity evoked by whisker deflection occurred in the motor cortex ~8 ms after the initial response in S1. It is evident that the map of the early M1 responses evoked by whisker stimulation is more compact, with a greater overlap of the representation of different whiskers than in the S1 map (Figures 3B–3D, 3G, and 3H). Nonetheless, the early localized whisker responses in M1 were spatially ordered and were found to occur in a mirror symmetric map to the somatosensory cortex (Figure 3H).

Quantitative analysis of the spatial properties of the early S1 and M1 responses after stimulation of C2 and E2 whiskers confirmed this mirror symmetric organization (Figures 3I–3K). The early S1 responses evoked by C2 and E2 whiskers were separated by 894 ± 25 μm (n = 5). The early sensory responses evoked by C2 and E2 whisker deflection in M1 were separated by 387 ± 59 μm (n = 5). The somatotopic map representation of these whiskers in S1 is therefore approximately two times larger than in M1.

We made a further quantitative analysis of the relative somatotopic separations of the whisker representations comparing arcs versus rows of the whisker pattern. Sensory responses were evoked by C1, C2, and C3 whisker deflections for studying the separation of whisker representation along the C-row; and whiskers B2, C2, and D2 were deflected to study arc-2 organization. In S1, adjacent whiskers in the arc-2 were separated by 475 ± 116 μm (n = 4), and in the C-row they were separated by 251 ± 31 μm (n = 4). In M1, arc-2 whiskers were separated by 221 ± 94 μm (n = 4), and the C-row whiskers were separated by 138 ± 22 μm (n = 4). Both in S1 and M1, the whiskers lying in the same row are therefore represented in cortical regions closer to each other than the whiskers lying in the same arc.

Stimulation of the forepaw did not evoke a second localized region of activity (Figure 3E), and we propose that the somatosensory cortex and the motor cortex representations of the forepaw are too close to be distinguished under these experimental conditions.

These data demonstrate a highly dynamic map of sensorimotor processing in the dorsal cortex of mice, and they indicate that a sensory whisker map representation exists in motor cortex.

Whisker Deflection-Evoked Sensory Responses Are Located in the Whisker Motor Cortex

In view of the whisker deflection-evoked sensory responses in motor cortex, we wondered whether these might colocalize with the region of motor cortex involved in controlling whisker movement. We therefore performed intracortical microstimulation in lightly anesthetized mice to functionally map this cortical area (Figure 4). Trains of extracellular electrical current pulses of 100 μA at 60 Hz for 1.5 s were delivered sequentially to different locations in the mouse motor cortex (Figure 4A). Depending on the location of the stimulus, whisker retraction, whisker protraction, jaw movement, or forepaw movement was evoked. Movement of the C2 whisker was quantified with millisecond precision using a laser micrometer (Figure 4B). A consistent functional map of evoked movements was obtained through alignment, relative to Bregma, of data obtained from four mice (Figures 4C and 4D). Electrical stimulation of an anterior region (roughly located between 1–2 mm anterior to Bregma and 1–1.5 mm lateral) reliably evoked whisker retraction. Intracortical microstimulation of an adjacent more medial and posterior region evoked whisker protraction. This region corresponds to the previously identified rhythmic whisking region in rat motor cortex (Haiss and Schwarz, 2005). Clearly, the region of M1 where we imaged C2 whisker deflection evoked sensory responses (located 1.4 ± 0.2 mm anterior and 1.1 ± 0.2 mm lateral relative to Bregma, n = 15) is within the whisker motor cortex (Figure 4C and 4D).

Sensory Processing in M1 Depends on S1

We next began to investigate the pathways involved in directing sensory information to the whisker motor cortex. Given that somatosensory cortex always responded earlier than motor cortex to a brief C2 whisker deflection, we wondered whether activity in somatosensory cortex might in fact drive the sensory response in motor cortex.

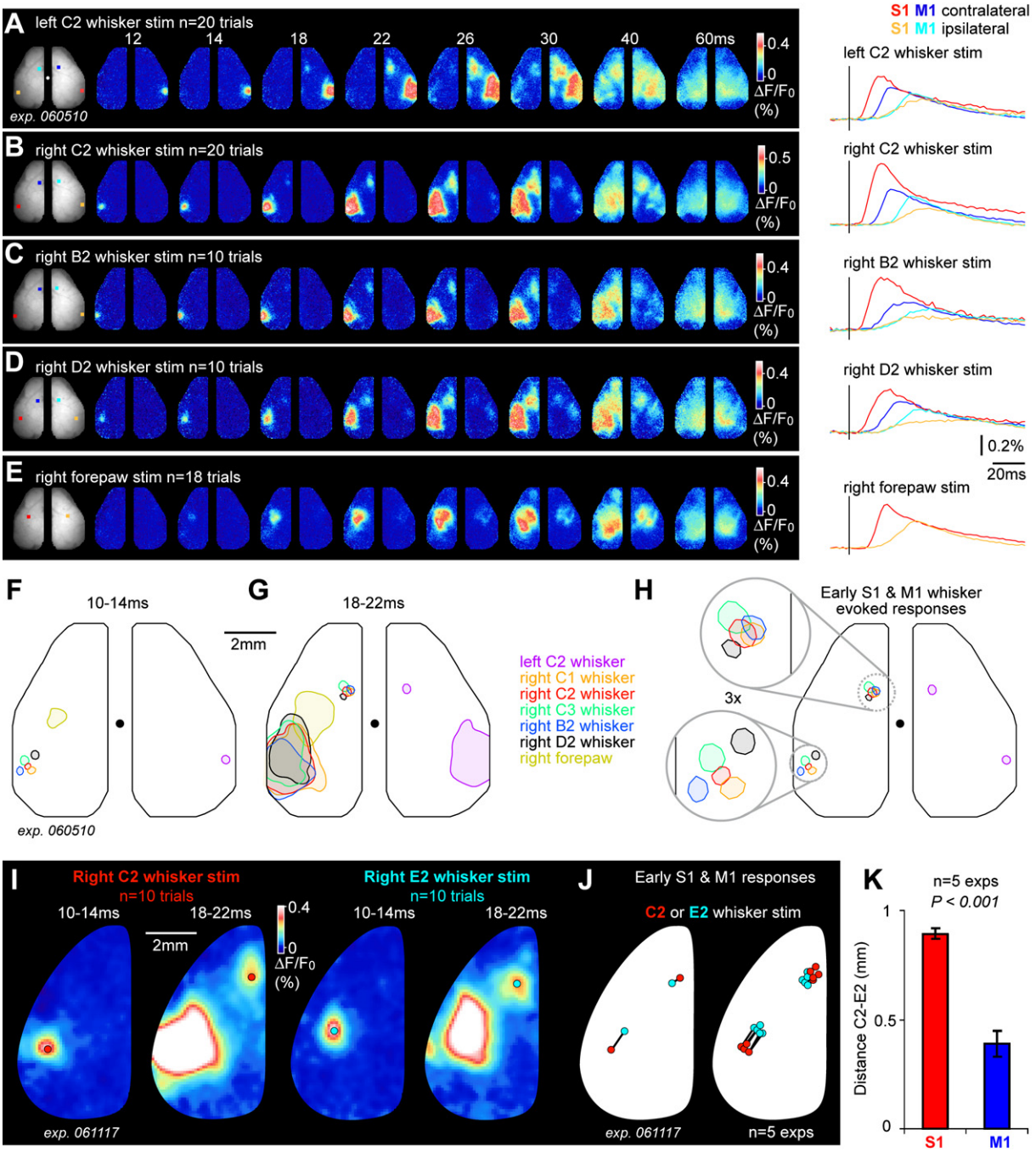


Figure 3. Bilateral VSD Imaging of Sensory Responses to Tactile Stimulation of Different Whiskers and the Forepaw

(A) Image of resting fluorescence of the VSD-stained bilateral craniotomies (far left). Bregma is indicated with a white dot. The red square indicates the location of the earliest response in contralateral somatosensory cortex, and the blue square the location of the first response in contralateral motor cortex. The orange and light-blue squares on the hemisphere ipsilateral to the stimulated whisker correspond respectively to somatosensory and motor areas. Their locations are symmetrical to the red and blue squares on the contralateral hemisphere. The spatiotemporal dynamics of VSD fluorescence changes evoked by a brief deflection of the left C2 whisker is shown in the adjacent panels. The time courses (far right) of responses to the whisker stimulus were measured within the regions of the red square (contralateral S1), blue square (contralateral M1), orange square (ipsilateral S1), and light-blue square (ipsilateral M1).

(B) The same experiment as in (A), but now with stimulation of the right C2 whisker.

(C) Stimulation of the right B2 whisker.

(D) Stimulation of the right D2 whisker.

We carried out two sets of experiments in urethane-anesthetized mice to test this hypothesis.

First, we used intracortical stimulation to directly excite the C2 barrel column (Figure 5A). Single brief current pulses evoked a response with short latency in the C2-related column, which spread across the barrel cortex and later depolarized the motor cortex. The pattern of the activity evoked by intracortical microstimulation is similar to that induced by the single deflection of the C2 whisker, suggesting that local excitation of the C2 column is sufficient to trigger the entire sensorimotor response (n = 3 experiments). These results from intracortical microstimulation are in good agreement with the rapid spread of depolarization observed upon intracortical microstimulation in the frontal eye field area of monkeys (Seidemann et al., 2002), although the late hyperpolarization evoked by the 500 Hz train of stimuli for 24 ms used in the monkey experiments was less prominent in our recordings, perhaps relating to our use of only a single stimulus.

In the second set of experiments, we investigated the effect of locally blocking synaptic activity upon the spreading VSD response (Figure 5B). Local injection of ~20 nl CNQX (250 μM) and APV (1 mM) to block ionotropic glutamate receptors in the C2 barrel column suppressed the entire C2 whisker deflection-evoked sensory response in both somatosensory and motor cortex (in S1 only 6% ± 4% and in M1 only 7% ± 4% of the control response remained, n = 10 experiments) (Farkas et al., 1999). Such local pharmacological blockade had little effect on the responses to deflection of the nearby E2 whisker (Figure S3). We conclude that activity in the C2 barrel column is necessary and sufficient for the depolarization in motor cortex evoked by C2 whisker deflection.

Monosynaptic Pathway from S1 to M1

Previous anatomical work in rats has provided evidence for a direct projection from septal regions of the barrel cortex to motor cortex (Miyashita et al., 1994; Izraeli and Porter, 1995; Hoffer et al., 2003). However, the mouse barrel cortex does not have an equivalent septal organization, with each barrel being tightly apposed to its neighbor. Nonetheless, it is clear that there is a projection from S1 to M1 in mice (White and DeAmicis, 1977; Porter and White, 1983), and here, we specifically investigated whether neurons in the mouse C2 barrel column project to M1 (Figure 6A). Pyramidal neurons located in the C2 barrel column were labeled with GFP expressed from a lentiviral vector driven by the αCaMKII promoter (Dittgen et al., 2004; Aronoff and Petersen, 2006). A strong axonal projection was found targeting motor cortex on the same cortical hemisphere (Figures 6B and 6D–6F). High-density axons with many boutons were found in the same location where sensory-evoked responses were imaged in motor cortex (Figure 6C). Despite strong labeling of the corpus callosum, the density of fibers in the somatosensory cortex of the other hemisphere was low (Figure 6G), in agreement with the weak long-latency ipsilateral VSD signals. The paucity of callosal axons terminating in the barrel cortex of the opposite hemisphere is also in good agreement with recent mouse data showing that only a small very lateral portion of the barrel field is innervated by supragranular callosal axons (Petreanu et al., 2007).

Our anatomical data provide evidence for a strong and direct glutamatergic connection from the C2 column of somatosensory barrel cortex to motor cortex. This monosynaptic pathway could mediate the sensory-evoked response in motor cortex. The ~8 ms latency difference between S1 and M1 activity is consistent with a pyramidal neuron axonal conduction velocity of ~450 μm/ms (Meeks and Mennerick, 2007). For the ~4 mm separation of S1 and M1, this would give an action potential propagation time of ~9 ms, consistent with our functional imaging results. However, we cannot exclude a contribution of other indirect pathways. Indeed, from previous studies, it is known that infragranular S1 neurons project to the posterior medial (POM) thalamic nucleus (Veinante et al., 2000), and POM, in turn, innervates M1 (Deschenes et al., 1998; Miyashita et al., 1994). Interestingly, under anesthesia, POM is inhibited by zona incerta (Lavallee et al., 2005), and activity in POM depends upon S1 cortex (Diamond et al., 1992), but during active whisking this sensory pathway could become important (Trageser et al., 2006).

(E) The same experiment as (A)–(D), but now with deflection of the skin on the right forepaw. In this case, there is not a clear spatial separation of the somatosensory and motor cortex response, which may, in fact, colocalize. Therefore, only two regions of interest are quantified.

(F) Contour plots showing the location of the early S1 responses. The half-maximal S1 response amplitude contours were computed from Gaussian-filtered VSD images at 10-14 ms after the stimulus.

(G) Equivalent half-maximal contour plots of the VSD responses 8 ms later than the previous panel, showing the early M1 responses and the spreading S1 responses.

(H) Superposition of the early S1 and M1 response components. The insets in the circles show three times magnified views of the early responses in S1 and M1. The somatotopic representation in S1 and M1 show a mirror symmetric organization. Thus the D2 whisker representations are closer to each other than the B2 representations.

(I) The VSD signals evoked by C2 and E2 whisker stimulation in a different experiment are shown on an expanded color scale to highlight the small early responses. The central region of the early responses (highlighted by filled circles in red for C2 and in cyan for E2) were quantitatively identified through Gaussian fitting. The primary sensory responses evoked by the C2 and the E2 whiskers are in different locations in both S1 and M1.

(J) Superposition of the primary response locations for the C2 whisker (red) and E2 whisker (cyan) for the example experiment (left) and from all five experiments (right). A line links the data from individual experiments. In S1 there is a large somatotopic shift in the primary representation comparing the C2 and E2 whiskers. In M1 there is also a clear shift observed in each of the five experiments, but the shift is smaller with a mirror symmetric displacement compared to the S1 shift.

(K) Quantification of the somatotopic shift comparing C2 and E2 whisker representations in S1 and M1. The somatotopic map in S1 is approximately twice as large as the M1 map. Bar graph shows mean ± SD.

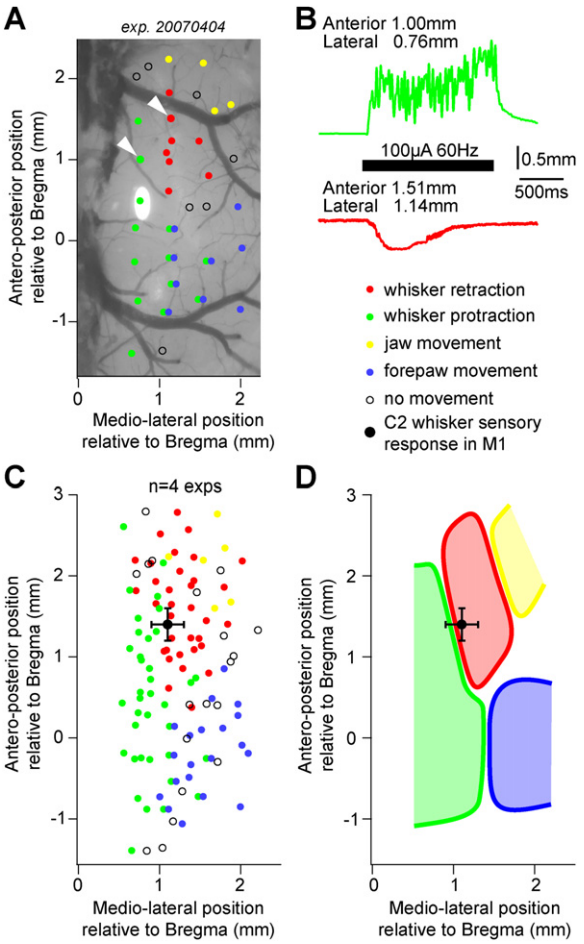


Figure 4. Functional Mapping of Mouse Motor Cortex with Intracortical Microstimulation

(A) Photomicrograph of the cortex with coordinates relative to Bregma. Each color-coded circle corresponds to a motor cortex location where intracortical microstimulation was tested in a lightly anesthetized mouse. Red points correspond to locations where intracortical microstimulation evoked whisker retraction. Green points indicate locations where stimulation evoked protraction. Blue points indicate evoked forepaw movement (in some cases both whisker protraction and forepaw movement were evoked, and in these locations the blue is displaced by ~0.05 mm to the right to allow both blue and green to be visible). The yellow points correspond to locations where stimulation evoked jaw movement. No movement was observed by stimulation at locations indicated by open black circles.

(B) Movements of the C2 whisker evoked by intracortical microstimulation at the locations indicated by white arrowheads in (A).

(C) Functional mouse whisker motor maps from four different mice were aligned at Bregma and superimposed. The location of the earliest sensory-evoked VSD signal (quantified in separate experiments) is superimposed as a black dot together with standard deviation bars (n = 15 experiments).

(D) Schematic drawing of the mouse whisker motor cortex map.

Sensory Responses in Awake Head-Fixed Mice during Behavior

In the above experiments, we investigated sensory processing in anesthetized mice. A critical issue is whether similar sensory-evoked responses occur in awake mice. We therefore trained mice for head fixation and performed VSD imaging experiments while filming whisker-related behavior. In order to deliver reproducible whisker deflections in awake and active mice, we attached a small metal particle to the C2 whisker and evoked whisker movement by generating brief magnetic fields (Crochet and Petersen, 2006; Ferezou et al., 2006). Such brief passive whisker deflections in awake mice (n = 13; Figure 7A) evoked the same stereotypical pattern of activity as observed in anesthetized mice. The highly distributed cortical sensory response to a single whisker deflection involving sequential activity in somatosensory cortex followed by motor cortex is therefore not induced by anesthesia, but appears to be a feature of normal brain function.

Sensory whisker information can also be actively gathered by mice as they move whiskers into contact with objects in their surroundings. We therefore combined VSD imaging of sensorimotor cortex and filming of whisker movements to identify the precise timing of individual whisker-object contacts. We observed the stereotypical sequence of cortical activity of S1 followed by M1 during the active touch of an object with the C2 whisker (Figures 7B and 7C and Movie S4). Although different whisker-object touches evoked variable responses, sequential depolarization of somatosensory cortex followed by motor cortex was observed in the averaged response of all 18 touches in this experiment (Figure 7D). Depolarization of both somatosensory and motor cortex evoked by active touch was observed in all five mice tested. In awake mice and under anesthesia, a single brief whisker deflection, be it active or passive, can therefore evoke depolarizing propagating sensory activity in both somatosensory and motor cortex.

Sensorimotor Processing Correlates with Behavior

The sensory responses in motor cortex could directly contribute to whisker motor control and thus we next analyzed the trial-by-trial correlations between cortical sensorimotor processing and behavior (Figure 8). The C2 whisker of awake head-fixed mice was passively deflected by magnetic pulses, while we simultaneously imaged VSD fluorescence and whisker movement. We distinguished between three different sequences of whisker-related behavior, which we analyzed separately. In the first case, the mice were quiet (without whisker movement) at the time of the stimulus, and following the stimulus, they began whisking (Figure 8A). In the second class of trials, the mice were quiet at the time of the stimulus and remained quiet, without whisker movement, after the stimulus (Figure 8B). In the third class, the mice were actively whisking at the time of the stimulus (Figure 8C), in which case they always continued to whisk after the stimulus. The whisker deflection induced by the magnetic field was oriented in a vertical plane and therefore not apparent on the images or the quantified traces of whisker movement (Figures 8A–8C). Sensory responses evoked by the

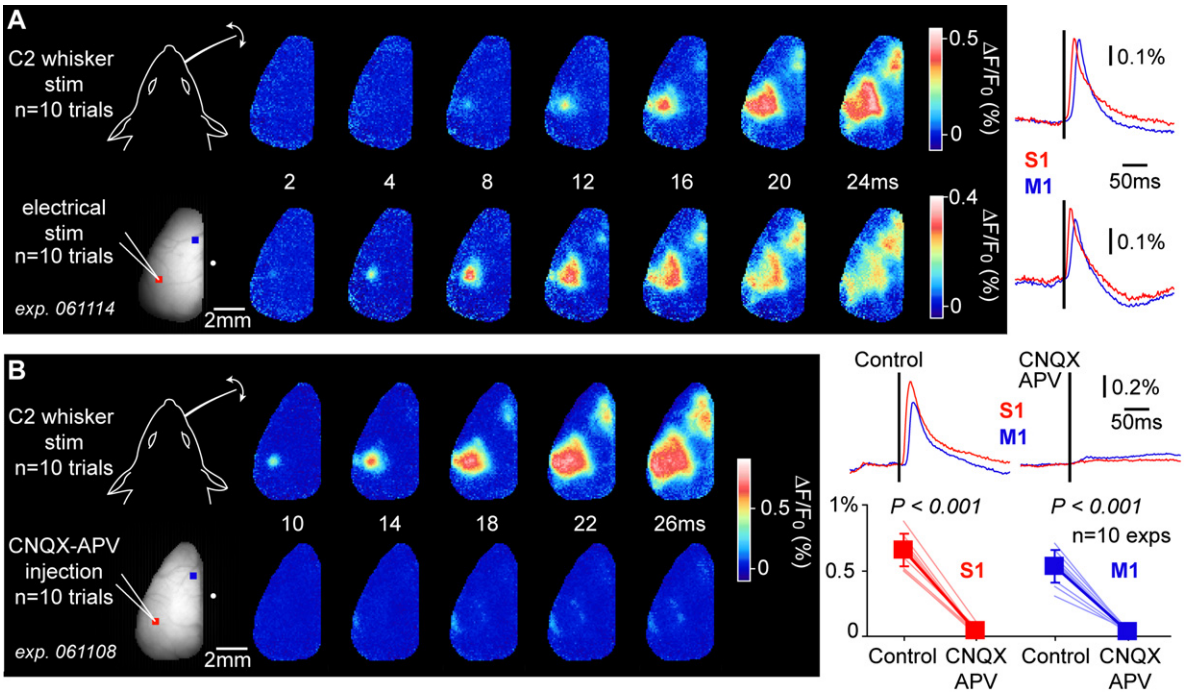


Figure 5. Sensory Processing in M1 Depends upon Activity in S1

(A) Intracortical microstimulation of the C2 barrel column evoked a similar response to that evoked by C2 whisker deflection, including sequential depolarization of S1 followed by M1.

(B) Local injection of ionotropic glutamate receptor antagonists into the C2 barrel column blocked the C2 whisker-evoked response in both S1 and M1. Time course of VSD responses before and after pharmacological blockade for this example experiment are shown in the upper right panel. The lower right panels indicate the effect CNQX and APV in all ten experiments carried out (light-red lines quantify responses in S1 in individual experiments, with bright red indicating the mean ± SD; light-blue lines show the effect in M1, with bright blue indicating the mean ± SD).

same magnetic C2 whisker deflection and imaged with VSD were highly variable, but correlated closely with the three different classes of whisker-related behaviors.

Large-amplitude spreading sensory responses were imaged in S1 and M1 on trials when the stimulus provoked the mouse to begin active whisking, whereas before the stimulus the whisker was not moving (Figures 8A and 8E and Movie S5). However, if the C2 whisker was neither actively moving before nor after the stimulus delivery, then smaller-amplitude localized responses were recorded in S1 with strongly reduced activity in M1 (Figures 8B and 8E). For stimuli that did not provoke whisking, responses were significantly reduced in S1 by 23% ± 42% (mean ± SD, $p = 0.026$) and in M1 by 45% ± 46% ($p = 0.034$), quantified across experiments in nine mice. This effect was significantly stronger in M1 than in S1 ($p = 0.042$, $n = 9$ mice). The stimulus-driven depolarization in M1 therefore correlates with the generation of sensory-evoked whisker movements. A passive whisker stimulus evoking a sensory response in the whisker motor cortex during quiet wakefulness might therefore be an important command signal for the mouse to begin whisking. The underlying mechanism for the variability of the responses evoked during quiet wakefulness is currently unclear. Strong responses were intermixed with weak responses, so it is unlikely to reflect slow changes in behavioral state. At least part of the variability is likely to result from interactions with spontaneous activity (Figure 2D), which could have a strong impact upon sensory processing in the same way that UP and DOWN states play profound roles in regulating sensory processing in the anesthetized rodent whisker sensory pathway (Petersen et al., 2003b; Sachdev et al., 2004). Further experiments in awake mice directly investigating the interactions of spontaneous cortical activity and sensory processing would be of great interest.

When the C2 whisker stimulus occurred during active whisking, smaller-amplitude sensory responses were evoked in somatosensory cortex (Chapin and Woodward, 1982; Fanselow and Nicolelis, 1999; Castro-Alamancos and Oldford, 2002; Hentschke et al., 2006; Ferezou et al., 2006; Crochet and Petersen, 2006). These small-amplitude sensory responses evoked during whisking were spatially restricted to a small part of the S1 barrel cortex, generating only very weak depolarization of motor cortex (Figures 8C and 8F). Across 11 experiments, the response evoked during whisking was decreased by 91% ± 36% in M1, and by 61% ± 18% in S1, relative to stimuli delivered at a time when there was no ongoing whisker movement. The decrease in evoked responses during whisking was significantly greater in M1 compared

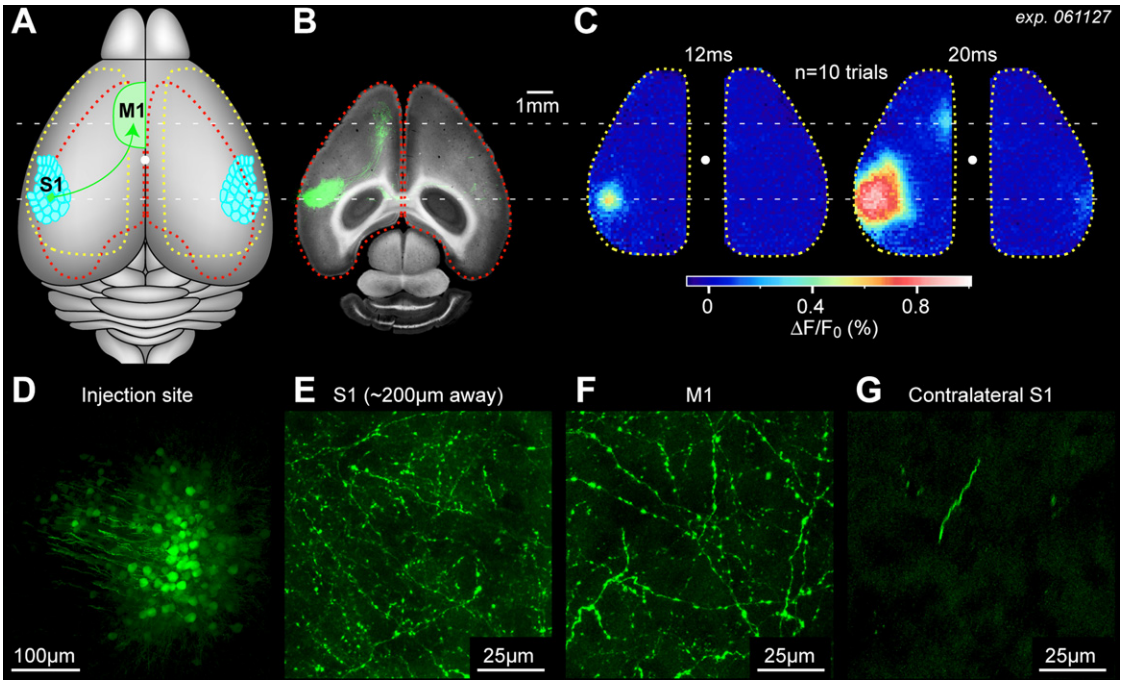


Figure 6. Monosynaptic Input from the Somatosensory Cortex Could Contribute to the Sensory Response in Motor Cortex

(A) Schematic drawing indicating location of the primary somatosensory barrel cortex (S1) and the whisker motor cortex (M1).

(B) Lentivirus encoding GFP under the control of the αCaMKII promoter was injected directly into the C2 barrel column, which had been functionally mapped by intrinsic optical imaging. Three weeks later, the brain was sliced horizontally in 100 μm sections and the GFP fluorescence imaged. The red dashed line (also in panel [A]) outlines a horizontal brain slice with superimposed GFP epifluorescence montage from three mice. A dense axonal projection from the C2 barrel column to motor cortex is evident.

(C) The yellow outline (also in panel [A]) indicates the area imaged by VSD showing early responses to C2 whisker deflection. The sensory evoked activity in the motor cortex colocalizes with the dense axonal projection from the C2 barrel column.

(D) Confocal maximal-intensity projection of the entire thickness of a horizontal brain slice showing the lentiviral injection site.

(E) As above, but for a region ~200 μm anterior to the injection site.

(F) As above, but for a region of the motor cortex.

(G) As above, but for a region of the barrel cortex on the opposite hemisphere symmetrical to the injection site.

to S1 ($p = 0.032$, $n = 11$ mice), demonstrating that sensory processing is more localized during active behavior. Ongoing behavior therefore plays a key role in dynamically gating cortical sensorimotor processing.

DISCUSSION

The data in this study provide the first images of the spatiotemporal dynamics of cortical sensorimotor integration, revealing highly dynamic and distributed processing, which correlates strongly with behavior.

Highly Distributed Processing of a Single Whisker Deflection

A single brief deflection of a single whisker evokes a somatotopically mapped cortical depolarization, which remains localized to its barrel column only for a few milliseconds. Pyramidal neurons then rapidly inform a large part of sensorimotor cortex about the whisker deflection. This dynamic highly distributed cortical depolarization provides a mechanism for the integration of sensory information. The corollary of such a spatially extended single whisker response across sensorimotor cortex is that individual neurons should be sensitive to a very broad range of stimuli, that is they should have broad subthreshold receptive fields. Whole-cell recordings in somatosensory barrel cortex show that supragranular neurons indeed have large receptive fields (Moore and Nelson, 1998; Zhu and Connors, 1999; Brecht et al., 2003) in good agreement with our VSD imaging results, which also relate primarily to subthreshold membrane potential changes in the superficial cortical layers (Ferezou et al., 2006). In fact, it is becoming increasingly clear that stimuli of one sensory modality can even affect processing in primary cortical areas of other sensory modalities (Fu et al., 2003; Wallace et al., 2004; Ghazanfar et al., 2005; Kayser et al., 2005; Martuzzi et al., 2006; Lakatos et al., 2007; reviewed in Schroeder and Foxe, 2005; Ghazanfar and Schroeder, 2006; Bulkin and Groh, 2006; Macaluso, 2006).

Such highly distributed processing of sensory information may be an important feature of the neocortex. Somehow, the brain must correlate tactile sensory input from different body parts. If a whisker has just been deflected by an approaching object or animal, it may well be that

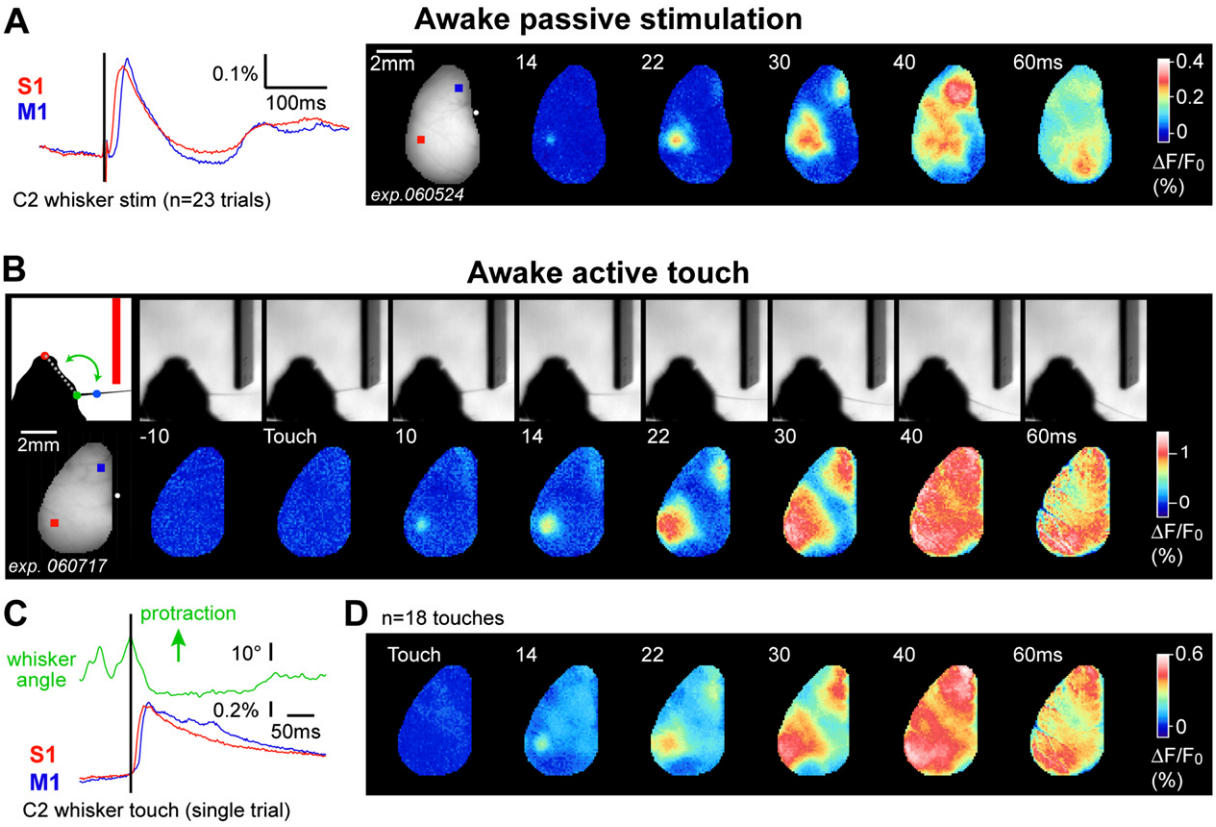


Figure 7. Both Passive and Active Deflections of the C2 Whisker Evoke Activity in Somatosensory and Motor Cortex of Awake Head-Fixed Mice

(A) Passive deflection of the C2 whisker in an awake mouse evoked VSD responses first in the somatosensory cortex followed by motor cortex, as observed in urethane-anesthetized mice.

(B) A single-trial example of active touch, imaged in an awake behaving mouse. The C2 whisker actively touched an object (upper image sequence), evoking a spreading VSD response, first in somatosensory and then in the motor cortex (lower image sequence).

(C) Quantification of whisker movement (green trace) and VSD fluorescence changes in S1 (red) and M1 (blue) from the same single trial.

(D) A similar sequence of sensorimotor cortex depolarization was observed on average across 18 different whisker-object touches in the same experiment.

the cause of the sensory stimulus will very soon affect input at other body locations. That a single whisker deflection can inform a large cortical area allows the modulation of sensory processing in these other regions, in a manner dependent upon the immediate previous sensory experience. In a quiet animal, the activity in the C2 barrel column has spread across sensorimotor cortex within tens of milliseconds, and the sensorimotor system may now be primed to respond to further input. Subsequent tactile stimuli occurring within the next tens or hundreds of milliseconds can therefore very easily become associated with the initial event. Such context-dependent processing of sensory information could be an important feature of the neocortex. Perhaps most importantly, such highly distributed processing is essential for associational plasticity and learning.

It should be noted that VSD imaging corresponds closely to subthreshold membrane potential changes in supragranular neurons, but not necessarily to action potential activity. Depolarization is of course necessary to evoke action potentials, but since neuronal membrane potential is often far from threshold, large depolarizations are possible without evoking action potentials even in awake mice (Crochet and Petersen, 2006). Current evidence in awake rodents would suggest that most of the electrical activity in the neocortex is in fact subthreshold with relatively low action potential firing rates (Crochet and Petersen, 2006; Lee et al., 2006). The VSD imaging technique thus provides information relating to depolarization, but not action potential firing. Indeed, we recently compared the spatiotemporal differences between sensory responses imaged with voltage- and calcium-sensitive dyes (which respectively reflect subthreshold and suprathreshold activity primarily) and found that the suprathreshold calcium signals remained more localized than the spreading VSD responses (Berger et al., 2007). The highly distributed sensory responses evoked by single whisker deflections are therefore likely to reflect subthreshold depolarizations rather than action potential firing.

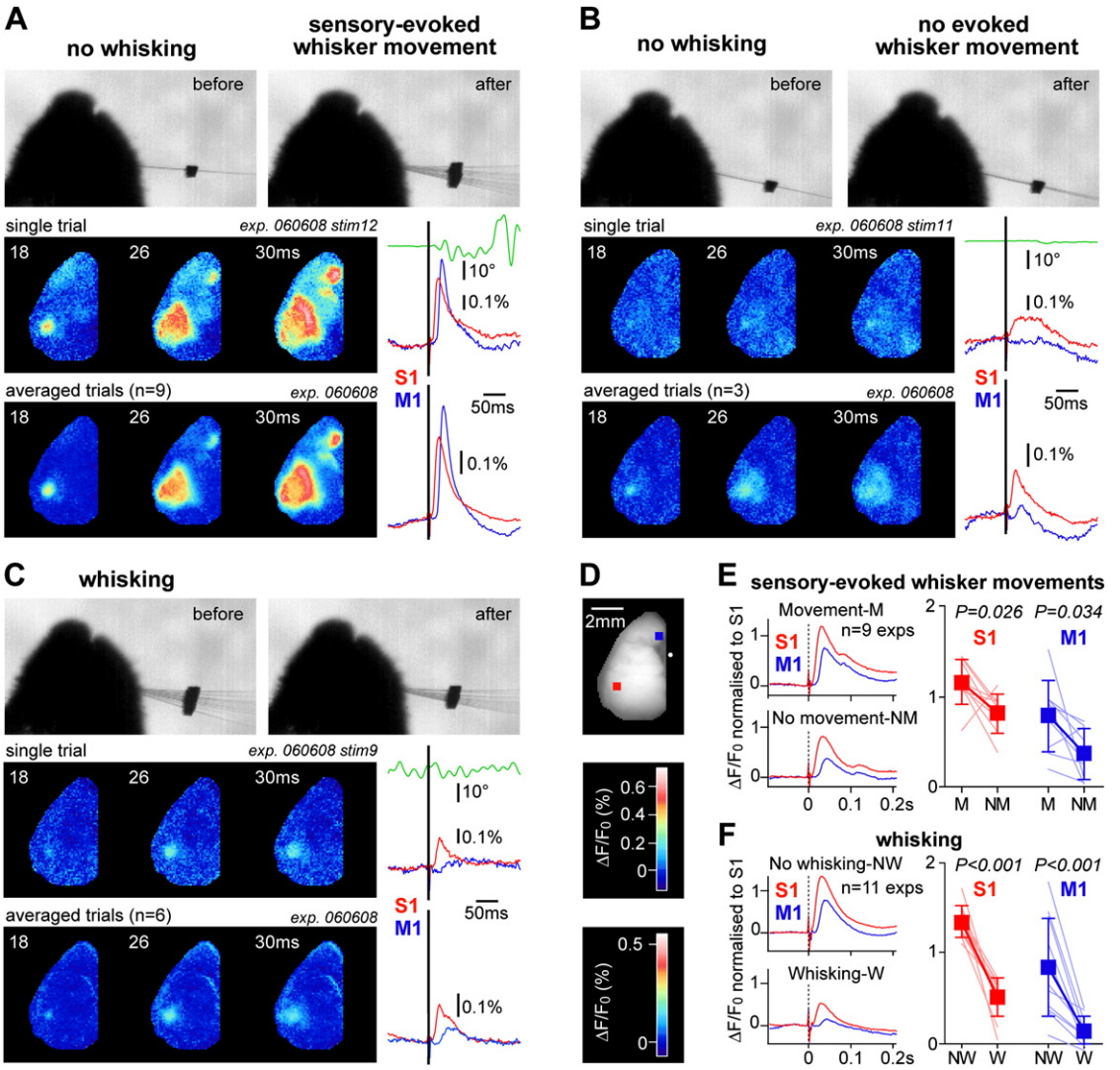


Figure 8. Trial-to-Trial Variability in Sensorimotor Processing Correlates with Behavior

(A) Individual trials were analyzed depending upon the whisker-related behavior. In trials during quiet wakefulness where the C2 whisker deflection drove a sensory-evoked whisker movement, a large spreading sensory response was evoked in both sensory and motor cortices. The upper images show 25 superimposed frames taken over a 100 ms period immediately before (left) and after (right) stimulation of the C2 whisker. The images show that in this trial the C2 whisker stimulus drives a sensory-evoked whisker movement. The middle panel shows VSD images and quantification from the same single trial. There is a large-amplitude spreading VSD signal that evokes a strong response in M1. The lower series of images shows the average of VSD images from all trials in this experiment where the stimulus was delivered during quiet wakefulness and evoked a whisker movement. The green trace (right) indicates whisker position; red and blue traces show the time course of the VSD signal in S1 and M1, respectively.

(B) In other trials from the same experiment, the same whisker stimulus was delivered during quiet periods as in (A), but in this case, the stimulus did not evoke a whisker movement. In these trials, only a small cortical response was observed, which remained localized to the somatosensory cortex.

(C) A small localized somatosensory response was also evoked during trials in the same experiment, when the mouse was spontaneously whisking at the time of the stimulus delivery.

(D) Location of regions of interest and color scales for the previous panels.

(E) Across 9 experiments, stimuli delivered during quiet wakefulness evoked significantly bigger responses in S1 and M1, if they were associated with a sensory-evoked whisker movement. This effect was significantly greater for the M1 response compared to the S1 response. The left panel shows the grand average time courses of the sensory responses with either evoked movement (M) or no movement (NM). The right panel indicates the data from individual experiments shown in light red for S1 and light blue for M1. The dark red and dark blue indicate mean ± SD.

(F) Across 11 experiments, the passively evoked sensory response in S1 and M1 was significantly smaller during active whisking compared to during quiet wakefulness. The smaller response evoked during whisking was more localized to S1, spreading little to M1. The left panel shows the grand average time courses of the evoked responses during no whisking (NW) or during whisking (W). The right panel indicates the data from individual experiments shown in light red for S1 and light blue for M1. The dark red and dark blue indicate mean ± SD.

There are many factors that regulate the spread of the VSD response. We previously demonstrated that very weak and brief whisker deflections can evoke VSD responses that remain localized to a single cortical column (Petersen et al., 2003a; Berger et al., 2007). Spontaneous activity also interacts strongly with sensory processing, with localized small-amplitude VSD responses being evoked during UP states (Petersen et al., 2003b). Equally, during early development, sensory responses to individual whisker deflections do not spread far, but rather remain localized to their specific cortical column (Borgdorff et al., 2007). Sensory experience has also been shown to profoundly regulate the extent of sensory processing (Polley et al., 1999, 2004). Finally, it is clear that sensory processing depends strongly upon ongoing behavior, as further discussed below.

Dynamic Control of Sensorimotor Processing

The large-amplitude sensory responses evoked during quiet wakefulness (Figure 8A), which propagate across sensorimotor cortex, may function as a sensitive detection system alerting the mouse to the presence of an unexpected sensory input. Sensory input to whisker motor cortex, together with lower-level sensorimotor loops (Nguyen and Kleinfeld, 2005), may be an important mechanism for the initiation of whisking—after a passive stimulus is perceived, the mouse can actively explore to uncover further sensory information relating to the stimulus.

On the other hand, small-amplitude localized sensory responses were evoked by passive stimuli during whisking (Figure 8C). Sensory processing is therefore dynamically gated by ongoing behavior (Chapin and Woodward, 1982; Fanselow and Nicolelis, 1999; Castro-Alamancos and Oldford, 2002; Hentschke et al., 2006; Ferezou et al., 2006; Crochet and Petersen, 2006). The barrel cortex during active whisking is in a different state compared to quiet periods without whisking. The slow large-amplitude cortical oscillations that propagate as waves during quiet periods (Ferezou et al., 2006) disappear during active whisking (Crochet and Petersen, 2006). Supragranular barrel cortex neurons depolarize during whisking with slight decrease of input resistance (Crochet and Petersen, 2006). These changes in brain state and in individual neurons likely contribute to the different sensory processing during active whisking compared to quiet wakefulness. Whereas the spreading responses to passive stimuli during quiet wakefulness may function to alert the animal to the presence of an unexpected stimulus, the localized processing of single whisker information in its cortical column may allow more specific sensory processing to occur. The more localized sensory processing during active whisking may be better suited to discriminate and perform fine-grain analyses of sensory stimuli rather than the large-amplitude propagating responses evoked during quiet wakefulness. Localized cortical processing of single-whisker information may be useful for texture discrimination or object location (Harris et al., 1999; Szwed et al., 2003; Arabzadeh et al., 2005; Knutsen et al., 2006; Mehta et al., 2007). Alternatively, the small-amplitude localized processing of the brief passive stimulus that we deliver during active whisking may simply go unnoticed by the mouse, making little impact upon brain or behavior. From this point of view, it is interesting to note that large-amplitude propagating sensory responses can occur during active touch of real objects (Figures 7B–7D; Ferezou et al., 2006; Crochet and Petersen, 2006). Active touch of real objects might be amplified by brain stem sensorimotor loops accelerating the whisker into the object (Nguyen and Kleinfeld, 2005), which would not occur for the brief passive stimuli applied in Figure 8. In order to address the functional roles of the dynamic gating of sensorimotor processing during behavior, future experiments must investigate the psychophysical detection thresholds (Stuttgen et al., 2006) for stimuli delivered during different behaviors.

Sensory Control of Motor Cortex

Sensory responses in M1 were prominent when a passive stimulus was delivered during inactive periods, and might therefore correspond to a "wake-up" call for the sensorimotor cortex (Figure 8A). Movements initiated through the sensory-evoked depolarization of the motor cortex might then be organized to actively gather further information relating to the tactile input. Equally, sensory responses were observed in motor cortex following active touch (Figures 7B–7D). Thus, in a similar way that we change our finger and hand movements during active contact with an object to obtain shape and textural information, the mouse might regulate whisker movements to help extract tactile information. The sensory map in whisker motor cortex (Figure 3) suggests that sensory information relating to individual whiskers is processed in specific regions of the whisker motor cortex. This might allow fine adjustments of individual whisker movements guided by specific sensory feedback related to that same whisker relayed from S1. Interestingly, the independent movement of individual whiskers has already been observed (Sachdev et al., 2002).

The sensory processing that we observe with VSD imaging could be gated in complex manners before resulting in alterations in motor output. The current VSD imaging technique provides information primarily relating to subthreshold membrane potential changes in layer 2/3. One interesting possibility requiring further investigation is that sensory processing in motor cortex might be most prominent in layer 2/3, which could be differentially regulated compared to the action potential firing of layer 5/6 pyramidal neurons that contribute more directly to motor control. It would therefore be of great interest to image the spatiotemporal dynamics of deeper cortical layers, which could potentially be achieved in future studies using dye injections combined with fiber imaging technology.

Future Perspectives

VSD imaging of sensorimotor cortex appears to be a promising technique for observing real-time integration

of information between sensory and motor cortex. Here, we studied processing related to passively and actively evoked whisker sensory input, but future studies should further investigate spontaneous activity (Figures 2B–2D) correlating both the activity patterns between brain areas and with behavior. The dynamic correlations between different cortical areas might provide information relating to the organization of the functional connectivity between cortical areas (Vincent et al., 2007) and how this is regulated by behavior. Equally, by making craniotomies extending further posteriorly, it might well be possible to image visual cortex and perhaps even cerebellar activity simultaneously with sensorimotor cortex. Over the next years, we plan to develop tapered fiber optic image bundles to visualize cortical dynamics in these large craniotomies in freely moving mice (Ferezou et al., 2006). This study, in which we imaged a large fraction of the mouse sensorimotor cortex at millisecond temporal resolution and subcolumnar resolution, is therefore a first step toward high spatiotemporal resolution imaging of the entire dorsal mouse brain during behavior.

EXPERIMENTAL PROCEDURES

Voltage-Sensitive Dye Imaging

All experiments were carried out with C57BL6J mice aged from 1 to 5 months in accordance with authorizations approved by the Swiss Federal Veterinary Office. Surgical and imaging procedures were largely as previously described (Petersen et al., 2003a, 2003b; Ferezou et al., 2006; Berger et al., 2007). Surgery for awake recordings (Figures 2D, 7, and 8) was carried out with isoflurane (1.5%), and a head-fixation post was glued onto the skull. For anesthetized recordings (Figures 1, 2A–2C, 3, 5, and 6), mice were injected with urethane (1.5 mg/g). The bone overlying the area to be imaged was removed. Extreme care was taken at all times not to damage the cortex, especially during removal of the dura. The VSD RH1691 was dissolved at 1 mg/ml in Ringer's solution containing (in mM) 135 NaCl, 5 KCl, 5 HEPES, 1.8 $CaCl_2$, 1 $MgCl_2$. This dye solution was topically applied to the exposed cortex and allowed to diffuse into the cortex for 1 hr. The cortex was subsequently washed to remove unbound dye and covered with agarose before placing a coverslip on top. The VSD was excited with 630 nm light from a 100 W halogen lamp gated by a Uniblitz shutter (Vincent Associates) under computer control via an ITC18 (Instrutech) communicating with custom software running within IgorPro (Wavemetrics). The excitation light was reflected using a 650 nm dichroic and focused onto the cortical surface with a 50 mm SLR camera lens (Nikon). Fluorescence was collected via the same optical pathway, but without reflection of the dichroic, long-pass filtered (>665 nm) and focused onto the sensor of a high-speed MiCam Ultima (Scimedia) camera via a 50 mm video lens (Navitar). This high-speed CMOS camera has a detector with 100 × 100 pixels. The field of view was 10 × 10 mm, and therefore every pixel collects light from a cortical region of 100 × 100 μm. Images were collected with 2 ms temporal resolution and analyzed offline using custom-written routines in IgorPro. Bleaching of fluorescence was corrected by subtraction of a best-fit double-exponential or, in experiments on anesthetized mice, by subtraction of heart-beat synchronized and averaged sweeps recorded without whisker stimulus. Time courses of fluorescence changes were quantified as $\Delta F/F_0$ from regions of interest covering 5 × 5 pixels, indicated by the colored squares in the images (corresponding to 500 × 500μm of cortex). In order to compare VSD signals from different animals, regions of interest were centered on the locations of the earliest responses in S1 and M1. Responses from these functionally identified regions were then compared or averaged across different experiments. Amplitudes of sensory-evoked responses were calculated as the change in the VSD signal ($\Delta F/F_0$) over a fixed time interval for each experiment (baseline time point was immediately before the stimulus; the response time point was chosen to be at the maximum of the averaged response).

Filming and Stimulating the C2 Whisker

In order to quantify whisker-related behavior, the large mystacial vibrissae were trimmed immediately before the recording sessions, leaving only the right-hand C2 whisker intact. The mouse was illuminated from below with infrared light and filmed through a 50 mm video lens (Navitar) with a high-speed MotionPro camera (Redlake). The behavioral images were obtained at 2 ms intervals between frames synchronized to the VSD imaging through TTL pulses. Custom-written routines running within ImageJ were used to automatically track whisker position. For the experiments involving passive whisker deflection in awake mice, reproducible whisker stimuli were evoked by attaching a small metal particle to the C2 whisker and generating brief magnetic pulses (Ferezou et al., 2006; Crochet and Petersen, 2006). For the experiments in anesthetized mice, 2 ms whisker deflections were generated by a computer-controlled piezoelectric bimorph (Ferezou et al., 2006).

Recording Local Field Potentials

The local field potential (LFP) was recorded in urethane-anesthetized mice by inserting a Ringer-filled pipette (~10 MΩ) into the supragranular cortex (~250 μm depth), successively in S1 and M1 locations corresponding to the regions of interest used to quantify the VSD fluorescence. The signal was amplified by a Multiclamp 700 amplifier (Axon Instruments) and filtered from DC to 500 Hz.

Intracortical Microstimulation of S1

A glass micropipette (~10 μm tip diameter) filled with Ringer's solution was introduced into the cortex to a depth of ~400 μm to target layer 4. The horizontal location of the pipette was targeted to the functional location of the C2 barrel column, as identified by precise colocalization of the cortical microstimulation evoked VSD response and the C2 whisker deflection-evoked VSD response. Electrical stimuli of duration 500 μs and amplitude four to eight times the threshold for evoking a VSD response (50–1000 μA) were applied using current injections delivered by a linear stimulus isolator (A395, World Precision Instruments).

Intracortical Microstimulation of M1

Surgery was carried out under 1.5% isoflurane, and anesthesia was subsequently switched to a continuous intravenous injection of ketamine at 3 mg/kg/min. Trains of 60 Hz stimuli with 100 μA bipolar current pulses, each of duration 200 μs, were delivered with glass-coated platinum-tungsten electrodes (80 μm shank diameter; 23 μm diameter of the metal core; free tip length, 8 μm; impedance, >1 MΩ; Thomas Recording). The C2 whisker position was quantitatively recorded using a laser curtain and a linear CCD array (RX 03, Metralight).

Local Pharmacology

A glass micropipette (~10 μm tip diameter) back-filled with mineral oil and tip-filled with the drug dissolved in Ringer's solution was slowly inserted to a depth of ~400 μm directly into the C2 column of the barrel cortex as identified by VSD imaging. By advancing a metal piston into the pipette by a known distance, we could inject a defined quantity of the drug directly into the C2 barrel column (typically ~20 nl, with each calibrated unit denoting 0.2 nl).

Lentiviral-Based Anatomy

Lentiviral vector was produced by transient calcium phosphate transfection of human embryonic kidney 293T cells with the Gag-Pol construct (pCMVΔ8.92), the Rev expression plasmid (pRSV-Rev), the

VSV-G protein envelope construct (pMD2.G), and the pFCK(1.3)GW transfer vector encoding GFP under the control of a 1.3 kb fragment of the αCaMKII promoter (Dittgen et al., 2004). Media was changed ~7 hr post-transfection and viral supernatant was harvested after ~40 hr, clarified by low-speed centrifugation, filtered at a 0.22 μm pore size, concentrated ~1000× by ultracentrifugation and resuspended in phosphate-buffered saline with 0.5% BSA. Lentivirus (~50 nl) was injected into both supragranular and infragranular layers of the C2 barrel column. The location of the C2 barrel column was identified by intrinsic optical imaging (Grinvald et al., 1986; Polley et al., 2004) following previously described procedures (Ferezou et al., 2006; Crochet and Petersen, 2006).

Statistical Tests

Data are expressed as mean ± standard deviation, and they were tested using SigmaStat (Systat Software) for statistical significance using Student's t test (paired when appropriate) or Wilcoxon signed rank test for data without a normal distribution.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/56/5/907/DC1/.

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REFERENCES

Ahrens, K.F., and Kleinfeld, D. (2004). Current flow in vibrissa motor cortex can phase-lock with exploratory rhythmic whisking in rat. J. Neurophysiol. *92*, 1700–1707.

Alloway, K.D., Zhang, M., and Chakrabarti, S. (2004). Septal columns in rodent barrel cortex: functional circuits for modulating whisking behavior. J. Comp. Neurol. *480*, 299–309.

Arabzadeh, E., Zorzin, E., and Diamond, M.E. (2005). Neuronal encoding of texture in the whisker sensory pathway. PLoS Biol. *3*, e17. 10.1371/journal.pbio.0030017.

Arieli, A., Sterkin, A., Grinvald, A., and Aertsen, A. (1996). Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. Science *273*, 1868–1871.

Aronoff, R., and Petersen, C.C.H. (2006). Controlled and localized genetic manipulation in the brain. J. Cell. Mol. Med. *10*, 333–352.

Benucci, A., Frazor, R.A., and Carandini, M. (2007). Standing waves and traveling waves distinguish two circuits in visual cortex. Neuron *55*, 103–117.

Berg, R.W., Friedman, B., Schroeder, L.F., and Kleinfeld, D. (2005). Activation of nucleus basalis facilitates cortical control of a brain stem motor program. J. Neurophysiol. *94*, 699–711.

Berger, T., Borgdorff, A.J., Crochet, S., Neubauer, F.B., Lefort, S., Fauvet, B., Ferezou, I., Carleton, A., Luscher, H.R., and Petersen, C.C.H. (2007). Combined voltage and calcium epifluorescence imaging in vitro and in vivo reveals subthreshold and suprathreshold dynamics of mouse barrel cortex. J. Neurophysiol. *97*, 3751–3762.

Borgdorff, A.J., Poulet, J.F.A., and Petersen, C.C.H. (2007). Facilitating sensory responses in developing mouse somatosensory barrel cortex. J. Neurophysiol. *97*, 2992–3003.

Brecht, M., Roth, A., and Sakmann, B. (2003). Dynamic receptive fields of reconstructed pyramidal cells in layers 3 and 2 of rat somatosensory barrel cortex. J. Physiol. *553*, 243–265.

Brecht, M., Schneider, M., Sakmann, B., and Margrie, T.W. (2004). Whisker movements evoked by stimulation of single pyramidal cells in rat motor cortex. Nature *427*, 704–710.

Bulkin, D.A., and Groh, J.M. (2006). Seeing sounds: visual and auditory interactions in the brain. Curr. Opin. Neurobiol. *16*, 415–419.

Carvell, G.E., Miller, S.A., and Simons, D.J. (1996). The relationship of vibrissal motor cortex unit activity to whisking in the awake rat. Somatosens. Mot. Res. *13*, 115–127.

Castro-Alamancos, M.A. (2006). Vibrissa myoclonus (rhythmic retractions) driven by resonance of excitatory networks in motor cortex. J. Neurophysiol. *96*, 1691–1698.

Castro-Alamancos, M.A., and Oldford, E. (2002). Cortical sensory suppression during arousal is due to the activity-dependent depression of thalamocortical synapses. J. Physiol. *541*, 319–331.

Caviness, V.S. (1975). Architectonic map of neocortex of the normal mouse. J. Comp. Neurol. *164*, 247–263.

Chapin, J.K., and Woodward, D.J. (1982). Somatic sensory transmission to the cortex during movement: gating of single cell responses to touch. Exp. Neurol. *78*, 654–669.

Civillico, E.F., and Contreras, D. (2006). Integration of evoked responses in supragranular cortex studied with optical recordings in vivo. J. Neurophysiol. *96*, 336–351.

Cossart, R., Aronov, D., and Yuste, R. (2003). Attractor dynamics of network UP states in the neocortex. Nature *423*, 283–288.

Cowan, R.L., and Wilson, C.J. (1994). Spontaneous firing patterns and axonal projections of single corticostriatal neurons in the rat medial agranular cortex. J. Neurophysiol. *71*, 17–32.

Cramer, N.P., and Keller, A. (2006). Cortical control of a whisking central pattern generator. J. Neurophysiol. *96*, 209–217.

Cramer, N.P., Li, Y., and Keller, A. (2007). The whisking rhythm generator: a novel mammalian network for the generation of movement. J. Neurophysiol. *97*, 2148–2158.

Crochet, S., and Petersen, C.C.H. (2006). Correlating whisker behavior with membrane potential in barrel cortex of awake mice. Nat. Neurosci. *9*, 608–610.

Deschenes, M., Veinante, P., and Zhang, Z.-W. (1998). The organization of corticothalamic projections: reciprocity versus parity. Brain Res. Brain Res. Rev. *28*, 286–308.

Diamond, M.E., Armstrong-James, M., Budway, M.J., and Ebner, F.F. (1992). Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus: dependence on the barrel field cortex. J. Comp. Neurol. *319*, 66–84.

Dittgen, T., Nimmerjahn, A., Komai, S., Licznerski, P., Waters, J., Margrie, T.W., Helmchen, F., Denk, W., Brecht, M., and Osten, P. (2004). Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring in vivo. Proc. Natl. Acad. Sci. USA *101*, 18206–18211.

Fanselow, E.E., and Nicolelis, M.A.L. (1999). Behavioral modulation of tactile responses in the rat somatosensory system. J. Neurosci. *19*, 7603–7616.

Farkas, T., Kis, Z., Toldi, J., and Wolff, J.R. (1999). Activation of the primary motor cortex by somatosensory stimulation in adult rats is mediated mainly by associational connections from the somatosensory cortex. Neuroscience *90*, 353–361.

Ferezou, I., Bolea, S., and Petersen, C.C.H. (2006). Visualizing the cortical representation of whisker touch: voltage-sensitive dye imaging in freely moving mice. Neuron *50*, 617–629.

Franklin, K.B.J., and Paxinos, G. (1996). The Mouse Brain in Stereotaxic Coordinates (San Diego, CA: Academic Press).

Fu, K.M., Johnston, T.A., Shah, A.S., Arnold, L., Smiley, J., Hackett, T.A., Garraghty, P.E., and Schroeder, C.E. (2003). Auditory cortical neurons respond to somatosensory stimulation. J. Neurosci. *23*, 7510–7515.

Gao, P., Hattox, A.M., Jones, L.M., Keller, A., and Zeigler, H.P. (2003). Whisker motor cortex ablation and whisker movement patterns. Somatosens. Mot. Res. *20*, 191–198.

Ghazanfar, A.A., and Schroeder, C.E. (2006). Is neocortex essentially multisensory? Trends Cogn. Sci. *10*, 278–285.

Ghazanfar, A.A., Maier, J.X., Hoffman, K.L., and Logothetis, N.K. (2005). Multisensory integration of dynamic faces and voices in rhesus monkey auditory cortex. J. Neurosci. *25*, 5004–5012.

Grinvald, A., and Hildesheim, R. (2004). VSDI: a new era in functional imaging of cortical dynamics. Nat. Rev. Neurosci. *5*, 874–885.

Grinvald, A., Lieke, E., Frostig, R.D., Gilbert, C.D., and Wiesel, T.N. (1986). Functional architecture of cortex revealed by optical imaging of intrinsic signals. Nature *324*, 361–364.

Haiss, F., and Schwarz, C. (2005). Spatial segregation of different modes of movement control in the whisker representation of rat primary motor cortex. J. Neurosci. *25*, 1579–1587.

Harris, J.A., Petersen, R.S., and Diamond, M.E. (1999). Distribution of tactile learning and its neural basis. Proc. Natl. Acad. Sci. USA *96*, 7587–7591.

Hattox, A., Li, Y., and Keller, A. (2003). Serotonin regulates rhythmic whisking. Neuron *39*, 343–352.

Hentschke, H., Haiss, F., and Schwarz, C. (2006). Central signals rapidly switch tactile processing in rat barrel cortex during whisker movements. Cereb. Cortex *16*, 1142–1156.

Hoffer, Z.S., Hoover, J.E., and Alloway, K.D. (2003). Sensorimotor corticocortical projections from rat barrel cortex have an anisotropic organization that facilitates integration of inputs from whiskers in the same row. J. Comp. Neurol. *466*, 525–544.

Izraeli, R., and Porter, L.L. (1995). Vibrissal motor cortex in the rat: connections with the barrel field. Exp. Brain Res. *104*, 41–54.

Jancke, D., Chavane, F., Naaman, S., and Grinvald, A. (2004). Imaging cortical correlates of illusion in early visual cortex. Nature *428*, 423–426.

Kayser, C., Petkov, C.I., Augath, M., and Logothetis, N.K. (2005). Integration of touch and sound in auditory cortex. Neuron *48*, 373–384.

Kenet, T., Bibitchkov, D., Tsodyks, M., Grinvald, A., and Arieli, A. (2003). Spontaneously emerging cortical representations of visual attributes. Nature *425*, 954–956.

Kleinfeld, D., Sachdev, R.N.S., Merchant, L.M., Jarvis, M.R., and Ebner, F.F. (2002). Adaptive filtering of vibrissa input in motor cortex of rat. Neuron *34*, 1021–1034.

Kleinfeld, D., Ahissar, E., and Diamond, M.E. (2006). Active sensation: insights from the rodent vibrissa sensorimotor system. Curr. Opin. Neurobiol. *16*, 435–444.

Knutsen, P.M., Pietr, M., and Ahissar, E. (2006). Haptic object localization in the vibrissal system: behavior and performance. J. Neurosci. *26*, 8451–8464.

Lakatos, P., Chen, C.M., O'Connell, M.N., Mills, A., and Schroeder, C.E. (2007). Neuronal oscillations and multisensory interaction in primary auditory cortex. Neuron *53*, 279–292.

Lavallee, P., Urbain, N., Dufresne, C., Bokor, H., Acsady, L., and Deschenes, M. (2005). Feedforward inhibitory control of sensory information in higher-order thalamic nuclei. J. Neurosci. *25*, 7489–7498.

Lee, A.K., Manns, I.D., Sakmann, B., and Brecht, M. (2006). Whole-cell recordings in freely moving rats. Neuron *51*, 399–407.

Lippert, M.T., Takagaki, K., Xu, W., Huang, X., and Wu, J.Y. (2007). Methods for voltage-sensitive dye imaging of rat cortical activity with high signal-to-noise ratio. J. Neurophysiol. *98*, 502–512.

Lovick, T.A. (1972). The behavioral repertoire of precollicular decerebrate rats. J. Physiol. *224*, 4P–6P.

Macaluso, E. (2006). Multisensory processing in sensory-specific cortical areas. Neuroscientist *12*, 327–338.

Martuzzi, R., Murray, M.M., Michel, C.M., Thiran, J.P., Maeder, P.P., Clarke, S., and Meuli, R.A. (2006). Multisensory interactions within human primary cortices revealed by BOLD dynamics. Cereb. Cortex *17*, 1672–1679.

Meeks, J.P., and Mennerick, S. (2007). Action potential initiation and propagation in CA3 pyramidal axons. J. Neurophysiol. *97*, 3460–3472.

Mehta, S.B., Whitmer, D., Figueroa, R., Williams, B.A., and Kleinfeld, D. (2007). Active spatial perception in the vibrissa scanning sensorimotor system. PLoS Biol. *5*, e15. 10.1371/journal.pbio.0050015.

Miyashita, E., Keller, A., and Asanuma, H. (1994). Input-output organization of the rat vibrissal motor cortex. Exp. Brain Res. *99*, 223–232.

Moore, C.I., and Nelson, S.B. (1998). Spatio-temporal subthreshold receptive fields in the vibrissa representation of rat primary somatosensory cortex. J. Neurophysiol. *80*, 2882–2892.

Nguyen, Q.T., and Kleinfeld, D. (2005). Positive feedback in a brainstem tactile sensorimotor loop. Neuron *45*, 447–457.

Petersen, C.C.H. (2007). The functional organisation of the barrel cortex. Neuron *56*, 339–355.

Petersen, C.C.H., Grinvald, A., and Sakmann, B. (2003a). Spatiotemporal dynamics of sensory responses in layer 2/3 of rat barrel cortex measured in vivo by voltage-sensitive dye imaging combined with whole-cell voltage recordings and anatomical reconstructions. J. Neurosci. *23*, 1298–1309.

Petersen, C.C.H., Hahn, T.T.G., Mehta, M., Grinvald, A., and Sakmann, B. (2003b). Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. Proc. Natl. Acad. Sci. USA *100*, 13638–13643.

Petreanu, L., Huber, D., Sobczyk, A., and Svoboda, K. (2007). Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. Nat. Neurosci. *10*, 663–668.

Polley, D.B., Chen-Bee, C.H., and Frostig, R.D. (1999). Two directions of plasticity in the sensory-deprived adult cortex. Neuron *24*, 623–637.

Polley, D.B., Kvasnak, E., and Frostig, R.D. (2004). Naturalistic experience transforms sensory maps in the adult cortex of caged animals. Nature *429*, 67–71.

Porter, L.L., and White, E.L. (1983). Afferent and efferent pathways of the vibrissal region of primary motor cortex in the mouse. J. Comp. Neurol. *214*, 279–289.

Sachdev, R.N., Sato, T., and Ebner, F.F. (2002). Divergent movement of adjacent whiskers. J. Neurophysiol. *87*, 1440–1448.

Sachdev, R.N., Ebner, F.F., and Wilson, C.J. (2004). Effect of subthreshold up and down states on the whisker-evoked response in somatosensory cortex. J. Neurophysiol. *92*, 3511–3521.

Schroeder, C.E., and Foxe, J. (2005). Multisensory contributions to low-level, 'unisensory' processing. Curr. Opin. Neurobiol. *15*, 454–458.

Seidemann, E., Arieli, A., Grinvald, A., and Slovin, H. (2002). Dynamics of depolarization and hyperpolarization in the frontal cortex and saccade goal. Science *295*, 862–865.

Semba, K., and Komisaruk, B.R. (1984). Neural substrates of two different rhythmical vibrissal movements in the rat. Neuroscience *12*, 761–774.

Shoham, D., Glaser, D.E., Arieli, A., Kenet, T., Wijnbergen, C., Toledo, Y., Hildesheim, R., and Grinvald, A. (1999). Imaging cortical dynamics at high spatial and temporal resolution with novel blue voltage-sensitive dyes. Neuron *24*, 791–802.

Shu, Y., Hasenstaub, A., and McCormick, D.A. (2003). Turning on and off recurrent balanced cortical activity. Nature *423*, 288–293.

Slovin, H., Arieli, A., Hildesheim, R., and Grinvald, A. (2002). Long-term voltage-sensitive dye imaging reveals cortical dynamics in behaving monkeys. J. Neurophysiol. *88*, 3421–3438.

Steriade, M., Nunez, A., and Amzica, F. (1993). A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. J. Neurosci. *13*, 3252–3265.

Stuttgen, M.C., Ruter, J., and Schwarz, C. (2006). Two psychophysical channels of whisker deflection in rats align with two neuronal classes of primary afferents. J. Neurosci. *26*, 7933–7941.

Szwed, M., Bagdasarian, K., and Ahissar, E. (2003). Encoding of vibrissal active touch. Neuron *40*, 621–630.

Trageser, J.C., Burke, K.A., Masri, R., Li, Y., Sellers, L., and Keller, A. (2006). State-dependent gating of sensory inputs by zona incerta. J. Neurophysiol. *96*, 1456–1463.

Veinante, P., Lavallee, P., and Deschenes, M. (2000). Corticothalamic projections from layer 5 of the vibrissal barrel cortex in the rat. J. Comp. Neurol. *424*, 197–204.

Vincent, J.L., Patel, G.H., Fox, M.D., Snyder, A.Z., Baker, J.T., Van Essen, D.C., Zempel, J.M., Snyder, L.H., Corbetta, M., and Raichle, M.E. (2007). Intrinsic functional architecture in the anaesthetized monkey brain. Nature *447*, 83–86.

Volgushev, M., Chauvette, S., Mukovski, M., and Timofeev, I. (2006). Precise long-range synchronization of activity and silence in neocortical neurons during slow-wave oscillations. J. Neurosci. *26*, 5665–5672.

Wallace, M.T., Ramachandran, R., and Stein, B.E. (2004). A revised view of sensory cortical parcellation. Proc. Natl. Acad. Sci. USA *101*, 2167–2172.

Welker, W.I. (1964). Analysis of sniffing of the albino rat. Behaviour *22*, 223–244.

White, E.L., and DeAmicis, R.A. (1977). Afferent and efferent projections of the region of mouse SmI cortex which contains the posteromedial barrel subfield. J. Comp. Neurol. *175*, 455–482.

Woolsey, T.A., and Van der Loos, H. (1970). The structural organisation of layer IV in the somatosensory region (SI) of the mouse cerebral cortex: the description of a cortical field composed of discrete cytoarchitectonic units. Brain Res. *17*, 205–242.

Xu, W., Huang, X., Takagaki, K., and Wu, J.Y. (2007). Compression and reflection of visually evoked cortical waves. Neuron *55*, 119–129.

Zhu, J.J., and Connors, B.W. (1999). Intrinsic firing patterns and whiskers-evoked synaptic responses of neurons in the rat barrel cortex. J. Neurophysiol. *81*, 1171–1183.