Facilitating Sensory Responses in Developing Mouse Somatosensory
Barrel Cortex

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Submitted 5 January 2007; accepted in final form 3 February 2007

Borgdorff AJ, Poulet JF, Petersen CC. Facilitating sensory responses in developing mouse somatosensory barrel cortex. J Neurophysiol 97: 2992–3003, 2007. First published February 7, 2007; doi:10.1152/jn.00013.2007. The sensory responses in the barrel cortex of mice aged postnatal day (P)7–P12 evoked by a single whisker deflection are smaller in amplitude and spread over a smaller area than those measured in P13–P21 mice. However, repetitive 10-Hz stimulation or paired pulse whisker stimulation in P7–P12 mice evoked facilitating sensory responses, contrasting with the depressing sensory responses observed in P13–P21 mice. This facilitation occurred during an interval ranging 300–1,000 ms after the first stimulus and was measured using whole cell recordings, voltage-sensitive dye imaging, and calcium-sensitive dye imaging. The facilitated responses were not only larger in amplitude but also propagated over a larger cortical area. The facilitation could be blocked by local application of pharmacological agents reducing cortical excitability. Local cortical microstimulation could substitute for the first whisker stimulus to produce a facilitated sensory response. The enhanced sensory responses evoked by repetitive sensory stimuli in P7–P12 mice may contribute to the activity-dependent specification of the developing cortical circuits. In addition, the facilitating sensory responses allow long integration times for sensory processing compatible with the slow behavior of mice during early postnatal development.

INTRODUCTION

The signaling pathway bringing tactile information from the mystacial vibrissae to the barrel cortex is a well-defined model system of sensory processing in the rodent brain (Kleinfield et al. 2006; Petersen 2003; Woolsey and Van der Loos 1970). Genetically specified programs during embryonic and early postnatal development are major driving force to the formation of cortical maps and patterns of synaptic connectivity. It is also clear that activity-dependent processes contribute substantially to the specification of cortical circuits during development (Feldman and Brecht 2005). Despite the intense interest in development and plasticity of this somatosensory signaling pathway, very few studies have investigated sensory processing in vivo in the barrel cortex of developing rodents (Bureau et al. 2004; Khazipov et al. 2004; Lendvai et al. 2000; Shoykhet et al. 2003; Stern et al. 2001).

In agreement with previous studies (Bureau et al. 2004; Shoykhet et al. 2003; Stern et al. 2001), we found that sensory responses to single whisker deflections were weak in young mice. However, repetitive whisker stimulation evoked facili-

tating sensory responses during early postnatal development as measured with whole cell membrane potential recordings, voltage-sensitive dye imaging, and calcium-sensitive dye imaging. This facilitation contrasts with the depressing sensory responses evoked by passive whisker stimulation characterized previously in anesthetized mature rodents (Ahissar et al. 2000; Brecht et al. 2003; Chung et al. 2002; Higley and Contreras 2006; Khatri et al. 2004; Moore 2004; Webber and Stanley 2006). The facilitating sensory responses in young animals could compensate for the weak responses observed after single whisker deflections and might therefore have a profound influence on the activity-dependent wiring of the cortex during early development.

METHODS

Animals and surgery

C57BL6J mice aged postnatal day (P)7–P21 were anesthetized with 1.5 mg/g urethane (except for implantation surgery for recording from awake animals, which was carried out under 1.5–2% isoflurane). Paw withdrawal, whisker movement, and eye blink reflexes were largely suppressed. A heating blanket maintained the body temperature at 37°C. The skin overlying the somatosensory cortex was removed, and the bone was gently cleaned. A custom-made head-holder was glued to the skull with dental cement. All experimental procedures were approved by the Swiss Federal Veterinary Office.

Intrinsic optical imaging

The location of the C2 whisker column was identified with intrinsic optical imaging (Grinvald et al. 1986). The cortical surface was visualized through the intact bone covered with Ringer solution sealed with a glass coverslip. The surface blood vessels were visualized using light of 530 nm to enhance contrast. The illumination was switched to 630 nm for functional imaging. The reflected light was imaged using a Qicam CCD camera (Q-imaging). Alternating sweeps were imaged with or without 10-Hz stimuli for 4 s delivered to the C2 whisker. The intrinsic signal functionally identified the location of the C2 whisker column and was mapped onto the blood vessel pattern to guide surgery for the craniotomy.

Voltage-sensitive dye and calcium-sensitive dye imaging

An ∼1.5 × 1.5-mm craniotomy was made centered on the location of the C2 whisker as determined by the intrinsic optical imaging. Extreme care was taken at all times not to damage the cortex, especially during the removal of the dura. Voltage-sensitive dye imaging [2992 0022-3077/07 $8.00 Copyright © 2007 The American Physiological Society www.jn.org

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(VSD) RH1691 (1 mg/ml) was dissolved in Ringer solution containing (in mM) 135 NaCl, 5 KCl, 5 HEPES, 1.8 CaCl₂, and 1 MgCl₂. This dye solution was topically applied to the exposed cortex and allowed to diffuse into the cortex for ~40 min. The cortex was subsequently washed to remove unbound dye, stabilized with 1.5% agar, and sealed with a coverslip. The voltage-sensitive dye was excited with 630-nm light from LEDs (L630, Epitex, Japan), reflected using a 650-nm dichroic, and focused onto the cortical surface with a 25-mm video lens (Navitar). Fluorescence was collected through the same optical pathway, long-pass filtered (>665 nm), and focused with another 25-mm video lens (Navitar) onto a NeuroCCD camera (Redshirt Imaging) running at 500 Hz under the control of IgorPro (Wave metrics). For calcium imaging, the calcium-sensitive dye (CaSD) OGB-1 AM was dissolved in DMSO with 20% pluronic acid and diluted 20-fold with Ringer solution and injected into layer 2/3 of the C2 barrel (~50 nl of a 500 μM solution). OGB-1 fluorescence gradually increased and stabilized after 1 h, covering an area of ~300 μm diameter. The dye was excited with 490-nm light from LEDs (L490-06U, Epitex, Japan) and reflected using a 500-nm dichroic mirror, and the emitted light was band-pass filtered at 535/15 nm. Cortical autofluorescence at these wavelengths was subtracted from the collected CaSD images. VSD and CaSD signals were quantified asΔFF or ΔF/FO to correct for differences in the spatial distribution of the fluorescent dye. Trials were triggered on a fixed phase in the ECG (electrocardiogram), and whiskers were deflected on alternate intercalated sweeps. Unstimulated trials were subtracted from trials with stimuli, and many trials (15–60) were averaged. These procedures aimed to reduce heart beat–related artifacts, bleaching artifacts, and the contribution of spontaneous cortical activity.

Fiber optic imaging

Flexible fiber optic image bundles were used to image VSD signals from freely moving mice (Ferezou et al. 2006). The craniotomy and cortical staining were performed under isoflurane anesthesia. After washing away the unbound RH1691, a fiber optic image bundle was placed in direct contact with the cortical surface and fixed in position using dental cement. The other end of the fiber was held in the focal plane of the epifluorescence microscope, thus allowing both excitation of the voltage-sensitive dye and imaging of the emitted fluorescence. A small piece of metal was attached to the C2 whisker, and the mouse was placed in an electromagnetic coil. The isoflurane anesthesia was withdrawn, and the mouse allowed to recover for over an hour. Brief whisker deflections were evoked by driving 1- to 2-ms current pulses through the electromagnetic coil.

Whole cell recordings

After performing a small craniotomy, pipettes were slowly advanced into the cortex with a positive pressure until the pipette resistance increased, and then suction was applied to establish a gigaseal followed by the whole cell configuration. Whole cell pipettes had resistances of ~5 MΩ filled with a solution containing (in mM) 135 potassium gluconate, 4 KCl, 10 HEPES, 10 phosphocreatine, 4 MgATP, 0.3 Na₃GTP (adjusted to pH 7.2 with KOH), and 2 mg/ml biocytin. Whole cell electrophysiological measurements were made with a Multiclamp 700 amplifier (Axon Instruments). The membrane potential was low-pass filtered at 5 kHz and digitized at 20 kHz in a sweep-based manner by ITC-18 (Intrutech Corp.) under the control of IgorPro.

Whisker stimulation

Whiskers were deflected backward by ~50 μm using piezoelectric bimorphs attached to the whisker ~1 mm away from the whisker pad. Each whisker stimulus was recorded by a fiber optic displacement sensor (Philtoc D64-QQT4, Philtoc). The voltage waveforms controlling the piezo movement were tuned to cancel resonances, and individual whisker movements evoked by the piezo lasted ~2 ms, followed by low-amplitude ringing <10% of the peak amplitude of the deflection decaying with a time constant of <10 ms.

Local cortical drug application

A glass micropipette (~10 μm tip diam) was filled with mineral oil, and a metal piston was inserted into the pipette expelling some of the mineral oil. The pipette and the metal piston were attached to a micromanipulator to allow placement of the pipette with micrometer precision. The metal piston was attached to a Narishige linear manipulator, which could move the piston in and out of the pipette. The drug dissolved in artificial cerebrospinal fluid (ACSF) was sucked into the pipette by retracting the piston. The piston movement could be regulated with micrometer precision, with each calibrated unit denoting 0.2 nl. The pipette 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 the metal piston into the pipette by a known distance, we could inject a defined quantity of the drug directly into the C2 barrel column.

Local cortical microstimulation

A large-diameter patch pipette (~10 μm tip diam) filled with ACSF 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 cortex as identified by precise colocalization of the cortical microstimulation–evoked VSD response and the C2 whisker deflection–evoked VSD response. Electrical stimuli of duration 200 μs and amplitude 4–8 times the threshold (maximal current injection amplitude was 100 μA) for evoking a VSD response were applied using current injections delivered by a linear stimulus isolator (A395, World Precision Instruments).

Analysis

VSD and CaSD images were analyzed using custom written routines in Igor Pro. VSD and CaSD time traces were calculated from mean values within an ~300-μm-diam circle (this analysis was centered over the functionally mapped C2 barrel column, except for experiments in Fig. 7, where responses in neighboring barrel columns were also analyzed). Paired-pulse ratios (PPRs) were calculated from the average response ~10 to 10 ms around the peak after subtraction of the average baseline value ~20 to 0 ms before each stimulus. Data are expressed as means ± SE.

RESULTS

Sensory responses to single whisker deflections

Intrinsic optical imaging was used to target whole cell (WC) recordings to the C2 column in the S1 barrel field of urethane anesthetized mice aged from P7 to P21. A controlled brief C2 whisker deflection evoked a depolarizing sensory response recorded in neurons located in cortical layers 2–5. We found marked differences in the evoked postsynaptic potentials (PSPs) recorded in mice aged P7–P12 compared with those recorded in mice aged P13–P21 (Fig. 1, A and B). Sensory-evoked PSPs in P7–P12 mice occurred at significantly longer latencies (P7–P12, 32 ± 2 ms, n = 43; P13–P21, 16 ± 2 ms, n = 22; Student’s t-test, P = 1.6 × 10⁻⁴), lasted significantly longer (half-width: P7–P12, 174 ± 24 ms, n = 43; P13–P21 86 ± 11 ms, n = 22; Student’s t-test, P = 0.0021), and had
significantly smaller amplitudes (P7–P12, 4.7 \pm 0.6 \text{ mV}, n = 43; P13–P21, 8.3 \pm 1.3 \text{ mV}, n = 22; Student’s t-test, \( P = 0.018 \)). These differences could also be observed when comparing PSPs recorded in the same class of neuron. Comparing a layer 5 pyramidal neuron recorded in a P7 mouse and a layer 5 pyramidal neuron recorded in a P13 mouse, latency and response duration are prolonged in the younger mouse, and PSP amplitude is smaller (Fig. 1A). In addition, spontaneous subthreshold activity observed in P13–P21 mice was strongly reduced in mice aged P7–P12. These results from developing mice are consistent with the previous in vivo studies of sensory processing in the developing rat (Bureau et al. 2004; Lendvai et al. 2000; Shoyket et al. 2003; Stern et al. 2001).

To measure sensory-evoked PSPs in large populations of neurons, we stained the barrel cortex with VSD RH1691 (Ferezou et al. 2006; Grinvald and Hildesheim 2004; Petersen et al. 2003a,b; Shoham et al. 1999) and imaged fluorescence changes evoked by the same brief C2 whisker deflections (Fig. 1, C and D). Similar to the findings observed with whole cell recordings, we found that sensory-evoked responses in mice aged P7–P12 occurred at significantly longer latencies (P7–P12: 28 \pm 2 \text{ ms}, n = 35; P13–P21: 16 \pm 1 \text{ ms}, n = 16; Student’s t-test, \( P = 1.4 \times 10^{-5} \)) and lasted significantly longer than in P13–P21 mice (half-width: P7–P12, 248 \pm 31 \text{ ms}, n = 35; P13–P21, 136 \pm 39 \text{ ms}, n = 16; Student’s t-test, \( P = 0.03 \)). In addition, the voltage-sensitive imaging technique provides spatial information relating to how far the sensory information propagates laterally across the cortex. As shown for individual example experiments of a P10 mouse compared with a P15 mouse (Fig. 1C), the evoked VSD response in mice aged P7–P12 remains more localized than the spreading response evoked in P13–P21 mice. The spatial extent at the peak of the response was quantified by fitting a Gaussian function to a linear profile of fluorescence changes oriented along the rows of the barrel map. The full-width at half-maximal was taken as a measure of the spread of the VSD signal and showed that the sensory response in P7–P12 mice (half-width of 446 \pm 53 \text{ \mu m}, n = 7) was significantly (Student’s t-test, \( P = 6.9 \times 10^{-5} \)) more localized compared with the spreading responses observed in P13–P21 mice (half-width of 838 \pm 54 \text{ \mu m}, n = 8).

A single brief C2 whisker deflection therefore evokes PSPs, which are delayed, longer-lasting, smaller in amplitude, and more localized in P7–P12 mice compared with P13–P21 mice. However, sensory input during behavior is rarely isolated to a single impulse, and we therefore studied the effects of applying repetitive stimuli to the C2 whisker.

**Sensory responses to 10-Hz stimulus trains**

Both WC (Fig. 2, A–C) and VSD (Fig. 2, D–F) measurements of responses in the anesthetized mouse somatosensory cortex of extended duration (Perez et al. 2005; Shoham et al. 1999) provide spatial information relating to how far the sensory information propagates laterally across the cortex. As shown for individual example experiments of a P10 mouse compared with a P15 mouse (Fig. 1C), the evoked VSD response in mice aged P7–P12 remains more localized than the spreading response evoked in P13–P21 mice. The spatial extent at the peak of the response was quantified by fitting a Gaussian function to a linear profile of fluorescence changes oriented along the rows of the barrel map. The full-width at half-maximal was taken as a measure of the spread of the VSD signal and showed that the sensory response in P7–P12 mice (half-width of 446 \pm 53 \text{ \mu m}, n = 7) was significantly (Student’s t-test, \( P = 6.9 \times 10^{-5} \)) more localized compared with the spreading responses observed in P13–P21 mice (half-width of 838 \pm 54 \text{ \mu m}, n = 8).

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cortex to 10-Hz whisker stimulation indicated qualitatively different behaviors in P7–P12 mice compared with P13–P21 mice. The sensory responses evoked in P13–P21 mice (Fig. 2, B, C, E, and F) exhibited depressing sensory responses that did not exceed the depolarization evoked by the first stimulus, as reported in previous studies of the mature barrel cortex (Ahissar et al. 2000; Brecht et al. 2003; Chung et al. 2002; Higley and Contreras 2006; Khatri et al. 2004; Moore 2004; Webber and Stanley 2006). For the P7–P12 mice, the second stimulus, occurring 100 ms after the first, also evoked a strongly depressed cortical response (Fig. 2, A, C, D, and F). However, for the P7–P12 mice, as the train of 10-Hz stimuli continued, the cortex responded with a growing depolarization. In the WC measurements from P7–P12 mice (Fig. 2C), the membrane potential depolarized by $8.0 \pm 2.3$ mV during the 10-Hz stimulation compared with $3.1 \pm 0.7$ mV for the first stimulus ($n = 15$). In VSD recordings (Fig. 2F), $\Delta F/F_0$ evoked by the first stimulus $0.61 \pm 0.18\%$ rose to $1.3 \pm 0.28\%$, peaking in the VSD recording at $610 \pm 60$ ms ($n = 9$) after the onset of the 10-Hz stimulus train. Repeated 10-Hz stimulation of the C2 whisker therefore evoked cortical sensory responses in P7–P12 mice, which were initially strongly depressed early in the stimulus train but continued stimulation-evoked massive depolarization. These dynamics in P7–P12 mice suggest that there are two processes underlying the responses to the train of stimuli: one being depression and the other facilitation.

**Paired pulse facilitation**

The early depression and the subsequent facilitation seem to be independent processes because the facilitation in P7–P12 mice can be evoked without going through the depression phase by simply delivering two well-timed whisker deflections instead of a train of stimuli. The PSP evoked by the second whisker deflection averaged over many trials in whole cell recordings from P7–P12 mice is approximately twice the amplitude of the first response [PSP paired pulse ratio (PPR) = $2.22 \pm 0.63$, $n = 23$] when evoked with an interstimulus interval (ISI) of 500 ms. The second stimulus also evoked significantly more action potentials (1st stimulus evoked $0.30 \pm 0.11$ APs/stimulus; 2nd stimulus evoked $0.69 \pm 0.20$ APs/stimulus; Student’s paired t-test, $P =$

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**FIG. 2.** Repetitive 10-Hz C2 whisker deflections evoke a growing depolarization during stimulus train in P7–P12 mice but not in P13–P21 mice. A: repetitive brief deflections of C2 whisker at 10 Hz evoked a depolarizing sensory response in a whole cell recording from a P7 mouse. The 2nd PSP evoked 100 ms after 1st whisker deflection was strongly depressed. However, continued stimulation led to a robust depolarization shown for an individual trial (black trace) and averaged across many trials (red). Dotted line indicates mean 1st response amplitude. B: in a whole cell recording of a neuron in a P13 mouse, repetitive stimulation evoked depressing PSPs (individual trial black and average across many trials; blue). Dotted line indicates mean 1st response amplitude. C: average PSP response was normalized to amplitude of 1st response and averaged across many experiments. Mice aged P7–P12 ($n = 15$) show a robust late depolarization evoked by prolonged 10-Hz stimulation, whereas repetitive stimulation evoked depressing PSPs in P13–P21 mice ($n = 5$). Dotted line indicates mean 1st response amplitude. D: VSD imaging from a P7 mouse indicated a strong late sensory response during 10-Hz C2 whisker stimulation. Dotted line indicates mean 1st response amplitude. E: VSD imaging of a P20 mouse reveals depressing sensory responses during a 10-Hz stimulus train. Dotted line indicates mean 1st response amplitude. F: average peak response to 1st stimulus was normalized and VSD responses averaged across many experiments; 10-Hz C2 whisker stimulation evoked a strongly depolarizing late response peaking at $-600$ ms after onset of stimulus train in P7–P12 mice ($n = 9$) but not in P13–P21 mice ($n = 11$). Dotted line indicates mean 1st response amplitude.
Averaged across all sweeps, there was a clear pulse facilitation with an ISI of 500 ms in a P8 mouse is shown in Fig. 3A. Averaged across all sweeps, there was a clear facilitation of the second response (Fig. 3A, black trace). Interestingly, when individual sweeps were analyzed, the responses evoked by the second whisker deflection were found to be highly variable from trial to trial. On some trials, the second response was strongly depressed (Fig. 3A, blue traces). In other trials, the second whisker deflection evoked a large response with either a slow rise-time (Fig. 3A, red traces) or a rapid rise-time (Fig. 3A, green traces). These highly variable responses to the second whisker deflection could reflect a contribution of positive feedback activity in recurrent excitatory circuits to the facilitated response.

We also analyzed the paired pulse ISI time-course of facilitation in our whole cell recordings. A strongly facilitating example experiment is shown in Fig. 3B. The ISI was varied in a pseudorandomized fashion (exploring a range from 100–1,000 ms in 100-ms steps) with different ISIs interleaved. The stimuli were applied many times, and the sweeps were averaged over all stimulus presentations of the same ISI. Only a very small response was evoked by the first stimulus and by the second stimulus at short ISIs of 100–200 ms. In the range of ISIs from 300 to 800 ms, the second stimulus evoked a substantial PSP, which was maximal at 600 ms ISI. At longer ISIs of 900–1,000 ms, the second stimulus again evoked only a very small response. Facilitation therefore only occurs during a specific time interval. In general, at a short ISI of 100 ms, the second response was strongly suppressed, and facilitation was observed at ISIs ~500 ms (but ranging 300–1,000 ms depending on the recording) with depression occurring again at longer ISIs.

To study the ensemble cortical membrane potential response evoked by paired pulse stimulation, we measured VSD responses under identical stimulus conditions (Fig. 3, C–F). In an example experiment from a P10 mouse (Fig. 3C), the time-course of facilitation observed with VSD imaging peaked at 700 ms. Facilitation was not observed in mice aged P13–P21 at any interstimulus interval (ISI) of 500 ms revealed a strongly facilitated PSP response to 2nd whisker stimulus (black trace, top, average of many trials). In individual trials, response to 2nd stimulus could either be depressed (blue), show a delayed large response (red), or show a fast large response (green). B: WC recording from a P9 mouse, which responded poorly to a single C2 whisker deflection showed a facilitated sensory response when stimulated with paired pulse C2 whisker deflections with an interstimulus interval (ISI) of 500 ms revealed a strongly facilitated PSP response to 2nd whisker stimulus (black trace). The time course of facilitation observed with VSD imaging from a P10 mouse showed a depressed sensory response at 100 ms, facilitating sensory responses at ISIs of 300, 500, 700, and 1,000 ms and depressing sensory responses again at ISIs of 1,300 and 1,600 ms. Dotted line indicates mean 1st response amplitude. D: VSD imaging in a P21 mouse showed depressing sensory responses at ISIs of 300 and 500 ms which recovered back to level of 1st response at longer ISI intervals. Dotted line indicates mean 1st response amplitude. E: overlaid ISI time-course of paired pulse ratios (PPRs) from VSD experiments in mice aged P7–P12 (red) and P13–P21 (blue). F: average time-course of facilitation and depression in P7–P12 (n = 10, red) and P13–P21 (n = 8, blue) mice.
Developmental switch from facilitation to depression

Facilitation measured with VSD with an ISI of 500 ms in mice P7–P12 gave a PPR of 1.97 ± 0.14 (n = 40). In mice aged P13–P21, we found a PPR = 0.85 ± 0.084 (n = 15). Normalizing the amplitude of the first response and averaging the time-course of the VSD signal across all experiments also reveals robust facilitation in the younger age group (Fig. 4A). To delineate the developmental profile of the facilitation, we subdivided the data obtained into five age groups of mice: P7–P8, P9–P10, P11–P12, P13–P15, and P17–P21 (Fig. 4B). We also plotted the PPR averaged across ISIs ranging 300–700 ms for each experiment as a function of age (Fig. 4C). Both analyses revealed that the facilitation switched to depression at P13, which interestingly coincides closely with the time that mice begin active whisking behavior (similar to the timing in rats; Welker 1964). This change in the dynamics of the sensory response over development was specific for the facilitation because neither the depression observed at 100-ms ISI nor the recovery at 1,500–1,600-ms ISI depended significantly on age over this time period (100-ms ISI: for P7–P12, PPR = 0.42 ± 0.14 (n = 21), for P13–P21, PPR = 0.35 ± 0.09 (n = 16); Student’s t-test, P = 0.34). 1,500–1,600-ms ISI: P7–P12, PPR = 0.90 ± 0.13 (n = 21), for P13–P21, PPR = 1.1 ± 0.11 (n = 15); Student’s t-test, P = 0.12; Fig. 4D).

Facilitating calcium responses

In our whole cell recordings from mice aged P7–P12, we found that the facilitated sensory response to the second stimulus not only gave larger PSPs, but also evoked more action potentials. Because action potentials evoke neuronal calcium signals, we also performed calcium imaging experiments. CaSD OGB1-AM was injected (Adelsberger et al. 2005; Berger et al. 2007; Kerr et al. 2005; Stosiek et al. 2003) into the C2 barrel column guided by intrinsic optical imaging (Fig. 5, A–D). Single C2 whisker deflections evoked calcium responses in the C2 barrel column (Fig. 5E). Paired pulse whisker stimuli were delivered with 100-, 500-, and 1,500-ms ISIs. In the example experiment from a P10 mouse (Fig. 5, A–F), the CaSD response was strongly depressed at 100 ms, strongly facilitated at 500 ms, and by 1,500 ms had recovered close to the response amplitude evoked by the first stimulus (Fig. 5F). Averaged across experiments in p8–p11 mice (Fig. 5, G and H), the sensory responses reported by the CaSD showed a strong facilitation at 500 ms ISI (PPR = 4.66 ± 0.72, n = 6; Student’s t-test, P = 0.0062).

Facilitation in awake mice

The facilitation observed under urethane anesthesia might not relate to cortical function under more physiological conditions. We therefore conducted VSD imaging experiments using fiber optic image bundles on awake mice (Ferezou et al. 2006). Sensory responses in freely moving mice (Fig. 6A) were evoked by gluing a small metal particle onto the C2 whisker and placing the mouse in an electromagnetic coil through which we drove 2-ms current pulses to generate a brief magnetic field (Crochet and Petersen 2006; Ferezou et al. 2006). VSD signals in an awake P10 mouse showed a facilitating sensory response at an ISI of 1,000 ms and depressing sensory responses at ISIs of 300 and 1,600 ms (Fig. 6B). In a freely moving P14 mouse, paired pulse stimuli evoked depressing sensory responses at the same ISIs of 300, 1,000, and 1,600 ms (Fig. 6C). Across the four P9–P11 mice tested, facilitation at 1,000-ms ISI was observed with a PPR = 1.52 ± 0.19 (n = 4; Student’s paired t-test, P = 0.027; Fig. 6D and E), whereas depression was observed in two older mice aged P14 and P15. The ISI time-course may be slightly delayed in awake animals, but it falls within the broad range of facilitation time-courses found under urethane anesthesia (Fig. 5E). Therefore we cannot exclude the possibility that the facilitation in awake mice might have resulted from a different and unrelated mechanism to the facilitation observed under anesthesia. Although further experiments in awake mice are clearly necessary, this data set does suggest that a developmentally regulated facilitating sensory response also occurs in the awake mouse, with an overlapping ISI time-course to that found under urethane anesthesia.

Spatial determinants of facilitation

VSD imaging revealed that the local cortical activity evoked by a brief single whisker stimulus in a P7–P12 mouse does not spread far across the developing somatosensory neocortex. However, the facilitated response evoked by a second whisker deflection at ISI of 500–700 ms clearly excited a larger area (Fig. 7, A and B). The spatial extent of the evoked responses in
seven mice was quantified by fitting Gaussian functions to the VSD response profile measured along the row orientation of the barrel cortex map. The full-width at the half-maximum amplitude of the response to the first stimulus (407 ± 29 μm) was significantly less (Student’s paired t-test, \( P = 0.037 \)) than the full-width of the response to the second stimulus (625 ± 91 μm) quantified at the same amplitude as for the first response. The facilitation is therefore useful both to generate large amplitude sensory signaling in the principal whisker barrel column and also to propagate this signal further across the barrel map into the representation of neighboring whiskers.

We further studied whether the stimulation of one whisker could facilitate the response of neighboring whiskers. In interleaved trials, we compared the effects of stimulating the same whisker twice or stimulating one whisker followed by an adjacent whisker. In the example experiment shown in Fig. 7, A–C, we first tested the repeated stimulation of the same whisker at 500-ms ISI. Repeated stimulation of the C2 whisker evoked robust facilitation, seen in VSD images (Fig. 7A),

**FIG. 5.** Sensory evoked calcium responses are facilitated in P7–P12 mice. A: image of changes in reflected light during intrinsic optical imaging of a P10 mouse when no stimuli were applied (A–F are from the same experiment). B: intrinsic optical image signal evoked by 10-Hz stimulation of the C2 whisker revealed a localized response. C: location of intrinsic signal was superimposed on blood vessel pattern (green dot). D: cell permeable calcium-sensitive dye OGB-1 AM was injected into C2 barrel column identified by intrinsic optical imaging. E: single brief C2 whisker deflection evoked a calcium signal in C2 barrel column (average of many trials). F: averaged time-courses of calcium-sensitive dye response in this P10 mouse evoked by a single C2 whisker deflection (black) and paired pulse C2 whisker deflections evoked at ISIs of 100 (green), 500 (red), and 1,500 ms (purple). G: averaged across many experiments normalized to 1st response amplitude, calcium-sensitive dye responses exhibited strong and robust facilitation in P8–P11 mice (\( n = 6 \)). H: PPR at 500 ms ISI for individual experiments (open circles) and means ± SE (red filled circle, P8–P11 mice, \( n = 6 \); blue filled circle, P13–P21 mice, \( n = 6 \)).

**FIG. 6.** Facilitating VSD responses in nonanesthetized freely moving mice aged P7–P12. A: flexible fiber optic image bundle was used to image VSD fluorescence in awake mice, and sensory responses were evoked by brief magnetic pulses to move a metal particle attached to the C2 whisker. B: in an awake P10 mouse, sensory responses facilitated at 1,000-ms ISI, but depressed at 300 and 1,600 ms. All traces are averages of many sweeps, and dotted line indicates mean 1st response amplitude. C: in an awake P14 mouse, sensory responses were slightly depressed at 300-, 1,000-, and 1,600-ms ISIs. All traces are averages of many sweeps, and dotted line indicates mean 1st response amplitude. D: PPR at different ISIs was computed for each experiment. E: averaged PPR showed facilitation for P9–P11 mice (\( n = 4 \)) but depression in P14–P15 mice (\( n = 2 \)).
space-time plots (Fig. 7B), and the time-course of fluorescence changes quantified in the C2 barrel column (Fig. 7C). Repeated deflection of the C3 whisker evoked a similar pattern of activity, except that the spatial focus of activity was now shifted somatotopically to a location ~200 μm away from the C2 response focus (center left column). Stimulating the C2 whisker 1st, followed by the C3 whisker 500 ms later, did not evoke a facilitated sensory response (center right column). However, deflection of the C3 whisker followed by the C2 whisker did evoke a facilitated response (right column). Spatial VSD profile is computed for a line crossing foci of C2 and C3 responses and plotted on the x-axis. Time is plotted on the y-axis. C: time-courses of VSD responses for the above stimuli measured at response focus. Strong facilitation is observed for repeated stimulation of the C2 whisker and C3 whisker. No facilitation is observed for sequence C2 followed by C3 stimulation, but facilitation was found for C3 followed by C2 stimulation. D: VSD responses were normalized to response amplitude evoked by single whisker deflection and averaged across 10 experiments. Repeated deflection of the same whisker evoked strong facilitation. Deflection of neighboring whiskers in a ventrally, dorsally, or rostrally oriented sequence did not evoke a facilitated sensory response. In general, facilitation was specifically observed on repetitive stimulation of the same whisker and did not cross from one whisker to another. However, one condition did evoke cross-whisker facilitation. Deflection of a rostral whisker followed by its caudal neighbor evoked facilitation. This sequence of whisker deflections would occur as the mouse advanced into an object, as schematically indicated by the drawing (bottom right). E: summary facilitation data from cross-whisker experiments with each black circle marking an individual experiment; red circles indicate means ± SE (C → R signifies 1st stimulus of a caudal whisker and then a rostral whisker; D → V signifies 1st stimulus of a dorsal whisker and then a ventral whisker; same signifies that same whisker was stimulated twice; V → D signifies 1st stimulus of a ventral whisker and then a dorsal whisker; R → C signifies 1st stimulus of a rostral whisker and then a caudal whisker).
whisker activation is interesting, because this corresponds to the order of whisker deflections evoked as a mouse approaches an object. The long time-course for facilitation (300–1,000 ms) would require head/whisker velocities of ~5 mm/s or head-turns of ~20°/s for this facilitation along a row of whiskers to contribute to sensory processing of object detection.

In summary, the facilitation is therefore rather specific to repeated stimulation of the same whisker. This result argues against an overall change in brain state evoked by the first stimulus, which would be expected to induce facilitation of any subsequently stimulated whisker.

**Activity in cortical circuits contributes to generating facilitation**

We next began to study the locus of this robust facilitation in P7–P12 mice. We reasoned that if the facilitation was generated at a subcortical level, the facilitation should persist on local infusion of pharmacological agents inhibiting cortical activity. The C2 barrel column was functionally mapped by VSD imaging and a glass micropipette was inserted directly into the C2 barrel column, allowing injection of pharmacological agents. Infusion of 50–70 nl ACSF had no effect on response amplitude or PPR (Fig. 8A). Injection of 50–70 nl of the N-methyl-D-aspartate (NMDA) receptor antagonist D-APV at 200 μM decreased the duration of the response evoked by the first stimulus with only a small decrease in the first response amplitude, and D-APV completely blocked the facilitation in response to the second stimulus (Fig. 8B). Injection of large volumes of the GABAA receptor agonist muscimol at 40 μM completely abolished the sensory evoked VSD responses (data not shown), but when small volumes were injected (Fig. 8C), the facilitation was completely and specifically disrupted, whereas having only a minor effect on the response to the first stimulus. During control ACSF infusion, we observed PPR = 2.22 ± 0.20 (n = 5); during APV application, PPR = 0.89 ± 0.09 (n = 7); and during muscimol application, PPR = 0.90 ± 0.10 (n = 6). These pharmacological manipulations were tightly localized to the injected C2 barrel column, because they had no significant effect on responses evoked by A2 whisker stimulation measured in the A2 barrel column (drug application of D-APV or muscimol in the C2 column reduced PPR in the A2 column by only 7 ± 2%, P = 0.18). If the incoming thalamic sensory input in itself showed facilitation, the injection of D-APV or muscimol would not affect the cortical facilitation. These observations therefore suggest that facilitation requires local cortical activity.

We next tested whether intracortical electrical microstimulation could evoke an activity able to substitute for the first whisker stimulus. A glass micropipette filled with ACSF 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 location of the C2 barrel column as identified by VSD imaging. Electrical stimuli of duration 200 μs and an amplitude 4–8 times threshold for evoking a VSD response were applied. Electrical stimulus of the C2 cortical column preceding a C2 whisker stimulus by 500 ms was found to facilitate the sensory response (Fig. 8D). These experiments suggest that local cortical activity can evoke facilitation of the sensory response.

**FIG. 8.** Recurrent cortical activity is necessary to generate facilitation. A: repeated stimuli delivered to the C2 whisker evoked facilitating VSD responses that were quantified in the C2 barrel column, normalized to peak amplitude of 1st response, and averaged across n = 5 mice (black traces). In control experiments, we injected artificial cerebrospinal fluid (ACSF) and found that this did not on average affect response amplitude or facilitation. B: injection of the N-methyl-D-aspartate (NMDA) receptor antagonist D-APV (200 μM) directly into the C2 barrel column had little effect on amplitude of 1st response but completely blocked facilitation (n = 7 mice, red trace). After 30 minutes for D-APV to diffuse away, facilitation recovered (blue trace). C: GABAA receptor agonist muscimol (40 μM) was injected in an amount that left amplitude of response to 1st stimulus almost unaltered (n = 6 mice). At this concentration, muscimol strongly inhibits facilitation (black trace control; red trace after muscimol injection) and can be fully washed out (blue trace). Agents that suppress cortex block facilitation, suggesting that cortical activity is necessary for facilitation. D: repeated stimulation of the C2 whisker evoked a facilitating sensory response (blue trace). Substituting 1st whisker stimulus with an electrical stimulus (n = 7) delivered directly to the C2 barrel column also facilitated response evoked by a subsequent C2 whisker stimulus (red trace). Red trace on left shows rapid time-course of response to electrical stimulus. Red trace on right shows response to a single whisker stimulation delivered 500 ms after electrical stimulus. Single whisker stimulus responses after electrical stimulus was strongly facilitated compared with response to just a single C2 whisker stimulus (black trace). Cortical activity evoked by intracortical microstimulation can therefore facilitate sensory responses.
DISCUSSION

Single brief deflections of the C2 whisker evoked smaller, slower, and more localized sensory responses in P7–P12 mice compared with the larger, faster, and spreading sensory responses observed in P13–P21 mice. However, repetitive stimuli evoked facilitating sensory responses in P7–P12 mice, whereas depressing responses were observed in P13–P21 mice. Facilitation in P7–P12 mice was observed as increased PSP amplitude, increased AP firing, increased VSD response amplitude, and increased CaSD response amplitude. The facilitation was specific to repeated stimulation of the same whisker and local cortical pharmacological manipulations could block it. The facilitation of whisker-evoked sensory responses in P7–P12 mice is therefore a robust phenomenon, although entirely unexpected because sensory responses in general depress during repetitive stimulation.

Functional role of facilitation in the developing mouse barrel cortex

Sensory responses facilitate in mouse barrel cortex of P7–P12 mice. The facilitation increases the amplitude of the sensory responses evoking more action potentials in cortical neurons. The facilitation may therefore compensate for the relatively weak sensory responses that are evoked by single whisker stimuli. The highly localized activity evoked by single whisker stimuli in P7–P12 mice is also changed in the facilitated responses, which propagate over a much larger cortical area. The facilitation therefore serves two functions: first to amplify the sensory response and second to spread the sensory information across a larger part of the somatotopic map within barrel cortex. Both of these features of the facilitated sensory responses may contribute to the activity-dependent specification of the developing cortical circuits (Feldman and Brecht 2005). We propose that the long-lasting facilitation evoking sensory responses that excite a large cortical area may be involved in inducing associational plasticity of sensory events.

The time scale of the facilitation from 300 to 1,000 ms suggests that this process may also be involved in perception of prolonged sensory input. For young animals with slow behavior and slow brain processing, this may provide an important mechanism for the cortical integration of sensory information.

Developmental switch from facilitation to depression

Sensory responses facilitate in mouse barrel cortex during the second postnatal week switching to depression at ~P13. Our results indicating depressing sensory responses in P13–P21 mice is in good agreement with results from previous studies of this sensory pathway in the mature anesthetized rodent (Ahissar et al. 2000; Brecht et al. 2003; Chung et al. 2002; Higley and Contreras 2006; Khatri et al. 2004; Moore 2004; Webber and Stanley 2006). Depression is therefore by far the most common observation of the result of repetitive stimulation in this pathway or indeed in any other sensory pathway to the mature mammalian brain. However, recently, Derdiman et al. (2006) observed facilitating AP responses in mature rats induced by touch in layers 4 and 5A (but not layer 2/3) during electrically evoked whisker movements. This is in contrast with the depressing responses in mature rodents evoked by passively applied stimuli, and these results suggest that stimuli may be processed dynamically in different ways depending on how the sensory information is acquired. Depression of sensory responses is also less prominent in awake mature rodents during active behaviors (Castro-Alamancos et al. 2003; Crochet and Petersen 2006). This behavioral modulation of depressing sensory responses is likely to be mediated in part by activity-dependent depression of thalamocortical synapses (Castro-Alamancos and Oldford 2002; Chung et al. 2002).

The switch from facilitation to depression in our experiments was clearly related to postnatal age. This switch in the short-term dynamics of sensory processing occurred at around P13. This age (similar to the development in rats; Welker 1964) corresponds closely with the age at which mice begin active exploration of their environment by making large rhythmic whisker movements at ~10–Hz frequency. Mature rodents will often rhythmically touch objects with their whiskers at high frequency, evoking repetitive sensory input. Because this is often combined together with head movements or indeed the object may be moving, it is likely to be important for the animal to rapidly process the sensory information on a whisk-by-whisk basis. The slow time-course of facilitation ranging 300–1,000 ms that we observed in P7–P12 mice would therefore not be compatible with rapid processing of whisking-related sensory input occurring at ~10 Hz. The disappearance of facilitation at P13 may therefore be well timed for the cortex to deal with the more rapid temporal sequences of information arriving in the more mature rodent.

Mechanisms underlying the facilitation

The repeated stimulation of the same whisker was required to evoke facilitation (except for the one case of a caudally oriented sequence of whisker deflections). That facilitation did not in general cross to other whiskers suggests that it is unlikely to result from a generalized change in brain state or arousal evoked by the first whisker stimulus. Instead, these results point to a role of activity in the specific sensory pathway. Furthermore, local injection of N-APV or muscimol into the C2 column to reduce cortical circuit activity completely blocked the facilitation evoked by repetitive C2 whisker deflection. This would argue that localized cortical activity is necessary to amplify the thalamic input to give rise to the facilitated sensory response. The involvement of high gain recurrent cortical excitatory circuits would also be consistent with the highly variable dynamics of the facilitated response when single trials were analyzed (Fig. 3A).

In vitro, short-term plasticity examined at cortical excitatory synaptic connections depress strongly at young ages (Abbott et al. 1997; Bender et al. 2006; Finnerty et al. 1999; Galarreta and Hestrin 1998; Petersen 2002; Reyes and Sakmann 1999; Tsodyks and Markram 1997; Varela et al. 1997). Depressing synapses have also been found in vitro at thalamocortical synapses (for P5–P9 mice: Laurent et al. 2002; for P14–P21 mice, Gil et al. 1997) and at the lemniscal excitatory input onto thalamic VPM neurons (for P7–P24 mice, Arsenault and Zhang 2006). Based on this in vitro data, one would have predicted finding depressing sensory responses in both P7–P12 mice and P13–P21 mice. Our observation that whisker-evoked sensory responses fa-
cilitate in P7–P12 mice and switch to depression at ~P13 is therefore rather unexpected.

Other brain areas may also contribute to generate the facilitation through reciprocal synaptic connections. Our local pharmacological manipulations of cortex showed that the principal whisker representation in barrel cortex is necessary for facilitation, but other brain areas downstream of primary somatosensory cortex and reciprocally connected through long-range excitatory loops could also contribute to generating the facilitation in P7–P12 mice. Reciprocal synaptic connectivity with motor cortex or secondary somatosensory cortex provides the most abundant excitatory feedback loops, but little is known about their development. Equally, corticothalamic synapses create thalamocortical loops that could also contribute to facilitation. Indeed, corticothalamic synapses facilitate in response to repetitive stimulation, but this facilitation is present in P13–P24 mice (Golshani et al. 2001). It is therefore not clear whether such long-range loops fulfill the necessary requirements to contribute substantially to the rapid switch from facilitating to depressing sensory responses we observed at ~P13. One further point to consider is that spontaneous activity also increases dramatically during early development. It is therefore possible that the dynamic state of synapses with respect to short-term plasticity in older mice is significantly affected by the higher levels of spontaneous activity.

In addition to a potential role for long-range excitatory synaptic connections, it is important to consider that the circuits in the barrel cortex responding with facilitation to sensory stimuli at P7–P12 are composed of both excitatory and inhibitory neurons. Excitatory synapses onto other excitatory neurons during this developmental age depress, but the situation regarding the postnatal development of GABAergic neurons for both their input and output synapses has not been studied in detail yet. However, it is clear that profound changes occur in the inhibitory circuits during the first postnatal weeks. GABAergic transmission is depolarizing and excitatory during very early postnatal development (Ben-Ari 2002), and these excitatory effects of GABA may persist at a subset of synapses during the second postnatal week. This could contribute to our observation of facilitation, although our sensory responses were blocked by muscimol application, suggesting that the overall effect of GABA is inhibitory in P7–P12 mice. Additionally, Long et al. (2005) recently reported the abrupt development at ~P13 of a glutamate-dependent spike-synchronizing mechanism, involving neocortical low-threshold spiking GABAergic interneurons. Such a rapid developmental maturation of local network activity (or similar concomitant developmental events) based on changes in GABAergic neurons could be involved in the mechanisms underlying our facilitating sensory responses. Immaturity of the GABAergic inhibitory neuronal network (Ben-Ari 2002; De Felipe et al. 1997; Hensch 2005; Micheva and Beaulieu 1995) together with enhanced NMDA receptor–dependent synaptic transmission (Crair and Malenka 1995; Mierau et al. 2004) or kainate receptor mediated current (Kidd and Isaac 1999) might also contribute to the prolonged sensory responses we observed in P7–P12 mice. Long-lasting sensory responses favor the summation of PSPs, which at a network level could contribute to generating a facilitated sensory response. Consistent with these previously described developmental changes, we indeed observed that sensory responses lasted almost twice as long in P7–P12 compared with P13–P21 mice (Fig. 1). However, through a mechanism based on only summation, one would expect a maximal effect at 100-ms ISI, where we instead find strongly depressed responses to the second stimulus. Interestingly, in a few experiments, a single whisker deflection evoked not only the early first response, but also a small late secondary depolarization occurring in the same period of time when facilitation could be evoked by applying a second whisker stimulus. Such a late depolarization could reflect the enhanced recurrent network activity of cells primed to respond to the subsequent stimulus with a facilitated sensory response, but this was only observed in a subset of recordings. Because facilitation could be evoked without this secondary depolarization, it is not clear if it contributes significantly.

Intrinsic ionic currents such as low-threshold calcium channels, persistent sodium currents, and dendritic regenerative currents may also play a role in the facilitation. Clearly, further study is necessary to uncover the mechanisms responsible for this facilitation and why it is not found in mice older than P13.

We conclude that repetitive sensory whisker stimulation evokes an unexpectedly large cortical sensory response in P7–P12 mice. The facilitated sensory processing during repetitive or prolonged stimulation may contribute substantially to the experience-dependent specification of the developing cortical neuronal network. Our study focused exclusively on the mouse whisker somatosensory signaling pathway, and it will be of interest to study whether a similar facilitation occurs in other sensory pathways during early postnatal development.

ACKNOWLEDGMENTS

We thank S. Crochet and I. Ferezou for help, advice, and useful discussions. Present address of A. Borgdorff: Pelvipharm SAS, Domaine CNRS, Bat 5, 1 Avenue de la Terasse, 91190 Gif-sur-Yvette, France.

GRANTS

We thank the Swiss National Science Foundation, the Leenaards Foundation, Roche Research Foundation, and Human Frontiers in Science Program for generous support.

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