Spiking in primary somatosensory cortex during natural whisking in awake head-restrained rats is cell-type specific

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Sensation involves active movement of sensory organs, but it remains unknown how position or movement of sensory organs is encoded in cortex. In the rat whisker system, each whisker is represented by an individual cortical (barrel) column. Here, we quantified in awake, head-fixed rats the impact of natural whisker movements on action potential frequencies of single (identified) neurons located in different layers of somatosensory (barrel) cortex. In all layers, we found only weak correlations between spiking and whisker position or velocity. Conversely, whisking significantly increased spiking rate in a subset of neurons located preferentially in layer 5A. This finding suggests that whisker movement could be encoded by population responses of neurons within all layers and by single slender-tufted pyramids in layer 5A.

barrel cortex | identified cells | action potential | morphology

Active scanning of the environment for sensory signals increases resolution (1), but in turn affects signal representation due to modulatory influences linked to arousal (2, 3) and through representation of movement-related signals in the sensory pathway (4). The whisker system of rodents is convenient to study cortical representation of sensory stimuli, because individual whiskers on the face are represented functionally and anatomically by identifiable individual cortical columns (5, 6) and whisker movements are readily accessible for video tracking. Movement-related information of the whiskers arrives in sensory cortex through activation of the sensory pathway by mechanoreceptors in the whisker follicle (7, 8); although corollary discharge (or efferent copy) through a monosynaptic pathway from motor cortex (9) can also affect spiking in sensory cortex (7). With respect to the sensory pathway of the trigeminal system, it has been suggested that whisker movement and whisker contact are encoded by different parallel pathways (10). Movement is encoded via the paralemniscal pathway [and possibly through a recent described pathway involving ventro-posterior medial nucleus of thalamus (11)]. Contact is encoded via the extralemniscal pathway. The lemniscal pathway is thought to participate in encoding both movement and contact. These experiments, however, were conducted in anesthetized animals, and whisking was evoked by electrical nerve stimulation (10). In addition, recent thalamic recordings in anesthetized and awake animals challenged the view that the paralemniscal pathway is encoding whisker movement (12, 13). Finally, cortical recordings in awake animals during free whisking showed that movement-related information is only weakly encoded in most, but not all neurons in sensory cortex (7, 14). But because recorded neurons were not identified posthoc, the contribution of specific cell-types in encoding movement-related information during active whisking remains unknown. Thus, although cortical neurons excited by the paralemniscal pathway (e.g., Layer 5A) (15, 16) may be involved in encoding of whisker movement, this involvement actually remains to be established.

Results

Spiking Frequencies of Neurons in Awake Barrel Cortex. We recorded juxtassomally in different layers of barrel cortex of unanesthetized, head-fixed Wistar rats to record spiking frequencies of single neurons while tracking whisker position (Fig. 1, Fig. S1). Because cortical layers contain multiple cell types (17, 18), recorded neurons were labeled with biocytin for posthoc identification. Throughout the different layers (e.g., L2/3–L6), we recorded from neurons with irregular spiking patterns with average values in the range of 0.1–6 Hz (e.g., a supragranular pyramidal neuron in Fig. S1). The frequency of spiking was significantly lower than the whisking frequency, which typically showed a peak in the power spectrum in the range of 5–8 Hz (Fig. S1C). In addition, we did not find a correlation between occurrence of spikes and whisker position for this supragranular pyramid (Fig. S1B). However, we also recorded from pyramidal neurons that significantly increased spiking upon whisking (Fig. 1A). These neurons were preferentially located in the upper portion of the infragranular layer (L5A). In total, we recorded from 88 neurons throughout different cortical layers and identified 26 neurons posthoc. Labeled neurons were classified according to their location with respect to the cytochrome oxidase dense region, characteristic of granular L4 and were categorized as supragranular pyramids, granular spiny neurons, slender-tufted, thick-tufted, or L6 pyramids (Fig. 1B; for detailed description of classification criteria, see ref. 18; see Fig. 1C for all recording locations). Unlabeled neurons were also included and categorized into layers according to recording depth (18).

To study whether spiking correlated to behavioral state, we compared spiking in anesthetized animals with spiking in awake, head-fixed animals (Fig. 1D–G). In anesthetized animals, spiking frequency was low (0.1–1 Hz), with the exception of thick-tufted pyramids (Fig. 1D). Average spiking frequencies under urethane were (in Hz $\pm$ SD): supragranular pyramids 0.39 ± 0.56 ($n = 22$), granular spiny neurons 0.58 ± 0.36 ($n = 15$), slender-tufted pyramids 1.08 ± 0.38 ($n = 16$), thick-tufted pyramids 3.27 ± 1.62 ($n = 23$), and L6 pyramids 0.47 ± 0.46 ($n = 15$) (18, 19). During quiet (nonwhisking) episodes in awake, head-fixed animals, spiking frequencies of identified neurons were comparable to those in urethane anesthesia (Fig. 1E, in Hz $\pm$ SD): supragranular pyramids 0.31 ± 0.21 ($n = 5$), granular spiny neurons 1.93 ± 2.02 ($n = 9$), slender-tufted pyramids 1.62 ± 1.81 ($n = 3$), thick-tufted pyramids 4.12 ± 3.22 ($n = 5$), and L6 pyramids 0.52 ± 0.47 ($n = 4$). During episodes of whisker movement, individual neurons showed variable and layer specific changes in spiking frequency (up to 14 Hz, Fig. 1F). Average

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Fig. 4. Occurrence of spikes is weakly correlated to whisking cycle. (A) Neurolucida reconstruction of slender-tufted pyramid and example traces (B) showing correlation between spike times and whisker position. (C) Spike-triggered-average (STA) of whisker position during quiet (black) and whisking (red) episodes for pyramid shown in A. (D–F) Analogous to A–C, but for thick-tufted pyramid recorded at 1382 μm from pia (L5B) (basal dendrites were not recovered). Note that spiking frequency was increased during whisking, but spiking was very weakly correlated to whisker cycle. (G) The fit on the STA (blue line in C and F) was used to determine the phase at $t = 0$ ms and plotted as number of neurons vs. phase of whisker cycle. (H) Scatter plot illustrating the modulation depth (log-normal scale) versus phase (linear scale) for individual units. Bold line indicates 1° amplitude.

Spike Times and Whisker Position Are Not Tightly Coupled. In L2/3 of sensory cortex, it has been shown that membrane potential changes correlate with whisker position (20). To study whether the occurrence of spikes is correlated to whisker position, we made cumulative distributions of whisker position at the time of spikes (Fig. S3*A* and *B*). In all layers, spikes occurred over a large range of whisker positions. The difference between distributions of whisker angle at spike times and whisker position for all times were small for both single experiments from different layers and on the population level when all experiment were grouped (Fig. S3*B*). We performed the same analysis for velocity (Fig. S3*C*), but we did not observe a strong correlation between whisker velocity and spike times. Because individual neurons did not show a strong bias for spiking at specific whisker positions or velocity, this result indicates that in cortex both whisker position and velocity are not consistently encoded by spike times in single neurons.

Finally, we computed spike-triggered-averages (STA) for individual units (Fig. 4 *A–F*) to determine the modulation depth of single units. The first example (slender-tufted pyramid; see also Figs. 1 and 2) illustrates that in addition to an increase in spiking during whisking (Fig. 2*A*), a large fraction of spikes occurred during the whisker protraction phase. We fitted a 4-parameter sine wave to the STA to determine the whisker phase at which spikes preferentially occurred (Fig. 4*C*). For this particular example, spikes occurred preferentially just after maximal protraction although the small average amplitude at $t = 0$ ms (0.7° protraction) still suggests a large variability in spike

occurrence with respect to whisker position. The second example (thick-tufted pyramid) illustrates that an increase in spiking during whisking is not necessarily accompanied by correlated spiking with respect to the whisking cycle (Fig. 4 *D–F*). On the population level, the STA analysis showed that a significant number of neurons was tuned to spike during protraction (51%, Fig. 4*G*, KS, $P < 0.05$) compared with retraction (23%, remaining 26% did not correlate to whisker cycle). However, STA analysis also indicated that even when a neuron was tuned to spike at a particular whisker phase, the STA amplitude was small, suggesting broad tuning curves. A radial plot, to characterize the relationship between phase tuning and STA amplitude, showed that modulation depth for most neurons was in the order of 1° with most neurons biased toward a phase between π and 2π , i.e., during whisker protraction (Fig. 4*H*).

Discussion

In conclusion, our results indicate that behavioral state affects spiking activity of cortical neurons in a layer-specific and most likely cell-type-specific way. In awake but nonwhisking animals, we found that average spiking frequencies were comparable to spiking in anesthetized animals. Upon free whisking, a subset of neurons robustly increased average spiking and these neurons were preferentially located in the upper portion of the infragranular layer [i.e., L5A, containing predominantly slender-tufted pyramids (21)]. However, even when spiking activity increased robustly upon whisker movement, we found that correlations between occurrence of spikes and whisker position or whisker velocity were weak. These weak correlations were previously observed in similar experiments for neurons of the trigeminal ganglion (22) and in extracellular recordings from unidentified units in barrel cortex (7, 23). Weak correlations (or broad tuning curves) do not imply lack of information (7, 24, 25), particularly considering low background spiking rates, but the accuracy of encoding information (in our case movement or position of a whisker) just increases significantly taking into account populations of neurons rather than single neurons (26). An alternative mechanism to encode whisker use could be that whisker phase rather than absolute whisker position is the determinant for spiking (23), although we also did not find a robust relationship between whisker phase and spike times (see Fig. 4). Remarkably, even though our data suggests the presence of a population code, electrical stimulation of single neurons in both primary motor and somatosensory cortex can evoke behavioral responses (27, 28), indicating that activity of single neurons can still significantly affect behavioral output.

Spiking Frequencies. In awake, head-fixed animals, we found average ongoing spiking rates of 0.3–3 Hz, with obvious layer-specific differences as observed during anesthetized conditions (18). More specifically, in head-fixed animals, spiking was generally around 0.3 Hz for L2/3, 1.4 Hz for L4, and 0.5 Hz for L6. In L5, we found higher ongoing spike rates (L5A 1.9 Hz, L5B 2.9 Hz). Ongoing spike rates were affected by behavioral state only in L5A. This could be due to state-dependent changes in excitability of the medial division of posterior thalamic nucleus (POm), projecting to L5A (2).

Reported spike rates of L2/3 neurons are strikingly consistent among different studies in awake animals (20, 29, 30). We found an average spiking rate of 0.28 Hz using juxta-cellular recordings of barrel cortex L2/3 neurons in head-fixed rats. Whole-cell recordings of mice barrel cortex L2/3 neurons demonstrated an average rate of 0.61 Hz (8, 20), population imaging on rat visual cortex L2/3 neurons resulted in an average ongoing spiking rate of 0.44 Hz (30) and finally, whole-cell recordings from (hind-limb) motor cortex L2/3 neurons showed that spike rates were as low as 0.3 Hz in freely moving animals (29). Spiking frequencies in L2/3 neurons are therefore consistently low, independent of

recording approach and cortical area. Unfortunately, similar spike frequency data for granular and infragranular layers do not exist because either spike rates were not consistently quantified or recorded cell-types were not identified.

Intracortical Projections of L5A Neurons. Within the barrel column, L5A slender-tufted neurons project to L2/3 neurons (21, 31). The presence of this connection suggests that information on whisker motion, conveyed by the paralemniscal pathway to L5A, could be relayed onto L2/3 neurons. In fact, a clear correlation between membrane potential and whisker position was shown by electrophysiological recordings in barrel cortex of awake mice (20). This correlation could be caused exclusively by the L5A–L2/3 projection. Because L2/3 also receives direct input from motor cortex (9), another possibility is that the correlation between membrane potential and whisker position in L2/3 is due to integrated input from the paralemniscal pathway and input from motor cortex. Because only membrane potential and not spiking correlates to whisker position in L2/3, the L5A–L2/3, and motor cortex–L2/3 projections may act in concert to facilitate spiking in L2/3 during for instance whisker contact (20).

Paralemniscal Pathway Encodes Whisker Motion. Finally, recent evidence from extracellular thalamic recordings challenged the view that the paralemniscal pathway is encoding whisker movement (12). In this previous study, no evidence was found for increased spiking in the paralemniscal part of the thalamus (medial division of POm) during free whisking in awake rats. However, whisker trajectories were not consistently quantified or POm recording sites identified histologically (13). We recorded spiking of identified neurons in different layers of primary somatosensory cortex while tracking whisker position and velocity simultaneously and found that slender tufted neurons in L5A could participate in encoding whisker movement. This view is in support of the hypothesis that the paralemniscal pathway is encoding whisker movement (10, 13, 32). Our data shows that this is the case for cortical neurons of the paralemniscal pathway. The next question will be whether movement related spiking is conveyed from (i) motor cortex directly (efferent copy), from (ii) activation of the paralemniscal pathway through mechanoreceptors in the whisker follicle, or indirectly (iii) by modulation of the sensory pathway by motor cortex (33).

Methods

Awake Wistar rats (28 animals, male and female, P31–46, weight 144.7 ± 30.9 g) were housed in an enriched environment and trained for 12 days to accustom to head-fixation. During surgical procedure, the animal was anesthetized with ketamine/xylazine (10 mg ketamine, 0.05 mg xylazine/100 g body weight). Depth of anesthesia was checked by both foot and eyelid reflex and vibrissae movements. The animal's temperature was monitored with a rectal probe and maintained at 37 °C by a thermostatically controlled heating pad. Experiments were performed on the left primary somatosensory (barrel) cortex (2.5 mm posterior, 5.5 mm lateral from Bregma). The skull was cleaned using alcohol (70%) and H_2O_2 (1%, in H_2O), the lateral muscle was partly

detached, and a metal pole was fixed to the skull using dental cement (Kerr Orange, USA). The craniotomy was protected by a small plastic cylinder with a screw-cap. Juxtacellular recordings were performed using patch pipettes (6–8 M Ω) filled with (in mM): 135 NaCl, 5.4 KCl, 1.8 CaCl_2 , 1 MgCl_2 , 5 HEPES, pH 7.2 with NaOH, and 20 mg/mL biocytin. The pia was kept covered with 0.9% NaCl. Duration of head-fixation was <60 min to maintain stable recording conditions. Single units were searched for by monitoring electrode resistance while advancing with 1- μm steps. Using this method, unit isolation was independent of spiking frequency.

Recordings were made using an Axoclamp 2B amplifier (Axon Instruments) in combination with a Lynx 8 amplifier, band filter settings 300 and 9,000 Hz (Neuralynx). Data were acquired using the Ntode Virtual Instrument (custom written software, R. Bruno, Columbia University, New York, NY) for Labview (National Instruments). Whisker movement and position was recorded at 60 Hz using an ETL-200 system modified for whisker tracking (ISCAN) and read into the Ntode program. After physiological properties were measured, neurons were filled with biocytin using current pulses (34). All experimental procedures were carried out according to the animal welfare guidelines of the Max-Planck Society and the Erasmus Medical Center in Rotterdam.

Histological Procedures and Reconstruction. All histological procedures were as described previously (18, 19). Cells were reconstructed using Neurolucida software (Microbrightfield,) or visually identified using a binocular microscope (CX31, Olympus).

Data Analysis. Data sets were analyzed using multiple custom-made Matlab routines (courtesy of R. Enchev, Institute of Cancer Research, London, U.K.). Quiet episodes (no whisker movements) and whisking episodes were sorted manually, but the experimenter was blind for spiking data during the sorting procedure. Subsequently, spiking frequencies were calculated for specific behavioral states. To determine the correlation of spike timing with respect to whisker movement, spike-triggered-averages were determined. Spikes were separated into quiet-spikes and whisking-spikes, and whisker position was determined at the time of spike firing (in addition to 100 ms before and after the spike). Correlation to whisker position (modulation depth) was determined by fitting a 4-parameter sinusoidal curve [$y = y_0 + a \sin(2\pi x/b + c)$] to the spike-triggered-average trace for whisker movement using Sigmaplot (Systat Software), where “ y_0 ” represents the baseline whisker position determined as the average angle during the entire recording, “ a ” represents modulation depth, “ b ” represents the whisking period as determined from the spike-triggered-average, and “ c ” represents the phase at time = 0. Data in Fig. 4H were plotted with modulation depth (a) as radial coordinate and phase at time 0 (c) as polar coordinate.

Anesthetized Conditions. Recordings from urethane (1.6 – 1.7 g $\cdot\text{kg}^{-1}$) anesthetized Wistar rats (P26–42, male/female) were previously published (18, 19). In summary, experiments were performed on the primary somatosensory cortex of the left hemisphere (2.5 mm posterior, 5.5 mm lateral from Bregma). Spontaneous AP frequency was measured through continuous recording for 125 s.

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