Intracortical Pathways Mediate Nonlinear Fast Oscillation (>200 Hz) Interactions Within Rat Barrel Cortex

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Staba, Richard J., Tyler D. Ard, Alexander M. Benison, and Daniel S. Barth. Intracortical pathways mediate nonlinear fast oscillation (>200 Hz) interactions within rat barrel cortex. J Neurophysiol 93: 2934–2939, 2005. First published December 8, 2004; doi:10.1152/jn.01101.2004. Whisker evoked fast oscillations (FOs; >200 Hz) within the rodent posteromedial barrel subfield are thought to reflect very rapid integration of multiwhisker stimuli, yet the pathways mediating FO interactions remain unclear and may involve interactions within thalamus and/or cortex. In the present study using anesthetized rats, a cortical incision was made between sites representing the stimulated whiskers to determine how intracortical networks contributed to patterns of FOs. With cortex intact, simultaneous stimulation of a pair of whiskers aligned in a row evoked supralinear responses between sites separated by several millimeters. In contrast, stimulation of a nonadjacent pair of whiskers within an arc evoked FOs with no evidence for nonlinear interactions. However, stimulation of an adjacent pair of whiskers in an arc did evoked supralinear responses. After a cortical cut, supralinear interactions associated with FOs within a row were lost. These data indicate a distinct bias for stronger long-range connectivity that extends along barrel rows and that horizontal intracortical pathways exclusively mediate FO-related integration of tactile information.

INTRODUCTION

During exploration, rats typically employ a “whisking” motion that rhythmically moves whiskers through a rostral-caudal plane, contacting an object either simultaneously or sequentially with multiple whiskers (Carvell and Simons 1990). Evidence for the integration of multiwhisker stimuli has come from unit studies that show simultaneous whisker stimulation may evoke enhanced suprathreshold responses in barrel cortex (Ghazanfar and Nicolelis 1997; Shimiegi et al. 1999), while sequential whisker deflections with delays between ~10 and 50 ms are thought to be mediated by inhibitory interactions that suppress unit activity (Higley and Contreras 2003; Simons 1985; Simons and Carvell 1989).

In addition to unit studies, epipial mapping studies show that multiwhisker stimulation evokes bursts of rhythmic field potentials termed “fast oscillations” (FOs; >200 Hz) within the posteromedial barrel subfield (PMBSF) (Barth 2003; Jones and Barth 1999; Jones et al. 2000). Results from these studies show that FOs are largest in amplitude within cortical areas representing the stimulated whiskers, yet may propagate 1–2 mm within the PMBSF, all the while remaining in close phase alignment over these distances. In addition, simultaneous stimulation can evoke enhanced FO responses, but FOs intentionally brought out of phase by asynchronous whisker stimulation of several milliseconds can attenuate FO responses (Barth 2003). While these data suggest that FOs may also play a prominent role in the integration of multiwhisker stimuli, the basis for FO interactions remain unclear.

Within the ventral posterior medial thalamus, a major subcortical site that projects to barrel cortex, multiwhisker stimulation may evoke unit discharge that summates nonlinearly (Ghazanfar and Nicolelis 1997), activity that may contribute to, if not exclusively produce, FO interactions measured at the cortex. However, other evidence suggests FOs are generated within cortical networks (Grenier et al. 2001; Staba et al. 2003), with horizontal intracortical pathways that could support long range interactions between barrel-related columns (Bernardo et al. 1990; Hoefinger et al. 1995). In view of these data, in the present study using anesthetized rats, cortical incisions were made between sites representing pairs of whiskers that were stimulated simultaneously to determine what effect(s) disrupting intracortical connections had on spatiotemporal patterns of FO.

METHODS

Animals and surgery

All procedures were conducted within the guidelines established by the University of Colorado Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (n = 14, 200–250 g) were anesthetized using a mixture of ketamine HCl (64 mg/kg), xylazine (13 mg/kg), and acepromazine (2 mg/kg), and a unilateral craniotomy was performed over the right hemisphere extending from bregma to lambda and from the midsagittal suture to the lateral aspect of the temporal bone, exposing a large area of parietotemporal cortex. The dura was retracted and the exposed cortex regularly wetted with Ringer solution [composed of (in mM) 135 NaCl, 3 KCl, 2 MgCl₂, and 2 CaCl₂]. At the conclusion of the experiment, animals were killed with an overdose of anesthesia without regaining consciousness.

Stimulation and recordings

Whiskers on the left mystacial pad were displaced ~300 μm in the dorsoventral direction (0.1 ms in duration, delivered at 1 Hz) using a laboratory-built solenoid (Barth 2003; Jones and Barth 1999). Separate stimulators were positioned at each whisker to stimulate whiskers simultaneously. Epipial recordings of whisker evoked field potentials were made using a 64 contact electrode array (100 μm tip diameter, 500 μm inter-contact spacing) arranged in an 8 × 8 grid that was placed flush against the cortical surface. The array was aligned within the right PMBSF based on single-whisker evoked responses. Surface
field potentials were referenced to a silver ball electrode placed over the contralateral frontal bone. Field potentials were amplified ($\times 500$), filtered (1–3,000 Hz), and digitized at 10 kHz.

Data collection and analysis

Responses were computed by averaging multiple sets (2–3) of individual trials ($n = 64$, 100 ms) of the evoked response during single and paired whisker displacements before and after a cortical incision was made either between ($n = 6$) or parallel ($n = 4$) to sites representing C4 and C1 whiskers. All cuts were made using a blade (4.3 $\times$ 0.2 $\times$ 1.0 mm) fashioned from a flattened 26-gauge hypodermic needle. With the aid of a surgical microscope, the pointed end of the blade was inserted into the cortex to a depth that aligned a calibration mark located on the shaft of the blade even with the surface of the cortex. The blade was then drawn upward to minimize the damage to surface vessels. Prior to the incision, surgical suture thread was placed along each of the four sides of the array, creating an outline of the array. The array was retracted, a cut was made, and the array was repositioned within area delimited by the suture thread. A multiple signal classification algorithm was used to estimate spectral power in wideband evoked responses. Peak frequency associated with the P1/N1 and FOs were defined as the peak in power $<80$ and $>200$ Hz, respectively. Evoked responses were band-pass filtered (200–600 Hz) using a fourth-order Butterworth filter, and FO amplitude was computed as the root-mean-square (RMS) for all subsequent analyses. To better illustrate patterns of FOs, using the evoked responses recorded from the array, color maps were generated using a bicubic spline interpolation algorithm and normalized to the maximum RMS value across a series of maps. This interpolation method utilizes global electrical gradients computed from a two-dimensional field potential distribution (i.e., grid of evoked responses) that yields the most accurate estimates of FO amplitude maxima within a given map. Color maps were used for graphical presentation only. Nonlinear summation was evaluated by subtracting the sum of evoked responses during stimulation of each individual whisker alone from the evoked response during simultaneous whisker stimulation for each contact on the 64 contact array. Differences were considered significant if the response amplitude exceeded the upper 95% confidence limit computed from the baseline amplitude; otherwise, the difference was considered 0 at that electrode site. Statistical analysis was performed on the total response amplitude, computed as the sum of RMS values of the evoked responses recorded from all 64 contacts on the array, excluding contacts within the incision area, which were identified by inspection of evoked response maps. Analyses used ANOVA or paired t-test with $\alpha = 0.05$.

Histology

A cytochrome oxidase (CO)-stained tangential section through layer IV of the flattened right hemisphere of one animal was obtained to determine the position of the array within the PMBSF. In addition, cresyl-violet (CV)-stained coronal sections through the PMBSF were obtained from a different animal to verify the depth of a cortical incision. After epipial mapping, both animals were deeply anesthetized and perfused intracardially with 0.1 M phosphate buffer ($100$ ml), followed by a 6% paraformaldehyde buffered fixative ($500$ ml). The brain was removed and immersed in 30% sucrose buffered solution kept at 7°C for 48 h. For CO-stained sections, the right cortex was flattened and secured between two glass slides prior to immersion. Sections were cut tangential to the pia with a cryostat at a thickness of 40 $\mu$m and incubated at 37°C for $\sim 2$ h in a medium that consisted of 50 mg dianaminobenzidine tetrahydrochloride, 0.25 ml dimethylsulfoxide, 2 mg catalase, 20 mg cytochrome C, and 5 g sucrose dissolved in 100 ml 0.1 M phosphate buffer. Sections were rinsed in six changes of 0.1 M phosphate buffer, mounted, and coverslipped. For CV staining, sections were cut coronally with a cryostat at a thickness of 40 $\mu$m, mounted on gelatinized slides, stained, and coverslipped.

Results

Transient displacement of a rodent’s whiskers evoked somatosensory-evoked potentials (SEPs) that consisted of both slow and fast components (Fig. 1A). Power spectral analysis of wideband (1–2,000 Hz) recorded SEPs showed the initial biphasic slow wave, labeled P1 and N1 to denote polarity and sequence of occurrence, had maximum power $<80$ Hz [26 ± 10 (SD) Hz], while the fast component, i.e., fast oscillation (FO), had peak power $>200$ Hz (277 ± 32 Hz). Band-pass (200–600 Hz) filtered traces of the SEP showed the FO was superimposed primarily on the P1 slow wave (Fig. 1A, indicated *).

Using a 64-contact electrode array that was placed flush against the cortex, stimulation of individual whiskers-evoked patterns of FOs that were consistent with the somatotopic organization of the PMBSF. In the example shown in Fig. 1 derived from a single animal, histological analysis confirmed that stimulation of a single whisker (C4 in this example) evoked FOs that were largest in amplitude within areas representing the stimulated whisker, i.e., principal barrel (Fig. 1, B and C). Interpolated color maps derived from the grid of FO traces (see Methods for computation) more clearly show the evoked FO profile overlapping the principal barrel (Fig. 1D). Based on the reliability of the single whisker-evoked response, the array was consistently aligned within the PMBSF across animals.

It has been shown that combined stimulation of paired whiskers evokes phase aligned FOs that increase nonlinearly (i.e., response amplitudes exceed the sum of responses evoked by stimulation of each whisker alone), suggesting that FO-related neuronal interactions, as opposed to linear summation of field potentials due to volume conduction, contribute to enhanced FO responses (Barth 2003). Here, we first explored whether such neuronal interactions were sensitive to spatial orientation by stimulating pairs of whiskers aligned within the same row or same arc.

Stimulation of whiskers C4 or C1 evoked FOs that were largest in amplitude within their respective representations in the PMBSF (Fig. 2A, “C4” and “C1”). In the example shown in Fig. 2 that was derived from the same animal and hemisphere as illustrated in Fig. 1B, when these same two whiskers were stimulated simultaneously (Fig. 2A, “Simultaneous C4,C1”), the amplitude of the response was supralinear and thus greater than a linear model of the combined response computed as the sum of responses during individual whisker stimulation (Fig. 2A, model). Subtracting the response to combined stimulation from the linear model yielded a difference map, reflecting the magnitude of supralinear interactions (Fig. 2A, difference). Mean response amplitudes during simultaneous stimulation of C4 and C1 whiskers were 141 ± 29% (mean ± SD; $n = 4$) greater than the linear model across animals.

Stimulation of whiskers D3 or B3 also evoked responses corresponding closely to their respective representations within the PMBSF (Fig. 2B, D3 and B3). However, simultaneous stimulation evoked FOs nearly the same amplitude as the linear model (Fig. 2B, simultaneous D3, B3 vs. model). In this example, the difference map (Fig. 2B, difference) showed little
supralinear interaction and the mean response amplitude across animals was 105 ± 29% (n = 4) of a linear model. In contrast to supralinear responses during paired stimulation of widely separated whiskers within a row, similar responses within an arc were restricted to adjacent whiskers (Fig. 2C). Simultaneous stimulation of whiskers D3 and C3 produced supralinear interactions that were 151 ± 30% (n = 4) of a linear model. Statistical analysis of these data revealed significant differences in the magnitude of response facilitation between whiskers within a row versus arc [ANOVA, F(2,11) = 9.4, P = 0.01]. Response facilitation during simultaneous stimulation of either C4, C1 or D3, C3 was greater compared with the amount of facilitation during stimulation of nonadjacent D3, B3 whiskers (both P < 0.05), while equivalent magnitudes of response facilitation were observed during simultaneous C4, C1 or D3, C3 whisker stimulation (summarized in Fig. 5A).

To determine if intracortical pathways contributed to supralinear interactions, an incision was made between cortical areas representing a pair of stimulated whiskers during a series of separate experiments. We chose widely separated whiskers (C4 and C1) to minimize damage to principle response areas in the PMBSF. Similar to the previous experiment, simultaneous stimulation of whiskers C4 and C1 evoked supralinear FOs (Fig. 3A). Based on the evoked response profile during individual stimulation of these whiskers, an incision was made between sites representing C4 and C1 whiskers that extended diagonally across the cortex equivalent in length to the size of the surface array (Fig. 3B, dashed line). In all animals, the cut resulted in little surface bleeding that subsided within 1–2 min and evoked responses typically returned within 10 min. In the example shown in Fig. 3B (inset), the depth of cut was 1.65 mm as measured from a perpendicular line drawn from the cortical surface to the bottom of the cut that reached the border of the gray matter. Analysis of responses after the cut showed that during individual whisker stimulation evoked response profiles were nearly identical to those prior to the cut (Fig. 3B, C4 and C1). No difference was observed in the amplitude of single-whisker-evoked responses before versus after the cut across animals (paired t-test, t = 1.8, df = 5, P = 0.13). However, simultaneous stimulation evoked responses that were indistinguishable from the linear model (Fig. 3B, simultaneous vs. model). Overall, supralinear response facilitation was significantly larger before the cut compared with after (t = 4.3, df = 5, P = 0.006; Fig. 5B).

To determine whether nonspecific effects of the cortical cuts may have contributed to elimination of supralinear FO interactions, within a separate group of animals an incision was made that was placed parallel to sites representing the C4 and C1 whiskers. Prior to an incision, as shown before, supralinear responses were observed during simultaneous C4, C1 whisker stimulation (Fig. 4A). Using the evoked response profiles to place the incision below sites representing the C4 and C1
whiskers (Fig. 4B, cut location indicated by - - -), analysis of evoked responses after the cut showed there was no difference in evoked response profiles (Fig. 4B, C4 and C1) or magnitude of supralinear response facilitation (Fig. 4B, difference) compared with before the cut. Incisions made parallel to sites representing the C4 and C1 whiskers in four animals found no difference in evoked response amplitude before versus after the cut ($t = H11005 0.13$, df = $H11005 3$, $P = H11005 0.9$), and values of supralinear response facilitation were not significantly different before compared with after the cut ($t = H11005 0.81$, df = $H11005 3$, $P = H11005 0.48$; Fig. 5C).

**DISCUSSION**

Morphological studies in rats and other mammalian species suggest that “rows” are the basic processing unit of the whisker pad (Brecht et al. 1997). Support for a row-oriented organization within barrel cortex derives from studies that show greater
densities and longer connectional tendencies between barrel-related columns within the same row versus different rows (Bernardo et al. 1990; Hoeflinger et al. 1995). Based on these anatomical data, one might expect an analogous functional bias along rows in barrel cortex, and in fact, some electrophysiological data do show that both supra- and subthreshold receptive fields tend to spread along barrel rows (Armstrong-James et al. 1992; Moore and Nelson 1998; Petersen et al. 2003).

Consistent with these latter studies, the present data show that values of supralinear FO responses are significantly larger during stimulation of nonadjacent whiskers aligned within a row compared with nonadjacent whiskers within an arc. However, some studies show no difference between unit responses during stimulation of adjacent whiskers within a row compared with an arc (Ghazanfar and Nicolelis 1997; Mira bella et al. 2001). Consistent with these latter studies, the present data show that values of supralinear FO responses are significantly larger during stimulation of nonadjacent whiskers aligned within a row compared with nonadjacent whiskers within an arc. FO responses after cortical incisions suggest that the basis for these neuronal interactions are mediated primarily by intracortical connections. Response facilitation is largely abol-

FIG. 4. A cut placed parallel to sites representing C4 and C1 whiskers had no effect on supralinear FO responses. A: similar to Fig. 3A but from a different animal. Simultaneous stimulation of C4, C1-evoked responses that were larger in amplitude compared a linear model of the sum of individual responses. A difference map shows the area of nonlinear response facilitation. B: using the evoked response profiles to position an incision, a cut made parallel and below sites representing C4 and C1 whiskers (- - -) had no effect on nonlinear FO interactions.

FIG. 5. Summary of results. A: supralinear responses were significantly larger during stimulation of C4, C1 whiskers and C3, D3 whiskers compared with responses during stimulation of B3, D3 whiskers (*P < 0.05; n = 4). B: supralinear responses during paired whisker stimulation were significantly larger before (Bef) vs. after (Aft) a cortical cut between sites representing the stimulated whiskers (*P = 0.006; n = 6). C: no difference was found in nonlinear summation of FOs before compared with after a cut placed parallel to sites representing the stimulated whiskers (n = 4).
ished after a cortical incision presumably due to a disruption of horizontal connections between sites representing the stimulated whiskers. Cortical cuts parallel to these same sites had no effect on FO interactions. If nonlinear interactions within the ventral posterior medial nucleus of the thalamus (Ghazanfar and Nicolelis 1997), a major station along the trigeminal pathway projecting to barrel cortex, do contribute to nonlinear interactions of FOs within barrel cortex, these thalamic contributions appear to be small because FO responses after the cut were not significantly different from baseline values. Indeed, this conclusion is consistent with much of the evidence that suggests the generation of FOs resides within cortical networks (Grenier et al. 2001; Kandel and Buzsaki 1997; Staba et al. 2003).

Recent evidence suggests that both excitatory and inhibitory cells within supra- and infragranular layers contribute to the generation of FO field potentials (Jones et al. 2000; Staba et al. 2004). Thus both excitatory and inhibitory pathways are candidates for FO propagation within the barrel field. Although horizontal projections from pyramidal cells may be farther reaching than projections from GABAergic cells (Aroniadou-Anderjaska and Keller 1996; Gottlieb and Keller 1997), functional evidence indicates that GABAergic projections also extend beyond single barrels and contribute to inter-barrel inhibition (Salin and Prince 1996). The present data indicate that this propagation supports FO interactions with sub-millisecond accuracy over distances of several millimeters. While similar phase-locking of 80- to 200-Hz oscillations with delays <2 ms have been observed in cat neocortex and attributed to excitatory chemical synaptic connections between pyramidal cells (Grenier et al. 2001), the speed and temporal precision of gap junctional connections recently demonstrated between inhibitory interneurons in neocortex (Galarreta and Hestrin 1999) present an alternative substrate for fast horizontal interactions within the barrel field that is worthy of further investigation.

GRANTS

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REFERENCES


