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Spatial structure of multiwhisker receptive fields in the barrel cortex is stimulus dependent

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Le Cam J, Estebanez L, Jacob V, Shulz DE. Spatial structure of multiwhisker receptive fields in the barrel cortex is stimulus dependent. J Neurophysiol 106: 986–998, 2011. First published June 8, 2011; doi:10.1152/jn.00044.2011.—The tactile sensations mediated by the whisker-trigeminal system allow rodents to efficiently detect and discriminate objects. These capabilities rely strongly on the temporal and spatial structure of whisker deflections. Subthreshold but also spiking receptive fields in the barrel cortex encompass a large number of vibrissae, and it seems likely that the functional properties of these multiwhisker receptive fields reflect the multiple-whisker interactions encountered by the animal during exploration of its environment. The aim of this study was to examine the dependence of the spatial structure of cortical receptive fields on stimulus parameters. Using a newly developed 24-whisker stimulation matrix, we applied a forward correlation analysis of spiking activity to randomized whisker deflections (sparse noise) to characterize the receptive fields that result from caudal and rostral directions of whisker deflection. We observed that the functionally determined principal whisker, the whisker eliciting the strongest response with the shortest latency, differed according to the direction of whisker deflection. Thus, for a given neuron, maximal responses to opposite directions of whisker deflections could be spatially separated. This spatial separation resulted in a displacement of the center of mass between the rostral and caudal subfields and was accompanied by differences between response latencies in rostral and caudal directions of whisker deflection. Such direction-dependent receptive field organization was observed in every cortical layer. We conclude that the spatial structure of receptive fields in the barrel cortex is not an intrinsic property of the neuron but depends on the properties of sensory input.

somatosensory cortex; vibrissa; touch; direction selectivity; rat

Layer IV of the primary somatosensory cortex (S1) of rodents contains discrete cytoarchitectonic modules called “barrels” (Killackey 1973; Woolsey and van der Loos 1970). Each barrel is in anatomic correspondence with one specific mystacial vibrissa on the snout of the animal (Simons 1985). Functionally, neurons localized in a particular cortical barrel respond preferentially, with shortest response latency, to one whisker, called the principal whisker (PW). However, whole cell and intracellular recordings of synaptic responses to individual whisker deflections showed that the convergence of information onto single neurons of layers II to V of the barrel cortex was extensive, spanning several adjacent whiskers (AWs) from the center of the receptive field (Brecht and Sakmann, 2002b; Brecht et al. 2003; Manns et al. 2004; Moore and Nelson 1998; Zhu and Connors 1999). These large receptive fields represent a potential substrate for response modulation by the context of the peripheral stimulation (Jacob et al. 2008). Here we propose that several basic functional properties of the multiple-whisker receptive fields are affected by simple changes in stimulus properties, specifically along the caudo-rostral axis.

The caudo-rostral axis is important for the whisker-to-barrel system, behaviorally, anatomically, and functionally. Whiskers on the mystacial pad are arranged in a precise geometric pattern of caudo-rostral rows and dorso-ventral arcs. During exploratory behaviors, rats move their vibrissae rostrally, creating a functional asymmetry between rows and arcs. Anatomically, studies have revealed a bias toward within-row connectivity in the intracortical circuitry (Bernardo et al. 1990a, 1990b; Hoeflinger et al. 1995; Kim and Ebner 1999). Additionally, cortical activity patterns induced by single-whisker deflections are elongated along rows (Armstrong-James and Fox 1987; Kleinfeld and Delaney 1996; Simons 1978), and suppressive two-whisker interactions are more prominent when the stimulated whiskers belong to the same row than to the same arc (Ego-Stengel et al. 2005). Consequently, we tested here whether properties of multiwhisker receptive fields change when activating the system with deflections in different directions of movement along the caudo-rostral axis.

In the visual cortex, changes in nonspatial properties of the stimulus, like contrast polarity (ON or OFF light transitions), allow characterization of contiguous but spatially segregated subfields in a subset of neurons called simple cells (Hubel and Wiesel 1962). In the barrel cortex, neurons respond with different magnitudes and latencies to different directions of deflections of the PW (Bruno and Simons 2002; Puccini et al. 2006; Simons 1978; Simons and Carvell 1989; Wilent and Contreras 2005). Neurons with multiwhisker receptive fields do not necessarily respond to the same angle of deflection of the different whiskers. If a neuron shows different directional selectivity to the PW and the AWs, the structure of the receptive field, its center of mass, and its preferred whisker will change with the stimulus direction. Evidence for this dependence is limited and contradictory in the literature. Kida et al. (2005) have shown similar direction preference for the PW and AWs, whereas Hemelt et al. (2010) observed insignificant angular tuning consistencies across vibrissae. Here we tested in the barrel cortex whether a change in the direction of whisker deflection (rostral vs. caudal) can unmask changes in receptive field mapping. Using a new stimulator composed of 24 independent piezoelectric actuators (Jacob et al. 2010) adapted to the five rows and the five most caudal arcs of the rat whisker pad, we have characterized cortical receptive fields using...
whisker deflections caudally and rostrally from resting position. When comparing one direction of deflection to the other we observed, in most cortical regular spiking neurons, modulations of the spatial structure of the receptive field. These changes included a shift in the center of gravity of the receptive field, differences in the latency of responses to PW and AWs, and even changes in receptive field size.

MATERIALS AND METHODS

Experiments were performed in conformity with French (JO 87-848) and European (86/609/CEE) legislation on animal experimentation. All authors have been granted a license from the French Ministry of Agriculture to conduct the animal research described here.

Animal Preparation

Male Wistar rats ($n = 29$, weight $= 306 \pm 23$ g, mean $\pm$ SD) were anesthetized with urethane ($1.5$ g/kg ip). Atropine methyl nitrate ($0.3$ mg/kg ip) was injected to reduce secretions in the respiratory path. Supplementary doses of urethane ($0.15$ g/kg ip) were administered when necessary throughout the experiment in order to maintain an adequate level of anesthesia, as indicated by the absence of eye blink reflex, the lack of response to hind paw pinch, and the absence of spontaneous vibrissa movements. ECG and EEG monitoring was performed throughout the experiment. Body temperature was maintained at $37^\circ$C by a regulated heating pad. The animal was placed in a stereotaxic frame, and the skull was cemented to a metal bar fixed rigidly to the frame. The snout was held by a modified head holder (Haidarliu 1996) allowing free access to the right vibrissae. The left posteromedial barrel subfield (P0 – 4, L4 – 8 from bregma; Chapin and Lin 1984) was exposed. Once the electrode had been inserted into the cortex, the craniotomy was covered with a silicon elastomer (Kwik-Cast, WPI).

Electrophysiological Recordings

Neural activity was recorded extracellularly from 202 neurons with a custom program [Elphy, G. Sadoc, Centre National de la Recherche Scientifique Unité de Neurosciences, Information et Complexité (CNRS-UNIC), www.unic.cnrs-gif.fr/software.html] and tungsten electrodes (FHC, 2–10 MΩ at 1 kHz) lowered perpendicularly into cortical columns. Signals were amplified (gain 5,000) and filtered for spike activity (0.3–3 kHz). For each recording site, up to two single units were isolated with a template-matching spike sorter (MSD, Alpha-Omega) and a multiunit signal was recorded simultaneously. Consecutive recordings were performed at least 100 μm away from each other, to avoid recording the same unit twice.

Whisker Stimulation

A recently developed whisker stimulation matrix based on piezoelectric benders (Jacob et al. 2010) was used to deflect independently the 24 most caudal whiskers of the right whisker pad (Fig. 1A). Whiskers were trimmed to 10-mm length and were inserted 3 mm into small plastic tubes of calibrated diameter glued on each bender. Benders were driven with RC-filtered (tau $= 2$ ms) voltage pulses (10 ms forward, 10 ms plateau, 10 ms backward motion, followed by a 20-ms rest period), producing resonance-free deflections of $14 \mu m$ at $7$ mm from the follicle ($93^\circ$/s initial velocity) delivered at $20$ Hz.
hundred and twenty sequences of randomized caudal and rostral deflections of the 24 whiskers were applied, each including a 50-ms blank with no whisker deflection, providing an estimate of the baseline firing rate of the neuron. Simultaneous recording of the spiking activity allowed the reconstruction of the linear receptive field of the recorded neuron with forward correlation techniques. Briefly, a peri-stimulus time histogram (PSTH, −50, 150 ms around stimulation time) was reconstructed for both caudal and rostral deflections of each of the 24 stimulated whiskers.

**Data Analysis**

All off-line data analyses were performed with the Python language (www.python.org) and its associated scipy and matplotlib scientific toolkits. 3D Weighted Linear regression and circular statistics were implemented with R and rpy (http://www.rpy.sourceforge.net).

Regular spiking unit/fast spiking unit classification. On the basis of bimodal distribution of spikes durations, neurons were classified as fast spiking units (FSUs) or regular spiking units (RSUs). In accordance with previous studies (Bruno and Simons 2002), 75% of the cells (n = 163) were classified as RSUs and 25% (n = 39) were classified as FSUs. The analysis presented here was focused only on RSUs.

**Determination of receptive field size.** As often reported in the barrel cortex (Armstrong-James et al. 1994), we frequently observed in our recordings a low baseline firing rate; 43% of the cells fired <1 spike/s. Classical methods for the detection of significant responses in PSTHs rely on the assumption that PSTHs are continuous Gaussian processes that can be modeled through their mean and variance. However, this hypothesis may lead to erroneous results when the firing rate of the cells is, as here, too low. Here, to correctly detect significant responses in low-firing rate conditions, we devised a method based on surprise analysis (Legény and Salcman 1985). This method takes into account the discrete nature of spiking activity by modeling it as a Poisson process. This is particularly important at low firing rates, a situation frequently found in the barrel cortex. A baseline firing rate was calculated by measuring the average firing rate count in the 150 ms starting at the beginning of “nonstimulation” intervals. The surprise (S)—a measure of the unlikeliness of the occurrence of a given firing rate, given the baseline firing rate—was then measured on the PSTH obtained for stimulations of each whisker and each direction. A high value of surprise corresponds to an unlikely activity of the neuron that is a probable functional response of the neuron. In addition, we took into account firing rate changes both below and above the baseline firing rate by combining the Poisson cumulative density function (CDF) and the Poisson survival function (SF), two functions parameterized by the baseline firing rate (fb): S(f) = −log10[minCDF(f, fb)SF(fb, f)].

Finally, in order to take into account different response dynamics (phasic and tonic) without an a priori on the timescale of the actual functional coding taking place, the surprise was estimated on integration windows spanning from 1 to 20 ms, with 1-ms steps.

To define a threshold above which neuronal responses are considered statistically significant, we built for each time bin a surprise threshold value corresponding to a 1% false positive ratio. We built the distribution of surprise values both with no stimulation and on 150 ms after stimulus onset. The threshold was computed such that false positive responses during the blank period would be below 1% of the count during actual whisker deflections (Fig. 2A). To obtain a comparable measurement of response strength across bin sizes, the surprise threshold for a given binning was finally subtracted from the corresponding surprise measurement. For a given neuron, we defined a whisker as triggering a significant response when the computed surprise was at any point of the time positive for at least one of the bin sizes. Detected significant responses were in good agreement with visual inspection of the PSTHs, for both cells with low and cells with high firing baseline levels (see Fig. 2B). We illustrated in two case studies (Fig. 2C) the differences between the surprise method (significant PSTHs are depicted in black) and three classical definitions of a significant sensory response: 1) the mean response of the neuron on a 30-ms window following stimulus onset goes above a threshold defined as the mean baseline firing rate + 3 SD (significant PSTHs are underlined with a dashed line), 2) activity in any 1-ms bin goes above mean + 3 SD threshold in a 30-ms window following stimulus onset (significant PSTHs are underlined with a dotted line); 3) the mean firing rate on a 50-ms window crosses a threshold defined as the mean + 3 SE (significant PSTHs are underlined with a continuous line) (see Jacob et al. 2008).

There are two main differences in the detection of significant responses by these methods versus the surprise method. First, all these classical methods are to some extent tied to the hypothesis that the PSTH can be modeled as a Gaussian process, thus allowing the use of SD or SE to evaluate the firing rate of the neuron. In many cases,
because of the low firing rate observed in barrel cortex neurons, this assumption does not hold, leading to the erroneous detection of significant responses (see, for example, the significant responses detected by method 2 in Fig. 2C).

Second, these different methods rely on a single binning (e.g., 1 ms, 30 ms, or 50 ms), thus missing responses that do not fit such specific timescales. For instance, very fast responses may be missed by a large bin size (note the response missed by method 1 in Fig. 2C). In contrast, low-amplitude long responses may be missed by small bin sizes. Such a variety of responses is captured by the multiple bin sizes used in the surprise method.

Response latencies. As in other studies (Armstrong-James et al. 1994; Petersen and Diamond 2000), the response latency was computed across whiskers previously identified as eliciting a significant response by using a dedicated method applied to the few milliseconds between the onset of the stimulation and the beginning of the significant response. To detect the onset of the response, we used the same surprise method as before, but instead of a stringent 1% threshold (which would have resulted in an overestimation of the latencies), we chose a 50% threshold. The latency time for a given bin size was defined as the end time of the first significant bin. The shortest of all these latency measurements was regarded as the whisker latency. Finally, to avoid detecting the onset of small baseline perturbations as the response latency, we made sure that every latency computed with the 50% threshold preceded with 5-ms precision the latency obtained with a 25% threshold (a more stringent measurement of the latency, which brought larger latency estimates). If not, the 25% threshold latency was preferred. Although these thresholds may appear high, they correspond to a false positive rate across the full width of a 150-ms window after stimulus. However, for the calculation of the response latency, the algorithm is used on a much shorter window, going from stimulus onset to response onset (generally <20 ms). Within this window, the 25% threshold translates into a 3.3% (20/150 × 25) false positive error rate.

We compared (Supplemental Fig. S1A) our surprise-based latency measurement with the latencies measured with the classical mean + 2 SD method (Foeller et al. 2005; Jacob et al. 2008 for a slightly different method) across all significant neuronal responses obtained in this study. The overall distributions of latencies were similar, although the SD-based latency method failed at low firing rates, resulting in many cases in exceedingly early latency measurements (see case study in Supplemental Fig. S1B).

Histology

At the end of the experiments, three small electrolytic lesions (30–50 pulses of 200-ms duration and 10–μA amplitude delivered at 0.3 Hz) were made at known depths, 500 μm apart. The animal was given a lethal dose of pentobarbital (Dolethal) and perfused transcardially with phosphate buffer (0.1 M, pH 7.4) followed by a fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). Coronal sections (80-μm width) were cut through the left postero medial barrel subfield and stained with cresyl violet (n = 10) or with cytochrome oxidase (n = 19) to visualize cortical layers and barrels. The denser staining of L4 combined with the relatively chromogen-free appearance of layers II/III and Vα provided a very clear demarcation of the borders between layers II/III and IV and layers IV and Vα. From histological examination these borders occurred at a mean depth of 480 μm and 940 μm, respectively. The border between layers Vα and Vβ was marked by a gradual increase in cytochrome oxidase stain, cell density, and the appearance of larger cell bodies. The transition from layers Vα to Vβ occurred on average at 1,300 μm.

Electrode tracking and lateral position of recorded cells. Coronal sections were done to unambiguously determine the recording layer.

As a consequence, the barrel identity from which the neurons were recorded could not be directly visualized. To recover this information, a functional positioning method was devised. This method relies on the fact that multiunit receptive fields were obtained simultaneously with each cell recording along the electrode track. Each of these multiunit receptive fields was used as the weights in a two-dimensional (2D) grid representing the barrel positions (colored spheres in Fig. 3, B and F). Such 2D barrel weight grids were positioned vertically in a three-dimensional (3D) space at the recording depth (Fig. 3, C and F) read on the motorized microelectrode driver (Luigs & Neumann). The weights used for this computation were obtained for a given whisker by taking the maximum of the corresponding surprise value over time (from 0 to 150 ms after stimulation onset) and binning range (1–20 ms) (summing caudal and rostral responses). The weights were then normalized between recordings (the sum of the weights equals 1 for each layer). To estimate the path of the electrode (straight purple line in Fig. 3, B, C, E, and F), a weighted least square regression was computed on the 3D distribution of weights. The position of each cell recording within the barrel cortex was finally inferred from this estimated “multiunit” electrode position.

This method was validated by a specific histological control performed on four animals (2 examples are shown in Fig. 3, A–C and D–F). We recorded multiunit activity at 10 different depths every 100 μm along the electrode track. Animals were then perfused, and the somatosensory cortex was flattened between two microslides. Tangential sections (100 μm) were stained with cytochrome oxidase (histological background in Fig. 3, A and D) to visualize the layer IV barrels (darker regions) and septa (Land and Simons 1985). Barrel cortices were reconstructed (black outlines in Fig. 3, A and D) with Neurilucida (MBF Bioscience), and the functional electrode track defined by the method described above was compared with the histologically reconstructed track (green outlines in Fig. 3, B and C and E and F). On all four animals and on all recordings, the computed and histologically defined barrel columns were the same.

This functional reconstruction permitted us to determine the anatomic position of our recordings. To avoid any misclassification of layer IV cells between barrel and septum, we considered only those recordings for which the electrolytic lesion was fully within the limits of the cytochrome-rich barrels. All cases in which the electrolytic lesion was in the border between barrels and septa were discarded from further analysis. We targeted the C2 and Straddler barrels, but in some cases, however, recordings were done in another barrel (17%).

Receptive Field Properties

Center of mass. The maximum-surprise map was computed. This grid of values obtained for a given direction of stimulation was used as the set of weights for the computation of the weighted center of the receptive field. This measurement was performed in the rows/arcs coordinates, with the distance between two adjacent barrels/whiskers in a same row or arc defined as the distance unit.

Eccentricity. An eccentricity vector was obtained by linking the center of mass of the receptive field to the whisker defined by the functionally reconstructed position of the recording electrode.

RESULTS

Using a 24-whisker stimulator, we applied deflections in caudal and rostral directions during extracellular recordings of well-isolated (Supplemental Fig. S2) RSUs (n = 163) and FSUs (n = 31). RSUs were subdivided according to their position in the different cortical layers. To characterize the receptive fields of these cells, we used forward correlation techniques and delineated the receptive field corresponding to both directions of stimulation (see MATERIALS AND METHODS and Fig. 1A). Among the 163 RSUs, 16% did not respond at all to
the sparse noise whisker stimulation (layer II/III: 32%, layer IV: 3%, layer Vα: 5%, and layer Vβ: 15%). Of the remaining 137 single units that showed significant responses to whisker deflections, 67% responded significantly to the deflection of 2–16 adjacent whiskers (see an example in Fig. 1B). The whiskers in multiple-whisker receptive fields were always contiguously located in the mystacial pad. The distribution of the number of whiskers for which a significant response was elicited per neuron was not different for caudal and rostral receptive fields (Fig. 1C, Kolmogorov-Smirnoff test, \( P = 0.89 \)). FSUs displayed receptive field size distributions almost identical to the RSU distribution (Kolmogorov-Smirnoff test, \( P = 0.99 \)). A quantitative examination of the receptive fields obtained with the two deflection directions showed that several of these functional properties were affected by the direction of whisker deflection, including the receptive field size, which is the number of whiskers eliciting a significant response, and the response latencies. Moreover, the well-established link between the functional representation of PW and the anatomic identity of the barrel cortex column from which the recordings were made was also influenced by the direction of whisker deflection.

Relation Between Receptive Field Center and Anatomic Identity of Recorded Barrel is Influenced by Direction of Whisker Deflection

S1 cortex is organized with such a strict anatomic topography (McCasland and Woolsey, 1988; Simons 1978; Woolsey and van der Loos 1970) that the center of a receptive field is thought to provide a reasonable estimate of the location of an electrode within S1 cortex. This observation is often considered as sufficient to conclude that a given cell is located within the barrel column that matches the functionally defined central or principal whisker. However, by comparing the responses to whisker deflection in one direction and the other we have observed that the center of the receptive field defined functionally is not such a good estimate of the anatomic location of the recording site, since it changes with the direction of whisker deflection. To study the link between the center of the receptive field and the anatomic identity of the recording site according to the direction of deflection of the whiskers, we localized the barrel column from which the cells had been recorded in each experiment (see MATERIALS AND METHODS). Cells did not systematically show a significant response to the stimulation of the
whisker corresponding to the recording barrel column (i.e., the anatomic whisker). Two examples are presented in Fig. 4A. In Fig. 4A1, a neuron recorded in layer IV of barrel C2 showed the strongest response for whisker C3 in the rostral direction and for whisker C2 in the opposite direction. Similarly, in the example shown in Fig. 4A2 of a neuron recorded in layer Va of the alpha cortical column, the whisker eliciting the strongest response was indeed alpha in the caudal receptive field but beta in the rostral receptive field. These examples illustrate that the PW defined functionally was shifted according to the angle of deflection in the rostral receptive field. These examples illustrate that the response was indeed alpha in the caudal receptive field but beta in the alpha cortical column, the whisker eliciting the strongest response for whisker C3 in the rostral direction.

To study this difference at the population level, we plotted the distributions of the number of whiskers eliciting a statistically significant response. Receptive field sizes were quantified for RSUs across all cortical layers as well as for FSUs. As reported previously (Armstrong-James and Fox 1987; Ghazanfar and Nicolelis 1999 Simons 1978), many SI cortical neurons exhibited a multiwhisker receptive field in our recording conditions. Here we quantified and compared statistically the receptive field size in response to caudal versus rostral deflections. Figure 5A shows three typical examples of layer IV and layer Va cells where the number of whiskers eliciting significant responses varied as a function of the direction of whisker movement. For example, cell 1 exhibited a caudal receptive field of four whiskers, whereas the rostral receptive field of the same neuron showed only two whiskers eliciting significant responses. In some instances, the receptive fields were only partially in correspondence (see cell 3 in Fig. 5A) and, as described before, the PW—the whisker that evoked the strongest response—was not necessarily the same for the two receptive fields of the same neuron. Cell 2 in Fig. 5A is an example of such a case (see also Fig. 1B).

Receptive Field Size is Modulated by Direction of Whisker Deflection

Receptive field size was measured by counting the number of whiskers eliciting a statistically significant response. Receptive field sizes were quantified for RSUs across all cortical layers as well as for FSUs. As reported previously (Armstrong-James and Fox 1987; Ghazanfar and Nicolelis 1999 Simons 1978), many SI cortical neurons exhibited a multiwhisker receptive field in our recording conditions. Here we quantified and compared statistically the receptive field size in response to caudal versus rostral deflections. Figure 5A shows three typical examples of layer IV and layer Va cells where the number of whiskers eliciting significant responses varied as a function of the direction of whisker movement. For example, cell 1 exhibited a caudal receptive field of four whiskers, whereas the rostral receptive field of the same neuron showed only two whiskers eliciting significant responses. In some instances, the receptive fields were only partially in correspondence (see cell 3 in Fig. 5A) and, as described before, the PW—the whisker that evoked the strongest response—was not necessarily the same for the two receptive fields of the same neuron. Cell 2 in Fig. 5A is an example of such a case (see also Fig. 1B).

To study this difference at the population level, we plotted the distributions of the number of whiskers eliciting a significant response for caudal and rostral deflections for every cortical layer and a joint 2D histogram (Fig. 5B). Deviations from the diagonal in this histogram indicate differences in the number of whiskers constituting the caudal and rostral receptive fields. A majority of responsive RSU neurons (93 of 137) displayed a difference in size between caudal and rostral receptive fields. This was the case for 63% (n = 24/38) of layer II/III RSU cells, 63% (n = 22/35) of layer IV RSU cells, 61% (n = 23/36) of layer Va RSUs, and 85% (n = 24/28) of layer Vb RSUs. Similarly, FSUs displayed in 61% (19/31) of the cases a different number of significant whiskers for rostral and caudal stimulations. The distributions of the size of caudal and rostral receptive fields were not significantly different, either for RSU or for FSU neurons (see Fig. 1C; RSU: Kolmogorov-
Smirnoff, $P = 0.89$; FSU: Kolmogorov-Smirnoff test, $P = 0.99$).

**Receptive Field Mismatch as Function of Direction of Whisker Deflection**

Classically, neurons encountered in a given electrode penetration are maximally activated by the deflection of the same vibrissa, the PW. Other AWs can also activate neurons, but the strength of the response is generally weaker (Simons 1985). This is indeed what we have observed here using one direction of whisker deflection. However, we also observed striking differences between receptive fields of a given neuron determined with caudal (C) and rostral (R) directions of whisker deflection. Same conventions as Fig. 1B. B: cross-distribution of the number of whiskers eliciting a significant response in caudal vs. rostral receptive fields for RSU neurons in each layer and for all FSU neurons grouped. The proportion of cells is indicated by the color scale.

We determined the position of the centers of mass of caudal and rostral receptive fields and calculated the distance between them (see Fig. 6A and MATERIALS AND METHODS). A value equal to 1 means that the centers of mass in the two receptive fields were separated by one whisker. The displacement was calculated only for cells showing at least one whisker eliciting significant responses in each receptive field (RSU, $n = 106$; FSU, $n = 29$). Figure 6A shows an example RSU neuron with a displacement of the center of mass of 1.13 whiskers. This is because the caudal receptive field includes only whisker C1, whereas the rostral receptive field includes whiskers C2 and C3, introducing an elongation of the receptive field away from C1. Several more examples of caudal and rostral receptive fields for neurons recorded in the different cortical layers are presented in Fig. 6B. This figure illustrates on one hand the diversity of multiwhisker cortical receptive fields and on the other hand several examples of shifts of the PW between caudal and rostral.
receptive fields (see cells 1, 2, 5, 9, 11, 13, and 14 in Fig. 6B). For the other examples presented in Fig. 6B the PW is the same for both directions of deflection, although neurons exhibit significant changes in the receptive field structure and span (see, for example, cells 7, 8, 15, and 16). The whiskers in multiple-whisker receptive fields were always contiguously located in the mystacial pad.

The distribution of the displacements of the center of mass across the population of recorded neurons is shown in Fig. 7 for the different cortical layers. We observed that 26% (n = 27) of layer II/III, 7% (n = 27) of layer IV, 32% (n = 27) of layer Va, and 18% (n = 25) of layer Vb cells exhibited a displacement of the center of mass of more than one whisker. The mean displacement for RSU neurons was 0.61 ± 0.74 in layer II/III, 0.37 ± 0.39 in layer IV, 0.68 ± 0.63 in layer Va, and 0.61 ± 0.49 in layer Vb. As expected, the displacement was significantly smaller in layer IV compared with layer V (Va and Vb merged vs. IV, Mann-Whitney test, P < 0.05). Finally, FSU displacement was also low (0.48 ± 0.55), although not significantly smaller than the displacement of RSU neurons. These results showed that the spatial extent of caudal and rostral receptive fields could be spatially separated. The spatial separation between responses to opposite directions of whisker deflections is reminiscent of the structure of simple cell receptive fields in the primary visual cortex (DeAngelis et al. 1995; see DISCUSSION). These results indicate that the receptive field can shift spatially as a function of the direction of whisker deflection. In addition, the displacement occurring in layer V, but not in other layers, was significantly (Rayleigh test P < 0.03) biased toward the horizontal direction, with a circular mean displacement angle of 3.8°. A polar distribution of the motion direction of the center of mass between caudal...
and rostral stimulations, with the caudal receptive field as the reference (Fig. 6C), illustrates the orientation bias: rostral receptive fields were often more rostrally positioned within the barrel cortex than the corresponding caudal receptive fields. This observation was true both for RSU and for FSU.

The direction-dependent changes in the receptive field structure observed here could have been explained by an “iceberg” effect where a given direction could activate a global inhibitory input that would reduce responses globally to a point where no significant response would be detected. If this were the case, the receptive fields defined with one direction of whisker deflection should always be a shrunken version of the receptive field defined with the other direction. However, we observed many instances where the receptive fields of a given neuron were very dissimilar in shape, such as cells 13 and 14 in Fig. 6B, thus ruling out this possibility as the sole explanation for the changes.

**Latency of Responses to PW and AWs Changes in Caudal and Rostral Receptive Fields**

Cortical neurons show responses that are selective to the angular direction of whisker deflection (Simons and Carvell 1989; Wilent and Contreras 2005). The angle of whisker movement might be represented also in the cortex by the temporal properties of responses, such as the minimal response latency. Here we determined and compared the response latency for two directions of whisker deflection for the PW and AWs. In this analysis, we took into account only cells with the anatomically matching whisker eliciting a significant response (RSU: n = 53; FSU: n = 16). For cells with multiple AWs, only the AW eliciting the strongest response was considered (RSU: n = 40; FSU: n = 14). Latencies were calculated in a time window corresponding to the response of the cells to the first ramp of the stimulus (from 0 to 35 ms). Overall, the latency of PW responses was significantly shorter for FSU than for RSU (Kolmogorov-Smirnoff, P = 0.007) when grouping across layers, suggesting that FSUs receive more direct thalamic input than RSUs (Fig. 8A). Looking at the effect of the direction of stimulation, we noted that it could impact latency. Two examples of responses to opposite directions of deflections of the PW are presented in Fig. 8B. In both cases the shortest response latencies were clearly different, with a gap of several milliseconds. For both PW and AW, large latency differences, up to 12 ms, were often observed (Fig. 8, D and E). However, differences in latency were highly variable both for PW and AW, and no systematic bias toward one direction was observed.

Finally, the relationship between response latencies for PW and AW pairs for caudal and rostral directions of deflection is depicted for RSUs in layer IV in Fig. 8C. PW latencies were significantly shorter than AW latencies for RSUs in layer IV (Mann-Whitney P = 0.001) and in layer Va (Mann-Whitney P = 0.002), as well as for FSUs grouped across layers (Mann-Whitney P = 0.02). In contrast, the difference in latency of response between PW and AWs was significant neither in layer II/III nor in layer Vb for RSU neurons. The noticeable difference in latency values for the PW against the AW in both directions correlates more consistently with the identity of the whisker than with the directions of deflections, even if there is a significant temporal shift in responses for caudal and rostral angles that might be used by the system to represent the direction of whisker movement (but see Kida et al. 2005).

**DISCUSSION**

Receptive fields of barrel cortex neurons were spatially characterized for two opposite directions of whisker deflection in the caudo-rostral axis by using randomized sequences of 24
whisker deflections (sparse noise) and forward correlation analysis. In agreement with previous studies (Armstrong-James and Fox 1987; de Kock et al. 2007; Ghazanfar and Nicolelis 1999; Simons 1978), the majority of cortical neurons (67%) across the different cortical layers, including layer IV, exhibited multiwhisker suprathreshold receptive fields. Moreover, the neuronal responses of RSUs to two opposite directions of whisker deflection show no consistency across the vibrissae that compose the receptive fields (see also Hemelt et al. 2010 for a similar observation in layer IV neurons). Thus the size and position of the group of whiskers on the snout eliciting significant spiking responses was modulated by the direction of whisker movement. In many instances (23% of RSUs), this modulation resulted in a clear spatial separation of caudal and rostral receptive fields. In those cases, we observed a mismatch between the anatomically and the functionally defined PW for one of the directions of whisker movement. In contrast, FSUs showed very similar receptive field structures for the two opposite directions of whisker deflection. The main finding of this work is that several properties of the receptive field of RSUs, such as the size, the response latency, and the center of mass, are stimulus dependent, meaning that they varied as a function of the direction of whisker deflection (see below).

Fig. 8. Latency of responses is affected by the direction of whisker movement. A: population histogram of the latency response of the PW for RSU neurons (top) and FSU neurons. B1: example of a layer II/III RSU cell showing a difference between caudal (C, 10 ms) and rostral (R, 14 ms) latencies for the deflection of the PW. Dashed line indicates the onset of the stimulation. Arrowhead indicates the minimal response latency. B2: example of a layer Vb RSU neuron showing a difference between caudal (21 ms) and rostral (14 ms) latencies for the PW. C: scatterplot of the PW response latency as a function of the AW response latency for the caudal and the rostral movement. D and E: scatterplots of the rostral response latency as a function of the caudal response latency for the PW (D) and for the AW (E).
White Noise Analysis and Receptive Field Mapping

In the visual system, a variety of methods have been used to map visual receptive fields quantitatively, from the plot of receptive fields by hand used by Hubel and Wiesel as early as 1962 (Hubel and Wiesel 1962) to line-weighting functions (Field and Tolhurst 1986) and response plane techniques (Palmer and Davis 1981). More recently, sophisticated receptive field mapping approaches have been developed based on the use of a modified version of white noise analysis (i.e., randomized spatiotemporal stimuli). In this approach, a rapid succession of impulse stimuli (spots of light for the visual system or short whisker deflections here) are presented randomly and the spiking activity that results from the stimulation is correlated to the stimulus sequence. This analysis leads to the definition of the transfer function of the recorded neuron and has the advantage of being very efficient in terms of the experimental time needed to explore the receptive field, since the stimuli are presented discontinuously at frequencies usually higher than 20 Hz (see review in DeAngelis et al. 1995 of an application of this analysis to the visual system). In principle, this analysis can provide a full characterization of input-output functions of linear and nonlinear systems (Marmarelis and Marmarelis 1978).

To our best knowledge this is the first attempt to apply such a system-analysis approach to the whisker/barrel cortex system. Applying this approach requires the use of a stimulation device that allows deflecting consecutively most whiskers in a random order. We dispensed such sparse noise stimulation in two opposite directions of whisker movements by using a 24-whisker stimulator (Jacob et al. 2010) centered on whisker C2. We established spatiotemporal receptive fields of well-isolated single neurons across different cortical layers from layer II/III to layer V. Since we integrated the spiking activity over a time window of several tens of milliseconds after the stimulation, we only considered here the 2D spatial projection of the spatiotemporal receptive field. Of particular concern was the possibility that the high-frequency stimulation of adjacent whiskers used here would produce a generalized lateral inhibition that would suppress the activity of the recorded neuron. However, we could determine significant receptive fields for the great majority of the recorded neurons, strongly suggesting that this approach is efficient in obtaining a complete description of the input-output relationship of cortical neurons.

Receptive Field Center is Modulated by Direction of Whisker Deflection

The classical anatomic description of the barrel cortex corresponds to a one-to-one mapping of facial whiskers into layer IV barrels (Woolsey and van der Loos 1970). This anatomic observation is reinforced by functional studies showing that the majority of cortical neurons in layer IV exhibit monowhisker receptive fields (Armstrong-James and Fox, 1987; Simons 1978).

The inverse relationship (the fact that neurons in a barrel column are more sensitive to the corresponding PW) has been often used as an indication of the anatomic identity of the recording site (see, e.g., Kida et al. 2005). At odds with this assertion, we have found that the PW, corresponding to the anatomic barrel column where the cell was recorded from, did not necessarily elicit a significant response. Our results are in agreement with Wright and Fox (2010), who showed that the whisker eliciting the strongest response may often differ from the anatomically defined PW, mainly in layer Vα. Similar observations were made by Armstrong-James and Fox (1987), showing by histological analysis that the principal vibrissa was not synonymous with the appropriate vibrissae in 14% of occasions. It must be taken into account that all of the recordings included in our study correspond to barrel column locations and not to septa, where strong influences of several surrounding whiskers are expected.

Difference in Direction Preference for PW and AWs

Barrel cortex cells exhibit direction selectivity in response to PW and AW stimulation (Bruno and Simons 2002; Puccini et al. 2006; Simons 1978; Simons and Carvell 1989; Wilent and Contreras 2005). Several models exist for the relation between the angular preference of the PW and of the AWs in the receptive field. Preferred angles can be correlated, meaning that all whiskers in a receptive field share the same preference, can be anticorrelated where whiskers show opposite angular preference, or may show no particular interdependence. Experimental support for these hypotheses is scarce but has been recently provided. In agreement with the first possibility, Kida et al. (2005) have shown that the direction preference of responses in the rostro-caudal axis to one or multiple AW stimulations is consistent with that of the PW. More recently, Hemelt and colleagues (2010) have reported, however, that most layer IV neurons that respond to several adjacent vibrissae show a wide range of tuning similarity across the receptive field, in support of the third hypothesis. In agreement with that report, we observed here in all cortical layers that the direction selectivity to stimulation of AWs often differed from the direction selectivity of the PW in the rostro-caudal axis. From our data, however, we cannot exclude the anticorrelated hypothesis because we did not study the full direction tuning curve.

Tuning to the direction of whisker deflection has been reported also in thalamic (Brecht and Sakmann 2002a; Minnery and Simons 2003; Shosaku et al. 1985; Timofeeva et al. 2003; Waite 1973) and subthalamic (Bellavance et al. 2010; Furuta et al. 2006) nuclei. Indexes of directionality for the PW are globally decreasing along the whisker-to-cortex pathway, while the impact of AWs is increasing.

Since the cortical direction selectivity in response to PW stimulation most probably derives from converging thalamic inputs (Minnery and Simons 2003) but that of AWs from combined thalamic inputs and intracortical connections (Armstrong-James and Callahan 1991; Fox et al. 2003; Goldreich et al. 1999; Wright and Fox 2010), the processing of information by the two sources do not seem to operate in a coordinate manner to produce similar direction selectivity. Interestingly, cortical FSUs are thought to receive convergent inputs from thalamic cells with various angular preferences (Swadlow and Gusev 2002). As a consequence, their angular tuning is broad and their receptive field is not modulated by the direction of whisker deflection. For RSUs, we observed a laminar difference with stronger modulation of the receptive field structure of nongranular neurons, suggesting that cortico-cortical connections are involved.
The spatial properties of receptive fields we explored here in the barrel cortex could serve, as in the visual cortex, to detect spatial contrasts between stimulations arising in AWs and, consequently, the detection of object edges. For example, it has been reported that the movement of AWs can diverge (Sachdev et al. 2002). During contact with objects, one whisker can move while the adjacent one remains stationary or the two whiskers can simultaneously move in opposite directions. Moreover, one whisker can be maintained in contact with an object while the other is retracted and protracted. In all these situations, responses of a cortical neuron to the movement of the PW in one direction and to an AW in the opposite direction will combine synergistically to produce a maximal response.

In conclusion, our results show that although the whisker-barrel system is somatotopically arranged in vertical modules (Woolsey and van Loos 1970) receiving inputs from the somatotopically corresponding whisker (Welker 1976), the functionally defined receptive field is stimulus dependent. Thus not only the response level but also the spatial structure of S1 receptive fields can differ in a significant way with different input patterns.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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