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Whisker Trimming Begun at Birth or on Postnatal Day 12 Affects Excitatory and Inhibitory Receptive Fields of Layer IV Barrel Neurons

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Shoykhet, Michael, Peter W. Land, and Daniel J. Simons. Whisker trimming begun at birth or on postnatal day 12 affects excitatory and inhibitory receptive fields of layer IV barrel neurons. *J Neurophysiol* 94: 3987–3995, 2005. First published August 10, 2005; doi:10.1152/jn.00569.2005. In rats, whisker trimming during development leads to persistent alterations in the function of cortical barrel circuits and to behavioral deficits later in life. Here we examined how whisker trimming begun either at birth (P0) or on postnatal day 12 (P12), around the onset of whisking behavior, affects receptive fields of layer IV barrel neurons. All whiskers on the left face were trimmed for 40–45 days and then allowed to regrow fully. Extracellular single-unit recordings and controlled deflections of principal and adjacent whiskers (PW and AW, respectively), individually or in paired combinations, were used to assess excitatory and suppressive effects of neighboring whiskers on barrel neurons. Results indicate that whisker trimming both from P0 and P12 leads to enlarged excitatory and weakened inhibitory receptive fields in layer IV neurons. PW- and AW-evoked responses are larger in magnitude in trimmed than in control animals; AW-evoked responses are disproportionately affected, decreasing the spatial focus of barrel neurons. Deprivation after P12 accounts for ~50% of the total effect observed in P0 trimmed animals. Suppressive interactions, evoked by two whiskers deflected in succession, are weaker in trimmed than in control animals. Suppressive caudal/rostral and ventral/dorsal gradients, however, seem unaffected by sensory deprivation. Thus the developmental period during which experience persistently modifies maturing barrel circuitry extends up to and likely beyond the onset of whisking behavior. Sensory deprivation during this time affects development of both excitatory and inhibitory receptive fields of barrel neurons and likely impairs cortical integration of sensory information from multiple whiskers.

INTRODUCTION

Sensory experience exerts particularly powerful and long-lasting effects on the developing nervous system (Hubel and Wiesel 1970; LeVay et al. 1980; Wiesel and Hubel 1963; for review see Hensch 2004). In the rodent somatosensory system, damage to whisker follicles or to the trigeminal axons permanently disrupts the development of whisker-related cortical barrels, provided the peripheral damage is produced within the first postnatal week (Van der Loos and Woolsey 1973). Simple whisker trimming from birth spares the gross morphology of the barrels but leads to increased responsiveness of deprived neurons when examined weeks to months after whisker regrowth in adulthood (Simons and Land 1987a; see also Rema et al. 2003). Functional plasticity in layer IV neurons in response to simple whisker removal has been proposed to decline rapidly to a comparatively low level during the first postnatal week (Fox 1992; see also Stern et al. 2001), corresponding to the decline in the ability to induce long-term potentiation and depression at thalamocortical synapses in vitro (Crair and Malenka 1995; Feldman et al. 1998). Whisker removal in rats older than 1 wk of age, even in adults, also affects responses of layer IV neurons to sensory stimulation but, as in many developmental studies, whiskers are allowed to regrow for only a few days (Armstrong-James et al. 1994; Diamond et al. 1993; Glazewski et al. 1998). Short periods of whisker regrowth may not be sufficient, however, to disambiguate transient use-dependent effects from permanent alterations in barrel circuit function (Allen et al. 2003; Armstrong-James et al. 1994). Importantly, this experimental paradigm cannot determine which, if any, of the trimming-induced effects persist beyond the deprivation and whisker regrowth periods.

Deprivation paradigms that do allow for a considerable period of whisker regrowth have shown that significant abnormalities in neuronal responses and behavior persist, although these studies have focused on whisker trimming from birth (Carvell and Simons 1996; Simons and Land 1987a). It is unknown whether whisker removal initiated later during development lastingly impacts the barrel circuitry. Substantial evidence indicates that this should be the case. Asymmetric synapse density in mouse barrel hollows continues to increase into the third postnatal week, as does the number of symmetric synapses made onto spiny stellate cell somata (White et al. 1997). Dendritic fields of layer IV neurons increase in size until P20–P25 (Greenough and Chang 1988). Zinc-containing glutamatergic inputs to layer IV barrels undergo continued development throughout the first month of life (Land and Shamalla-Hannah 2002). At the functional level, barrel neuron receptive field properties progressively diverge from those of their thalamic inputs during the first 4 postnatal weeks (Shoykhet 2003). The prolonged development of multiple processes in layer IV barrels therefore suggests that sensory experience should continue to affect circuit development well beyond the first postnatal week.

Whisker trimming from birth, followed by months of whisker regrowth during adulthood, leads to enlarged excitatory receptive fields of layer IV barrel neurons (Simons and Land 1987a) and to persistent behavioral deficits in tactile discrimination (Carvell and Simons 1996). Interestingly, deficits are observed in a task that normally requires two or more neighboring whiskers; performance is unimpaired on simpler discriminations that can be accomplished by normally reared

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animals using only a single whisker (Carvell and Simons 1996). These findings suggest that neonatal whisker trimming affects the integration of information from nearby whiskers, a process that operates differently in layer IV barrels than in subcortical relays (Simons and Carvell 1989; see also Kyriazi et al. 1994) and that appears to depend critically on strong intrabarrel inhibition (Goldreich et al. 1999; Pinto et al. 2000). Because neonatal sensory deprivation is known to impact the development of local inhibitory circuits in the barrels (Akhtar and Land 1991; Fuchs and Salazar 1998; Micheva and Beaulieu 1995a,b), we hypothesized that whisker trimming would also diminish between-whisker suppressive effects in layer IV.

In this study, we examined receptive field organization of barrel neurons in adult rats whose whiskers had been trimmed for 40–45 days beginning either at birth or on postnatal day 12, the time of onset of normal whisking behavior (Welker 1964). We show, first, that barrel circuit function remains susceptible to lasting, perhaps permanent, modification by tactile experience well beyond the first postnatal week. In addition, we show that deprivation-induced modifications include weakened inhibitory receptive fields of cortical neurons, an alteration which may underlie impaired integration of tactile information from adjacent whiskers in deprived animals.

METHODS

Experimental subjects were derived from untimed pregnant dams, which were monitored at 24-h intervals; the day of birth is designated as P0. Litters were culled to 8–10 pups to ensure uniform growth and development. Data are reported from two similar but independent series of experiments conducted several years apart. In both series, littermates were randomly assigned either to a normally reared, control group or to an experimental group whose whiskers were trimmed. In the first series, whiskers were trimmed for 45 days beginning at P0, and this set of subjects, denoted P0, consisted of four control and nine trimmed rats (male and female, Long-Evans black hooded strain, Charles River, Wilmington, MA). In the second series, whiskers were trimmed for 40 days beginning at P12, and the subject pool, denoted P12, included four control and four trimmed animals (female and male, Sprague-Dawley strain, Harlan Sprague -Dawley, Indianapolis, IN).

Sensory deprivation

In each trimmed group, all of the large whiskers (rows A–E, arcs 0–6) on the left side of the face were trimmed daily to within 1 mm of the skin surface using iridectomy scissors and a dissecting microscope; in each control group, the iridectomy scissors were used to gently ruffle the left mystacial pad. During the first postnatal week, rat pups were restrained by holding them in the experimenter's hand; subsequently, animals were briefly anesthetized using metofane or isoflurane. The pups were allowed to recover from anesthesia and returned either to their mother (before P35) or to their cage. On the last day of trimming in both P0 and P12 groups, the left whiskers were also cut in control animals. Rats were coded to permit experimenter-blind evaluation and were allowed to mature without further intervention for >30 days. Whiskers regrew equivalently in both control and trimmed animals. Thus until completion of all recording sessions, the experimenters remained blind to the trimming condition of the animals.

Surgical procedures

Preparation of the rats for cortical recording experiments was similar to that described previously (Simons and Carvell 1989). Under halothane anesthesia, a catheter for drug delivery was inserted into the external jugular vein, a blood pressure-monitoring catheter was inserted into the femoral artery, and the trachea was cannulated. After exposing the skull and inserting three small stainless steel EEG screws, a steel post for holding the animal's head was fixed to the bone with dental acrylic. A small craniectomy and dural resection were made over the whisker representation in the right somatosensory cortex. An acrylic dam was constructed around the craniectomy, and wound margins were sutured closed around the acrylic. During the recording session, halothane was discontinued, and the rat was maintained in a lightly narcotized and sedated state using fentanyl (Sublimaze, 5–10 μg/kg/h, iv; Janssen Pharmaceuticals, Janssen Biochimica, Berse, Belgium). To prevent spontaneous movements of the vibrissae, which would preclude use of our whisker stimulators, the rat was immobilized by neuromuscular blockade (pancuronium bromide, ~1.6 mg/kg/h, iv; GensiaSicor, Irvine, CA) and respired using a positive pressure respirator (Inspira ASV, Harvard Apparatus, Holliston, MA). Tracheal airway pressure waveform was continuously monitored to ensure airway patency. Throughout the experiment, the rat's condition was assessed by measuring the electroencephalogram, heart rate, expired CO_2 levels, and arterial blood pressure. At the conclusion of the experiment, the animal was deeply anesthetized with pentobarbital sodium (Nembutal; 100 mg/kg) and perfused for cytochrome oxidase (CO) and horseradish peroxidase (HRP) histochemistry. Experiments were similarly terminated if normative physiological conditions could not be maintained during the recording experiment.

Electrophysiological recordings

Double-barreled glass micropipettes were advanced perpendicularly into layer IV of the right hemisphere using a hydraulic microdrive equipped with a stepping motor (David Kopf Instruments, Tujunga, CA). One barrel, which was used for extracellular unit recordings, contained 3 M NaCl (~1-μm tip diam, 5–10 MOhms impedance), and the other, which was used to mark selected electrode positions, contained 10% HRP in 50 mM tris HCl (Simons and Land 1987b). During electrode advancement, whiskers were stimulated manually or with electromechanical stimulators. Stimulus-driven units were isolated using a waveform amplitude discriminator and a digital oscilloscope with time base delay. We only report data obtained from units discharging regular spikes (RSUs) (Bruno and Simons 2002; Simons 1978). A laboratory computer equipped with a PCI-MIO-16E-4 data acquisition board (National Instruments, Austin, TX) was used to store sequential interspike intervals, which were measured with a resolution of 100 μs. The computer also controlled the whisker stimulators. Simultaneous data acquisition and stimulus output were accomplished using custom software (Whisk3; M. Shoykhet and D. J. Simons) written in LabView 5.1.1 (National Instruments). In the P0 study, a DEC LS11 computer was used for stimulus control and data acquisition.

For electrode track reconstruction, the cortex was sectioned in the tangential plane, reacted for CO and HRP (Land and Simons 1985; Simons and Land 1987b; Wong-Riley 1979), and counterstained with thionin. Recording sites were determined using microdrive depth readings, tissue disruption around the electrode track, and HRP spots. Data are reported only for units recorded within the CO-rich barrel centers.

Whisker stimulation

At the outset of the experiment we identified the principal whisker associated with the small craniectomy site. This was defined as the vibrissa whose deflection evoked the most vigorous, or sole, excitatory response; on subsequent histological examination, the principal whisker (PW) was always found to correspond anatomically to the barrel in which recordings were obtained. Multiangle piezoelectric

stimulators (Simons 1983) were attached to the PW and to its immediately adjacent neighbors in the same whisker row or arc. Each stimulator could be activated independently. The stimulator held the vibrissa ~10 mm from its base. Stimulus waveforms consisted of ramp-and-hold trapezoids that produced 1-mm displacements of 200-ms duration; onset and offset movement velocities were ~125 mm/s.

Individual whiskers were deflected alone or in paired combinations. The former stimuli were used to assess excitatory components of a neuron's receptive field. The PW and one to four of its immediately adjacent neighbors were moved in eight angular directions, i.e., in 45° increments relative to the horizontal alignment of the whisker rows. Each stimulus direction was repeated 10 times with intervals ≥2 s. For each repetition of the battery, stimuli were randomized with respect to both whisker and direction. A condition-test paradigm was used in which the PW and an adjacent whisker (AW) were deflected sequentially 30 ms apart; the suppressive effect of the first deflection on the cell's response to the second deflection was taken as a measure of the former's contribution to the cell's inhibitory receptive field (Simons and Carvell 1989). This interval was chosen to coincide with the period of strong condition-test suppression observed previously in normal animals; because stimulus-evoked suprathreshold responses end within 20 ms of whisker movement, the 30-ms interdeflection interval ensured that spiking responses to conditioning and test stimuli did not overlap in time. The conditioning stimulus consisted of movement of a whisker in one of eight different deflection angles; the test stimulus was delivered at the maximally effective direction for the test whisker, determined first using the single-whisker deflection protocol. The condition-test stimulus battery also included 10 test stimuli delivered alone. An entire battery consisted of 18 stimuli (8 conditioning stimulus directions plus 1 test alone times 2 whiskers). Each battery was repeated 10 times, and stimuli were randomized within each battery.

In nine P0 animals (6 trimmed, 3 control), we stimulated the PW and its four immediately adjacent neighbors. In three P0 trimmed and one P0 control animal, we stimulated the PW and only its caudally adjacent neighbor. The caudal AW was chosen because, out of the PWs four immediate neighbors, it exerts the strongest inhibitory influences in normal animals (Brumberg et al. 1996; Simons and Carvell 1989). In all P12 animals, both excitatory and inhibitory receptive fields were examined using the PW and the caudal AW only.

Data analyses

Spike trains were reconstructed from the interspike interval data and converted into peristimulus time histograms (PSTHs) having 1-ms bins. From these, we derived the mean and variance of spike discharges during selected time periods before and during the whisker stimulus. Responses to stimulus onsets (ON) and offsets (OFF) were computed during a 20-ms period after the beginning of whisker movement away from or back to its resting position. These 20-ms time windows encompass the entire transient ON and OFF responses. Spontaneous activity was measured during a 100-ms period before stimulus onset. Suppression of whisker responses during the condition-test paradigm was evaluated using the condition-test ratio, which for each cell was calculated as the average response to the test stimulus when it is preceded by the conditioning stimulus divided by the average response evoked by the test stimulus presented alone. Data were incorporated in Excel spreadsheets and analyzed statistically using Excel, SPSSPC+ (SPSS), or Plot-It graphical software (Scientific Programming Enterprises, Haslett, MI). Unless stated otherwise, the probability values stated in RESULTS were obtained with a nonparametric Mann-Whitney test for two independent samples; two-sided $P < 0.05$ was accepted as statistically significant. All means are presented ±SE.

RESULTS

Only data obtained from layer IV regular spike units located within the CO-rich barrel centers are included in the analyses. We characterized responses of 31 and 77 neurons in P0 control and P0 trimmed rats, respectively. In the P12 cohort, analyses are based on 41 neurons in control animals and 37 neurons in trimmed rats.

Increased responsiveness to whisker stimulation and weakened suppressive interactions between adjacent whiskers distinguish units in trimmed animals from those in normally reared rats. Figure 1 qualitatively shows these differences using as examples two individual neurons from a P0 control and a P0 trimmed animal. In each panel, the central PSTH is comprised of spikes accumulated over 10 deflections of the PW (solid arrow) at the maximally effective (preferred) angle. Surrounding PSTHs show the PW-evoked response at that angle when each PW deflection occurs 30 ms after deflection of an adjacent whisker (condition-test paradigm). Each AW was deflected in eight different directions for a total of 80 repetitions (10 repetition/direction), and PSTHs represent responses accumulated over all angles and scaled with respect to the response evoked by deflection of the PW alone.

The neuron in the control animal responded well to PW deflections (central PSTH, solid arrow: 1.6 spikes/stimulus in the preferred direction; 1.1 spikes/stimulus averaged over all 8 directions; data not shown) and less robustly and less reliably to deflections of the AWs (surrounding PSTHs, open arrows: averaged over all 8 directions, rostral 0.1 spikes/stimulus,

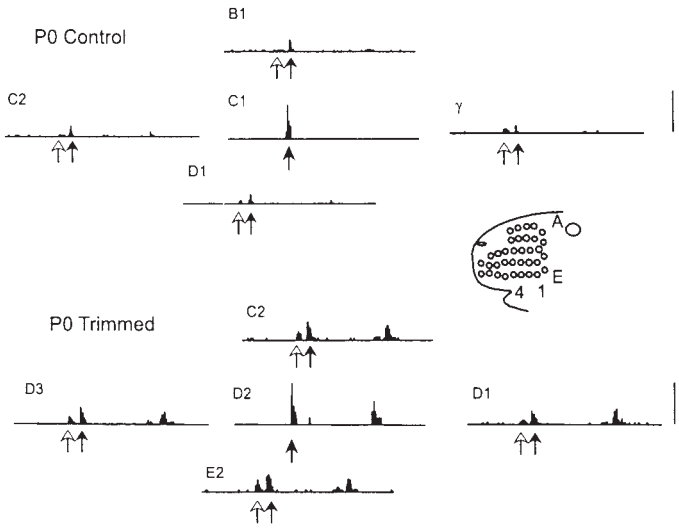

FIG. 1. Responses of individual neurons to single and paired whisker deflections in P0 control (*top*) and P0 trimmed (*bottom*) animals. Data are presented as peristimulus time histograms (PSTHs) with 1-ms resolution. In both *top* and *bottom panels*, the central PSTH represents accumulated responses to 10 ramp-and-hold deflections of the principal whisker (PW) in the maximally effective, preferred angle. Filled arrow indicates stimulus onset. Surrounding PSTHs represent responses during the condition-test paradigm, where the adjacent whisker (AW) is deflected 1st (open arrow), followed 30 ms later by deflection of the PW (filled arrow). AW is deflected 10 times in each of 8 angles, whereas the PW is deflected in the preferred direction. Surrounding PSTHs represent responses accumulated over 80 stimuli and are scaled with respect to the largest bin in the PW-alone response. Scale bars represent the probability of observing a spike in a 1-ms bin on a given trial and correspond to 0.7 spikes/1-ms bin. The position of surrounding PSTHs with respect to the center PSTH indicates position of the AW on the face with respect to the PW. Snout figurine indicates caudal/rostral and ventral/dorsal orientation, as well as the canonical whisker nomenclature.

caudal 0.9 spikes/stimulus, dorsal 0.1 spikes/stimulus, ventral 0.4 spikes/stimulus; 0.7 spikes/stimulus in the preferred directions averaged over all 4 AWs; data not shown). AW stimuli, nevertheless, strongly suppressed the PW-evoked responses in the condition-test paradigm (surrounding PSTHs, solid arrows: condition-test ratios: rostral 0.32, caudal 0.16, dorsal 0.46, ventral 0.26). In the trimmed animal, both PW- and AW-evoked responses are larger in absolute magnitude. In their respective preferred directions, PW deflections evoked 2.5 spikes/stimulus (center PSTH, solid arrow) whereas AW deflections, on average, evoked 1.5 spikes/stimulus (data not shown). Averaged over all eight deflection angles, PW responses were 2.1 spikes/stimulus (data not shown) compared with 0.9, 0.6, 1.1, and 0.8 spikes/stimulus for rostral, caudal, ventral, and dorsal AWs, respectively (surrounding PSTHs, open arrows). The trimming-induced increase in magnitude is more pronounced for AW responses, yielding an excitatory receptive field with less spatial focus in the trimmed than in the control animal. Despite evoking more robust responses, however, AW deflections in the trimmed animal suppress subsequent PW-evoked responses less effectively compared with the control rat (surrounding PSTHs, solid arrows; condition-test ratios: rostral 0.96, caudal 0.71, ventral 0.61, dorsal 0.92).

Heightened responsiveness of units in deprived barrels and weaker suppressive interactions were also observed in animals trimmed from P12, but the effects of whisker trimming appear less pronounced in P12 than in P0 animals. The population PSTHs in Fig. 2*A* show that trimming from P12 leads to enlarged PW-evoked OFF responses, whereas the PW-evoked ON responses are similar between trimmed and control rats. AW deflections in the preferred direction evoke more robust ON and OFF responses in P12 trimmed than in control animals (Fig. 2*B*). AW-evoked responses averaged over all deflection angles are also larger in P12 trimmed versus control animals (Fig. 2*C*, AW deflection), yet, on average, AW deflections suppress responses to the subsequent PW deflections less effectively in trimmed than in control rats (Fig. 2*C*). These findings are quantified below.

Mean response magnitudes of individual units in the P0 and P12 cohorts are plotted in the histograms of Fig. 3, where average responses were calculated from each unit's maximally effective PW or AW deflection angle. PW- and AW-evoked ON and OFF responses (Fig. 3*A*) as well as responses to sustained PW deflections (i.e., plateau, data not shown) are larger in P0 trimmed than in P0 control animals (all $P < 0.05$). Similar results were observed when responses were averaged over all eight deflection angles (data not shown; all $P < 0.05$). Thus whisker trimming from birth affected all evoked response components, i.e., ON, plateau, and OFF responses. In addition, spontaneous firing rates are higher in P0 trimmed than in P0 control rats (Fig. 3*A*; $P < 0.05$). These results in P0 animals confirm previous findings in neonatally trimmed (P0) rats (Simons and Land 1987a). More robust responses relative to control values were observed also in P12 trimmed animals (Fig. 3*B*). In contrast to the P0 trimmed cohort, however, deprivation from P12 affected some but not all response components. Specifically, PW-evoked OFF responses and AW-evoked ON and OFF responses were larger in trimmed animals than in controls (Fig. 3*B*; $P < 0.05$). On the other hand, PW-evoked ON (Fig. 3*B*) and plateau responses (data not shown) as well as spontaneous firing rates (Fig. 3*B*) were equivalent between trimmed and control rats in the P12 group ($P > 0.05$).

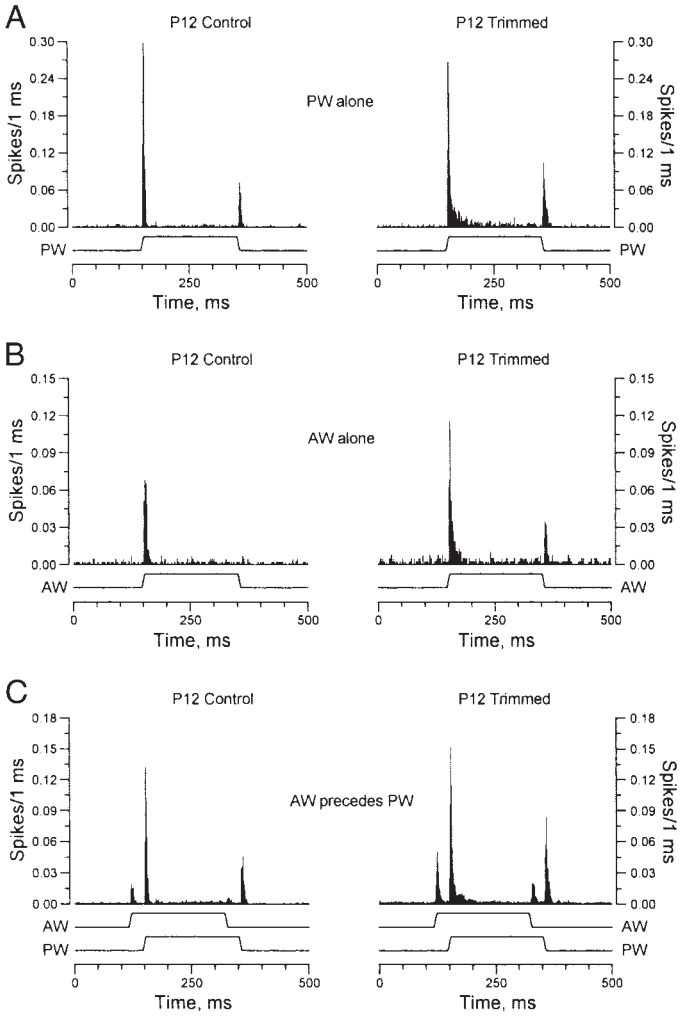

FIG. 2. Population PSTHs of responses to single and paired whisker deflection in P12 animals. All PSTHs are presented in 1-ms bins. Data from P12 control animals ($n = 41$ units) are on the *left* and from P12 trimmed animals ($n = 37$) on the *right*. Whisker stimuli are shown schematically underneath PSTHs. *A*: responses to deflection of PW alone. PSTH shows responses evoked by moving PW in each unit's preferred angle (10 stimulus presentations for each unit). *B*: responses to deflection of the caudal AW alone. Preferred angle responses are shown (10 repetitions). Note the scale difference between *A* and *B*. *C*: responses to the condition-test paradigm, where AW deflection precedes that of PW by 30 ms. Sequence of whisker movements is indicated schematically below the PSTHs. AW was deflected in all 8 directions, and responses averaged over all 8 deflection angles are shown. AW responses in *C* appear smaller than those in *B*, where preferred angle responses are plotted. PW was deflected in its preferred direction during the condition-test paradigm.

Deprivation disproportionately increases weaker responses

A consistent feature of the data shown in Figs. 1–3 is that the least robust responses in control animals are affected most by trimming. For example, mean AW ON responses are nearly twofold larger in P0 trimmed versus control animals, whereas mean PW ON responses are increased by only ~25%. Consequently, the ratio of the average AW to PW evoked response is larger in P0 trimmed (~0.42) than in control (~0.25) animals ($P < 0.05$; Fig. 4*A*); the larger ratio indicates that, on average, trimming decreases spatial receptive field focus. Similar effects

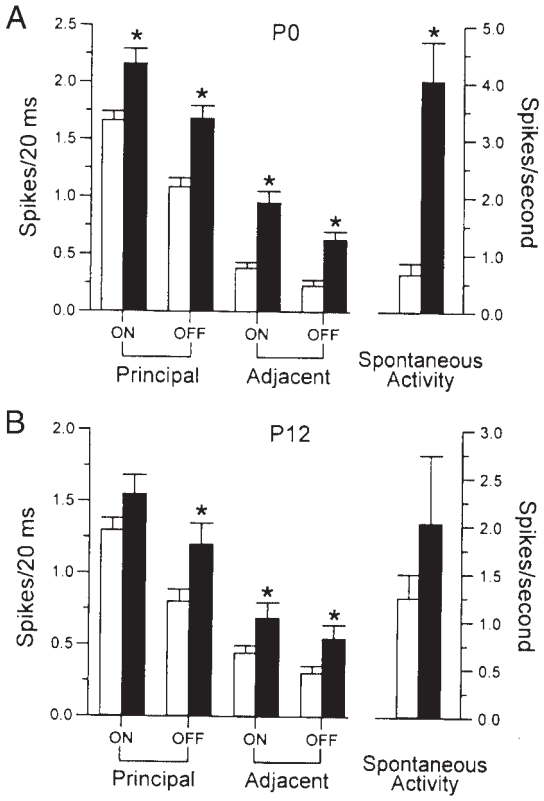

FIG. 3. Effect of whisker trimming on average response magnitudes. *A*: ON and OFF responses to PW and AW deflections in P0 control (open bar) and P0 trimmed (solid bar) animals. ON and OFF responses from deflections in the maximally effective directions are shown (max ON and max OFF). Spontaneous activity rates are shown on the *right*. *Significant statistical difference between trimmed and control values. *B*: PW- and AW-evoked max ON and max OFF responses and spontaneous firing rates in P12 control (open bars) and P12 trimmed (solid bars) animals.

were observed in P12 trimmed versus P12 control animals (Fig. 4*A*; $P < 0.05$).

In general, deprivation leads to a disproportionate increase in responses that, in control animals, are on average smaller, e.g., AW versus PW. This effect is also shown in OFF-ON ratios. In control animals, responses to deflections offsets (OFF) are less robust than responses to deflections onsets (ON); the OFF-ON ratio is thought to reflect the sensitivity of the cortical barrel circuit to the temporal features of its thalamic input (Kyriazi et al. 1994; Pinto et al. 1996, 2000; Simons and Carvell 1989). Whisker trimming begun at either P0 or P12 produces a greater relative increase in OFF versus ON responses and leads to larger OFF-ON ratios in trimmed than in control animals (Fig. 4*B*; $P < 0.05$).

Figure 4*C* shows the inverse relationship between control response magnitude and its deprivation-induced increase. We examined eight response components, including mean responses to PW and AW ON deflections at each cell's preferred direction and mean responses averaged over all deflection directions. For each evoked response component, a trimming-associated fold-increase was determined by dividing the average response magnitude from all cells in trimmed animals by the average response magnitude in control animals, e.g., Fig. 3. In both P0 and P12 populations, the trimming-dependent fold-increase in average response magnitudes decays exponentially as a function of control response magnitudes. As in the case of OFF and AW responses, responses to nonpreferred deflection angles increased more than those to preferred deflection angles. This resulted in a small decrease in angular tuning in both P0 and P12 animals. Angular tuning was quantified in two ways. In one we calculated the ratio of the maximum angle response to the response averaged over all deflection angles; the largest effect, observed in P0 animals, was an ~10% decrease in tuning ($P = 0.17$). In the second, we determined how many response angles were statistically smaller than the preferred angle using a one-tailed *t*-test with $P < 0.05$ (see Simons and Carvell 1989). In contrast to our previous study (Simons and Land 1987a), the reductions in angular tuning using this measure were not statistically significant, either.

Data from Fig. 4*C* are replotted in Fig. 4*D*, where control values are expressed on a logarithmic scale. With this transformation, the relationship between control values and deprivation-induced fold-increases is linear. High linear correlation coefficients (P0, $r^2 = 0.96$; P12, $r^2 = 0.95$) indicate that the association between response magnitude in trimmed animals and the initial value observed in control rats is quite robust. The slope obtained for the P0 data (−2.40) is twice that obtained from P12 data (−1.15). These findings suggests that *1*) whisker trimming between P0 and P12 accounts for ~50%

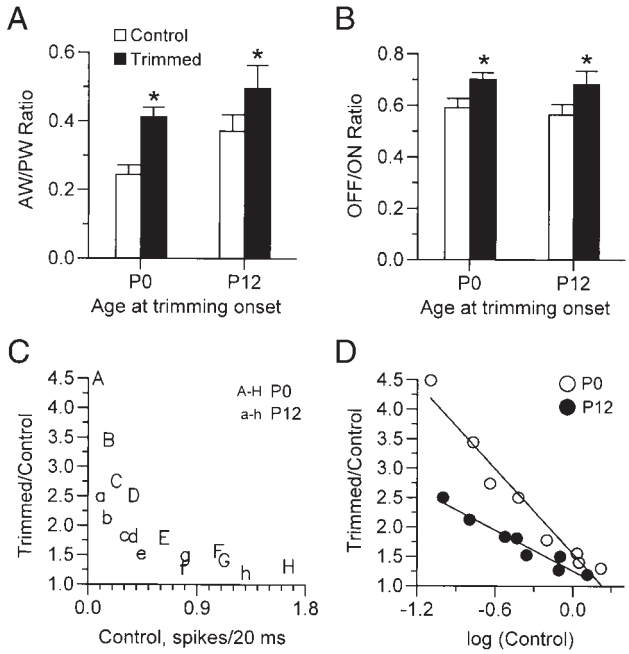

FIG. 4. Greater effect of trimming on smaller evoked responses. *A*: average ratio of AW-evoked to PW-evoked ON responses in P0 and P12 control (open bars) and trimmed (solid bars) animals. AW/PW ratio is computed for each individual neuron from ON responses averaged over all 8 directions. Ratios are averaged within each population, and the mean ratio ± SE for each population is shown. *Statistically significant differences between trimmed and control values. *B*: average OFF-ON ratios in P0 and P12 cohorts. For each neuron, the ratio of PW-evoked OFF response to the PW-evoked ON response, both averaged over all deflection angles, is computed. Ratios are averaged within each population. *C*: trimming-induced effects depend on the magnitude of evoked responses in control animals. Ratio of trimmed to control values is plotted as a function of the value observed in control animals. For all evoked response components, ratios obtained from mean response magnitudes both in preferred directions and averaged over all deflection angles are plotted (P0, capital letters; P12, small letters; A, AW mean OFF; B, AW mean ON; C, AW max OFF; D, AW max ON; E, PW mean OFF; F, PW max OFF; G, PW mean ON; H, PW max ON). *D*: values are replotted with *x*-axis transformed to a logarithmic scale. Solid lines indicate linear regression approximations of the data.

of the total deprivation effect observed in the P0 trimmed animals, with deprivation after P12 contributing the remaining 50%; and *2*) deprivation disproportionately enhances weaker responses.

Trimming weakens between-whisker suppressive interactions but preserves spatial gradients

We used paired whisker deflections in a condition-test paradigm to assess inhibitory components of receptive fields. In previous studies in lightly narcotized animals, suppression of the conditioned-test response has been shown to reflect intrabarrel processing of thalamic input (Carvell and Simons 1988; Simons and Carvell 1989). A given PW and its caudal adjacent neighbor were deflected in this paradigm because, of all AWs, the caudal AW evokes the most robust suppression (Brumberg et al. 1996; Simons and Carvell 1989). The *bottom panels* in Fig. 2 show neuronal population responses in P12 control and trimmed animals when AW deflections (the conditioning stimulus) precede PW deflections (the test stimulus). Prior AW deflection diminishes PW responses. Data from individual units were quantified by calculating a condition-test ratio (Fig. 5); smaller values indicate more suppression (see METHODS). On average, in control animals, PW-evoked responses are diminished by preceding AW deflections by ~40–50% (Fig. 5*A*; P0 control, $x = 0.62 \pm 0.04$, $n = 31$; P12 control, $x = 0.52 \pm 0.04$, $n = 39$). Suppression of the PW-evoked response by the AW conditioning stimulus is significantly weaker in both groups of trimmed animals than in their respective control groups (Fig. 5*A*; P0 trimmed, $x = 0.76 \pm 0.02$, $n = 77$; P12 trimmed, $x = 0.65 \pm 0.05$, $n = 36$; $P < 0.05$). Similar results were obtained when PW deflections preceded AW deflections, a stimulus protocol that normally evokes robust response suppression (Fig. 5*B*). AW-evoked responses are reduced, on average, by 90% after PW deflections in control animals but only by ~75% in trimmed rats (P0: control $x = 0.11 \pm 0.02$, $n = 31$; trimmed $x = 0.28 \pm 0.03$, $n = 77$. P12: control $x = 0.12 \pm 0.04$, $n = 41$; trimmed $x = 0.22 \pm 0.06$, $n = 36$; $P <$

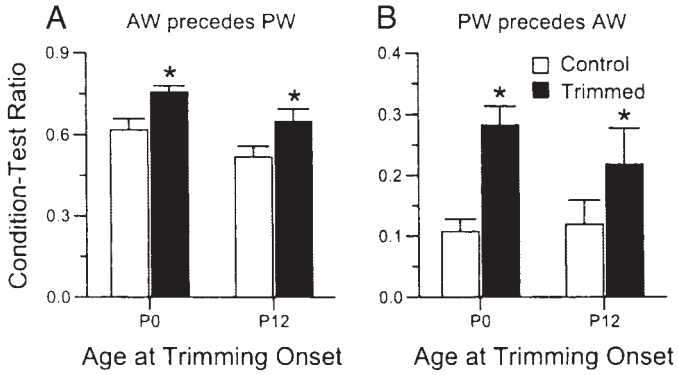

FIG. 5. Suppression of evoked responses by preceding whisker stimulation. *A*: paired whisker stimulation with the conditioning AW deflection preceding the PW deflection by 30 ms. AW is deflected 10 times in each of 8 directions, and the PW is deflected in the unit's preferred direction. Condition-test ratio is computed by dividing response to conditioned test stimulus by that evoked by test stimulus presented alone. Smaller values indicate greater response suppression. Condition-test ratios are larger in trimmed (solid bars) than in control (open bars) animals in both P0 and P12 cohorts. *B*: paired whisker stimulation with PW deflection preceding AW deflection. Note that PW stimulation suppresses responses to subsequent deflections to a greater extent than AW stimulation, which accounts for scale difference between *A* and *B*.

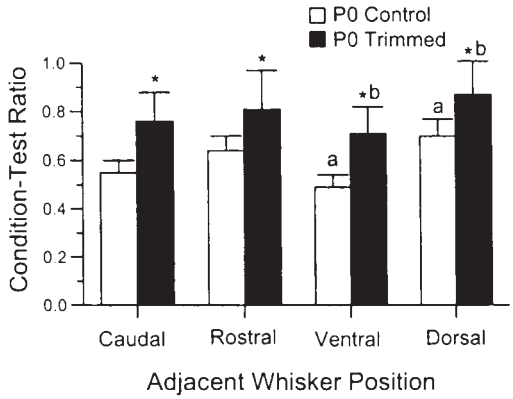

FIG. 6. Adjacent whisker suppressive gradients in P0 control and P0 trimmed animals. In both control (open bars) and trimmed (solid bars) cohorts, caudal AW evokes greater suppression than the rostral one, and the ventral AW evokes greater suppression than the dorsal one. Latter gradient is statistically significant in control and trimmed animals (a and b, respectively). For all AWs, condition-test ratios are significantly larger in trimmed than in control animals (*).

0.05). Thus whisker trimming either from birth or from P12 significantly weakens between-whisker suppression regardless of the sequence of AW and PW deflection.

The strength of adjacent whisker-evoked suppression depends on the location of the AW with respect to the PW. On average, the immediately caudal AW evokes greater suppression than the rostral AW, and the ventral greater than the dorsal (Brumberg et al. 1996; Simons and Carvell 1989). We examined whether these gradients are affected by whisker trimming by separately assessing the suppressive effects of caudal, rostral, ventral, and dorsal AWs in six P0 trimmed and three P0 control animals. Results, presented in Fig. 6, show that, for each AW tested, condition-test ratios were smaller in control than in trimmed animals (all $P < 0.001$). Ventral AWs evoked stronger response suppression than dorsally adjacent vibrissae in both control and trimmed rats (paired *t*-test; control: $n = 23$, $P = 0.004$; trimmed: $n = 38$, $P = 0.0001$). As observed previously (Brumberg et al. 1996; Simons and Carvell 1989; see also Bruno and Simons 2002), the ventral/dorsal gradient is more pronounced than the caudal/rostral one. In agreement with previous findings, the caudally AWs are more suppressive than the rostrally AWs, but in this sample, the difference reaches statistical significance only at a trend level in both control and trimmed animals (paired *t*-test; control: $n = 23$, $P = 0.11$; trimmed: $n = 40$, $P = 0.07$). Thus, although neonatal whisker trimming reduces the overall strength of surround inhibition, it does not disrupt adjacent whisker suppressive gradients.

Fast-spike units

A small sample of fast-spike units (FSUs; Bruno and Simons 2002; Simons 1978) were recorded in the P0 study ($n = 4$ control, 6 trim); only one FSU was sampled in the P12 group. The mean PW evoked response, averaged over all deflection angles, of the six FSUs in the P0 trim group was 3.01 ± 0.87 spikes/stimulus onset. In comparison, the four FSUs in the control group responded with 2.87 ± 0.69 spikes; a recent paper in normally reared rats reported a mean response of 2.68 ± 1.60 (Kwegyir-Afful et al. 2005). Thus trimming in P0

animals does not seem to lead to increases in FSU activity commensurate with those in regular-spike units (~40%; Fig. 4*C,* point G).

DISCUSSION

This study shows that whisker trimming during development leads to enlarged excitatory and weakened inhibitory receptive fields of barrel neurons. Alterations in receptive field structure considerably, and perhaps permanently, outlast the periods of deprivation and whisker regrowth. During development, the barrel circuit remains susceptible to experience-dependent modification for at least the first few weeks of life, only reaching a half-maximal point at the end of the second postnatal week.

Deprivation disrupts excitatory and inhibitory receptive fields

The barrel circuit consists of two extensively interconnected populations of neurons—excitatory cells and inhibitory cells. The receptive field properties of excitatory neurons in normal adult barrels are shaped by interactions between local feed-forward excitation and inhibition (for review, see Miller et al. 2001). Feed-forward excitation arises directly from thalamic input onto barrel excitatory cells and feed-forward inhibition from thalamic input onto barrel inhibitory interneurons (Bruno and Simons 2002). Where in this thalamocortical circuit do the trimming-induced changes occur? Responses of thalamic barreloid neurons in trimmed animals are essentially normal, suggesting that the effects of deprivation reside in the cortex or at the thalamocortical synapse (Glazewski et al. 1998; Simons and Land 1994). While in these studies, some whiskers were spared, these findings in P0 animals in which all whiskers were trimmed are highly similar to results obtained in deprived barrels using partial deprivation (Simons and Land 1987a). Cortical neurons in deprived animals have normal membrane potential and input resistance (Finnerty et al. 1999; Maravall et al. 2004), indicating that changes in passive membrane properties cannot account for the observed enlarged excitatory receptive fields. On the other hand, neonatal whisker trimming without regrowth does result in decreased numerical density of thalamocortical synapses and of symmetric, presumed inhibitory intracortical contacts in mouse barrels (Sadaka et al. 2003). Thus persistent trimming-induced effects likely reflect anatomical and/or functional alterations in thalamocortical and local corticortical synaptic connections in layer IV.

These findings indicate that whisker experience affects the development of functional inhibition within the barrel and/or its engagement by thalamocortical input (Akhtar and Land 1991; Fuchs and Salazar 1998; Micheva et al. 1995a,b; see also Knott et al. 2002). Three lines of evidence support this conclusion. First, neonatal whisker trimming leads to increased spontaneous and stimulus-evoked responses in deprived barrels. Second, neonatally deprived animals exhibit weakened between-whisker response suppression, which in lightly narcotized rats likely depends on intrabarrel inhibition. Notably, between-whisker suppression is stronger in layer IV barrels than in thalamic barreloids (Simons and Carvell 1989; see also Kelly et al. 1999), although in more deeply anesthetized animals, such suppressive effects observed in the cortex could reflect an already strongly suppressed thalamic input signal (Higley and Contreras 2005). Third, the disproportionate enhancement of normally smaller excitatory responses in deprived animals is similar to effects produced in normally reared animals by microiontophoretic application of $GABA_A$ or $GABA_B$ antagonists (Kyriazi et al. 1996a,b). For example, in both deprived barrels and those affected by bicuculline application (these results; Kyriazi et al. 1996b; Simons and Land 1987a), AW excitatory responses increase more than PW responses, and OFF responses more than ON responses. The similarity between the effects of whisker deprivation and those of bicucculine application suggests that whisker trimming leads to a persistent imbalance between intrabarrel inhibition and excitation in favor of the latter. A similar mechanism has been proposed for the rat visual system where a short period of sensory deprivation early in life has been found to increase the excitability of the layer IV circuitry by increasing net excitatory and decreasing net inhibitory synaptic drives (Maffei et al. 2004). Consistent with this interpretation, in trimmed animals, the evoked responses of FSUs, presumed inhibitory barrel neurons, do not appear to be markedly increased or increased commensurately with those of RSUs. The responsiveness of the small sample of FSUs recorded in this study was well within the range of normally reared animals. Data from a larger sample of FSUs (S. H. Lee and D. J. Simons, unpublished observations) indicates that FSU responsiveness may actually be somewhat diminished in trimmed animals.

Although these findings are consistent with weaker net intrabarrel inhibition, some organizational features of inhibitory circuitry within the barrel appear to develop independently of whisker experience. We found that caudal/rostral and ventral/dorsal suppressive gradients are maintained in deprived barrels, despite overall reduced levels of between-whisker suppression. In normally reared animals, thalamocortical inputs strongly engage inhibitory (fast-spike) barrel neurons, and such functional contacts are biased in favor of contacts by thalamic neurons that respond to caudally or ventrally AWs (Bruno and Simons 2002). These findings therefore suggest, in contrast to an earlier hypothesis (McCasland et al. 1991), that mechanisms underlying the development of inhibitory gradients in thalamocortical circuitry are robust enough to operate in the absence of normal whisker experience.

Deprivation effects persist after whisker regrowth

In this and other studies from our laboratory, trimmed whiskers are allowed to regrow fully. This paradigm permits a clearer distinction between persistent effects induced by sensory experience during development and acute effects that reflect use-dependent modification of synapses. The latter, use-dependent effects occur both during development and in adulthood (Allen et al. 2003; Armstrong-James et al. 1994; Glazewski and Fox 1996). Full whisker regrowth typically requires several weeks, and these findings were obtained from animals whose whiskers regrew for a minimum of 30 days, and in some cases, for several months. In other developmental deprivation paradigms, whiskers regrow only for a few days, which permits the vibrissal hair to achieve a minimal length for applying mechanical stimuli (Fox 1992; Glazewski et al. 1998). In these studies, neurons in layer IV barrels are found to be less responsive to trimmed whiskers, the opposite of what

we and others (Hand 1982; Rema et al. 2003) observe after whiskers regrow fully.

In these experiments, we trimmed all mystacial vibrissae and observed increases in evoked cortical responses to both PWs and AWs. Results are comparable with those found previously in deprived barrels that were located adjacent to barrels whose whiskers had remained intact throughout development (Simons and Land 1987a; see also Rema et al. 2003). Our findings therefore indicate that neonatal sensory deprivation leads to similar effects in deprived layer IV barrels regardless of the presence or absence of nearby, normally functioning whiskers. In the whisker/barrel system, an imbalance in activity arising from neighboring vibrissae (trimmed, intact) is not required for the development of permanent abnormalities in deprived barrels.

Critical periods in developing barrel circuitry

Abnormal tactile experience beginning as late as the end of the second postnatal week produces long-lasting and perhaps permanent alterations in barrel circuit function. This period of susceptibility extends well beyond the critical period for morphological development of the barrel pattern, which ends at P5 (Van der Loos and Woolsey 1973). It also extends past P7–P8, which marks the end of the period in which long-term potentiation (LTP) and long-term depression (LTD) of thalamocortical synapses can be induced by standard in vitro experimental procedures (Crair and Malenka 1995; Feldman et al. 1998). An earlier study by Fox (1992) indicates that in layer IV the most robust effects of whisker removal without regrowth occur when deprivation is initiated during the first four postnatal days. By P7–P8, effects are markedly reduced, and during the second postnatal week, short periods of trimming without regrowth have no effect on the amplitude of evoked excitatory postsynaptic potentials (EPSPs) in layer IV neurons (Stern et al. 2001). With our experimental paradigm, in which whiskers are allowed to regrow, barrels in P12 animals retain a substantial degree of developmental plasticity, inasmuch as alterations in receptive field properties reflect a midpoint (a 50% change) between normal adult values and those obtained with trimming initiated at P0.

Available evidence thus suggests multiple periods of susceptibility, wherein different forms of deprivation elicit various effects. Likely variables affecting the final outcome of deprivation include the extent, duration, and developmental timing of whisker removal and, possibly, the duration of whisker regrowth. These findings suggest an extended developmental period during which sensory experience produces effects on barrel function that outlast the experience of deprivation and may be permanent. This period of susceptibility extends beyond the time of onset of whisking behavior, which occurs at P12–P14 (Welker 1964). Extrapolation of our data from P0 and P12 trimmed groups would predict that normal whisker experience continues to influence the functional development of barrel circuitry at least into the third postnatal week. We propose that in the somatosensory system, tactile experience during the first month of life permanently impacts cortical function, albeit to a progressively diminishing degree.

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