Activity in the Barrel Cortex During Active Behavior and Sleep

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1Program in Neuroscience, Harvard Medical School, Boston; 2The Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences; 3RIKEN-MIT Neuroscience Research Center, 4McGovern Institute for Brain Research, and 5Massachusetts Institute of Technology-Harvard Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge; and 6Department of Anesthesia, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

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Vijayan S, Hale GJ, Moore CI, Brown EN, Wilson M. Activity in the barrel cortex during active behavior and sleep. J Neurophysiol 103: 2074–2084, 2010. First published February 17, 2010; doi:10.1152/jn.00474.2009. The rate at which neurons fire has wide-reaching implications for the coding schemes used by neural systems. Despite the extensive use of the barrel cortex as a model system, relatively few studies have examined the rate of sensory activity in single neurons in freely moving animals. We examined the activity of barrel cortex neurons in behaving animals during sensory cue interaction, during non–stimulus-related activity, during various states of sleep, and during the administration of isoflurane. The activity of regular-spiking units (RSUs: predominantly excitatory neurons) and fast spiking units (FSUs: a subtype of inhibitory interneurons) was examined separately. We characterized activity by calculating neural firing rates, because several reports have emphasized the low firing rates in this system, reporting that both baseline activity and stimulus evoked activity is <1 Hz. We report that, during sensory cue interaction or non–stimulus-related activity, the majority of RSUs in rat barrel cortex fired at rates significantly >1 Hz, with 27.4% showing rates above 10 Hz during cue interaction. Even during slow wave sleep, which had the lowest mean and median firing rates of any nonanesthetized state observed, 80.0% of FSUs fired above 1 Hz. During all of the nonanesthetized states observed 100% of the FSUs fired well above 1 Hz. When rats were administered isoflurane and at a depth of anesthesia used in standard in vivo electrophysiological preparations, all of the RSUs fired below 1 Hz. We also found that >80% of RSUs either upmodulated or downmodulated their firing during cue interaction. These data suggest that low firing rates do not typify the output of the barrel cortex during awake activity and during sleep and indicate that sensory coding at both the individual and population levels may be nonsparse.

INTRODUCTION

Although the barrel cortex has been studied extensively, little is known about the basic properties of neural activity in this region in freely behaving animals and about the behavior of distinct cell types in this context. The rate of firing in sensory cortex has several important implications for stimulus representation. Higher ongoing rates are believed to reflect the behavioral importance of a stimulus and the attentional focus of an animal (Bichot and Desimone 2006; Williford and Maunsell 2006). Lower firing rates (<1 Hz) would readily allow for binary coding schemes (DeWeese et al. 2003) and may come at a lower metabolic cost (Attwell and Laughlin 2001; Lennie 2003). More generally, the state of the cortex, either the neurally defined state such as alpha (Nicolescu and Fanselow 2002) or gamma (Cardin et al. 2009; Hasenstaub et al. 2005), or the behaviorally defined states of wakefulness and sleep, are typically correlated by different ongoing levels of activity.

The few studies that have examined neural activity in the barrel cortex of freely behaving animals (Fanselow and Nicolescu 1999; Fanselow et al. 2001; Fee et al. 1997; Kleinfeld et al. 2002; Krupa et al. 2004; Prigg et al. 2002) have not distinguished between different cell types. Although segregation among neural types is not possible between most of the anatomical and morphological groups known to exist in the neocortex, a basic distinction can be made between regular-spiking units (RSUs) and fast-spiking units (FSUs). The former correspond predominantly (but not exclusively) to excitatory pyramidal cells, whereas the latter correspond apparently exclusively to a subtype of inhibitory interneuron. These neural types have been shown in in vivo anesthetized studies to have unique properties. The FSUs in barrel cortex are typically more sensitive than RSUs to weak stimuli, fire at a shorter latency to sensory input, and show higher firing rates overall (Lee and Simons 2004; McCormick et al. 1985; Swadlow 1989; Swadlow and Gusev 2002).

An ongoing debate in sensory physiology is whether or not the firing of neurons in the primary sensory cortices is “sparse.” The notion of sparseness is debated at the single cell level and the population level. At the single neuron level, the claim has repeatedly been made that neurons in primary sensory cortex exhibit low firing rates, ~1 Hz or less. For example, in the barrel cortex, a common model of mammalian sensory processing, several groups have argued that almost all neurons fire at or below 1 Hz (Brecht et al. 2005; de Kock et al. 2003; de Kock et al. 2005; Fanselow et al. 2002), even during stimulus-evoked responses in anesthetized (Brecht and Sakmann 2002; Brecht et al. 2003, 2005; de Kock et al. 2007; Kerr et al. 2005; Petersen et al. 2003), during stimulus-evoked responses in awake (Brecht and Sakmann 2002; Brecht et al. 2003, 2005; de Kock et al. 2007; Moore and Nelson 1998; Petersen et al. 2003; Sato et al. 2007) and awake head-fixed animals (Crochet and Petersen 2006). Similar arguments for a sparse code have been put forth for the primary auditory cortex (DeWeese et al. 2003) and visual cortex (Olshausen and Field 2005). These single-cell observations about rate are complemented by a paired argument, that even if some neurons fire at higher rates, they constitute only a small fraction of the total population (DeWeese et al. 2003).

A related and often simultaneously discussed issue is what fraction of neurons modulate their firing rates in the presence of a stimulus (Barlow 1972). Recent studies using two-photon imaging of the barrel cortex have argued that a relatively small fraction of the total number of neurons in a barrel show a
superthreshold response (detectable by Ca\(^{2+}\) signal changes) during the processing of a sensory input (Kerr et al. 2007). These findings are a replication of a subset of studies using intracellular in vivo techniques that found most recorded neurons did not show superthreshold sensory responses, although most did show subthreshold responses to vibrissa deflection (Brecht 2005; Moore and Nelson 1998).

The majority of studies making claims as to the sparseness of cortical activity use preparations that are anesthetized or immobilized, suggesting that studies in awake behaving animals are necessary. Prior studies have shown increased firing rates in multiunit activity during contact with a textured surface (Prigg et al. 2002; von Heimendahl et al. 2007), increased firing rates in response to shock with a nerve cuff during performance of unrelated behaviors (Fanselow and Nicolelis 1999; Fanselow et al. 2001), increased and decreased firing rates during performance of a width-detection task (Krupa et al. 2004), and phase locking of neural activity to ongoing whisking (Fee et al. 1997). Although all of these studies have added significantly to our understanding of barrel cortex function, none of the prior studies have systematically quantified firing rates in the awake animal, reporting the distribution and summary statistics of firing rates across a large sample of neurons in and outside of sensory contact regions.

In this study, we describe recordings made from the barrel cortex of awake, freely behaving rats during running in the presence and absence of explicit tactile cues as well as during sleep and during the administration of isoflurane. During periods of sensory cue interaction and during periods of non-stimulus-related activity, $>75\%$ of RSUs fired at rates significantly $>1$ Hz. Furthermore, during cue interaction, $80\%$ of RSUs either upmodulated or downmodulated their firing. Even RSUs that showed significant negative modulation (decreased firing) during cue interaction showed rates significantly $>1$ Hz in the portion of the track containing the cue. During slow wave sleep, firing rates were lower but were still significantly $>1$ Hz. However, when rats were administered isoflurane and at a depth of anesthesia used in standard in vivo electrophysiological preparations, all of the RSUs fired below 1 Hz. All of the FSUs fired at rates well above 1 Hz in all of the nonanes-thetic states measured. Our results suggest that barrel cortex activity is nonsparse at the individual and population levels and that the majority of neurons are differentially engaged by sensory stimuli.

**METHODS**

**Subjects**

Data were collected from nine Long-Evans rats 4–9 mo of age. Seven of these rats were used to characterize the firing rates during awake activity and sleep, and the remaining two were used for the isoflurane studies. They were housed in individual cages and kept on a 12-h light-dark cycle. During testing, rats were maintained at 85% of their normal body weight.

**Surgical procedure**

While under anesthesia, each rat was implanted with a microdrive array, containing 21 independently adjustable microdrives. For five of the rats, nine electrodes targeted the CA1 region of the hippocampus (stereotaxic coordinates: 3.6–3.9 mm posterior to bregma and 2.2 mm lateral from the midline) and nine electrodes targeted the primary somatosensory cortex barrel field (stereotaxic coordinates: 3.3 mm posterior to bregma and 4.85–5.0 mm lateral from the midline). The cannulae that contained the tetrodes for these five rats had IDs of 1.19 mm, and the minimum separation between tetrodes was 0.3 mm. For two of the rats, six electrodes targeted the CA1 region of the hippocampus (stereotaxic coordinates: 3.6–3.9 mm posterior to bregma and 2.2 mm lateral from the midline), six electrodes targeted the primary somatosensory cortex barrel field (stereotaxic coordinates: 3.3 mm posterior to bregma and 4.5–5.0 mm lateral from the midline), and six electrodes targeted a nonbarrel field portion of the primary somatosensory cortex (stereotaxic coordinates: 0.75 mm anterior to bregma and 4 mm lateral from the midline). The cannulae that contained the tetrodes for these two rats had IDs of 1.07 mm, and the minimum separation between tetrodes was 0.3 mm.

For an additional two rats 18 electrodes targeted the primary somatosensory cortex barrel field (stereotaxic coordinates: 3.3 mm posterior to bregma and 4.75 mm lateral from the midline). The cannulae that contained the tetrodes for these two rats had IDs of 1.6 mm. A bipolar electrode was implanted into the rat’s neck muscle to record the EMG, except for the two rats with cannulae that targeted only the primary somatosensory cortex barrel field. All surgical and behavioral procedures met institutional guidelines.

**Behavioral paradigm and electrophysiological recordings**

After fully recovering from surgery, rats were trained to run around an elevated circular track (344.8 cm diam), with tactile cues, for a food reward. The rat was initially placed at a consistent starting location on the track. The rat’s task was to run 270° in the clockwise direction from where it was placed. Once the rat reached the 270° mark, it was given a food reward placed in a removable food well. Once the rat consumed its food reward, the food well was removed. The 270° mark served as the new starting point. The rat ran 270° clockwise from its new starting point and was given a food reward. This procedure continued for the remainder of the training session. The rat’s traversal of 270° from its starting point to the food reward was considered a trial. All rats ran in a relatively smooth fashion, which was consistent from trial to trial; however, the speed at which trials were run varied across rats.

The tactile cue consisted of a regularly repeating pattern of rectangular protrusions on the wall of the maze. The rectangular protrusions were located on both sides of the wall and were aligned with one another. They were 3 cm high and protruded by 2.5 cm. The rectangular protrusions were $\sim$2.7 cm wide on the inner wall of the circular track and $\sim$3.2 cm wide on the outer wall of the track and were separated by 1.2 cm. The length of cue was 31 cm. The representation of the textured region in all figures depicts the region of the maze in which the rat could interact with the texture; the length of this region is greater than the length of the texture.

During the behavioral task, the location of the rat was monitored using two sets of infrared diodes attached to the microdrive array. The location of these diodes was sampled by an overhead camera at 30 Hz, and this provided data giving the rat’s location to a resolution of $\sim$0.65 cm.

While the rat ran the circular track, unit activity from each tetrode was recorded. Unit activity was filtered between 300 Hz and 6 kHz and sampled at 32 kHz per channel. If the signal from any channel of a tetrode exceeded a preset threshold, a 1 ms window of the waveform around the time of the threshold crossing was stored for each channel. The local field potential (LFP) was also recorded. It was filtered between 0.5 and 475 Hz, sampled at 2 kHz per channel, and continuously recorded. Unit activity and LFP were also recorded while the rat was in the sleep box before maze running and after maze running.

Electrodes were adjusted at the end of the recording day. Tetrodes were advanced only if no cells were observed on that day and were not advanced again until the end of the following day, if necessary. Units were clustered during the sleep period before maze running using the
The intensity function of a given cell was modeled as a function of the coordinates of the cell and the activity of the neuron at that location. The calculation of GLM rate maps involves estimating the parameters of the conditional intensity function underlying the spike train using the glmfit function in Matlab (Fig. 1).

Cluster quality

To check the quality of our clusters, we calculated both the L-ratio and isolation distance (Schmitzer-Torbert et al. 2005). The mean L-ratios for sleep before maze running, during maze running, and during sleep after maze running were 0.058 ± 0.072, 0.079 ± 0.096, and 0.056 ± 0.061, respectively. The mean isolation distance for sleep before maze running, during maze running, and during sleep after maze running were 24.127 ± 18.345, 24.707 ± 24.632, and 24.298 ± 18.071, respectively.

Sleep classification

Sleep was classified at 1 s resolution into one of four states: slow wave sleep (SWS), rapid eye movement (REM) sleep, intermediate sleep, or awake. The classification scheme relied on EMG power, power in the neocortical delta band, power in the hippocampal ripple band, and the power ratio of the hippocampal theta band and hippocampal delta band. The above power measurements were used to classify sleep in a fashion similar to that of Ji and Wilson (2007). In brief, periods with low EMG power, low neocortical delta, and low theta/delta power were classified as REM. Periods with low EMG power, high neocortical delta and ripple power, and high theta/delta power were classified as SWS. Periods with low EMG power, which met neither the criteria for SWS nor that for REM sleep, were classified as intermediate sleep. Periods with high EMG power were classified as awake.

Behavioral paradigm and electrophysiological recordings when administering isoflurane

Immediately after the postmaze running session in the sleep box, the rat was transferred to a clear plastic box. A tube was connected to one side of the box through which isoflurane (1.5–2.5% in 100% O2) was administered. Another tube was connected on one end to the opposite side of the box, and the other end was connected to a fan filter. Once the rat had ceased moving, its position was adjusted such that the whiskers were not deflected. When the rats were at a depth of anesthesia used in standard in vivo electrophysiological preparations, as assessed by breathing rate and the hindpaw withdrawal reflex, administration of isoflurane was stopped.

Calculation of GLM rate map

The generalized linear model (GLM) calculations were done using methods detailed in Truccolo et al. (2005). The spike rate (conditional intensity function) of a given cell was modeled as a function of the rat’s position and as a function of the cell’s spiking history

\[ \log(\lambda(t|H_t)) = \sum_{j=1}^{3} a_j n_{j,t} + \sum_{k=1}^{S} \theta_k g_k(t) \]

In the preceding equation, \( g_k(t) \) is a cardinal spline and captures the position of the rat. In our case, the cardinal spline had 33 control points, and the set of control points was separated by ~11.5 cm. Also, in the preceding equation, \( n_j \) captures the spiking history of the cell. In our case, there were spiking history bins from 1 to 10 ms in increments of 1 ms and from 11 to 341 ms in increments of 10 ms. We estimated the parameters of the above equation by maximum likelihood using the glmfit function in Matlab (Fig. 1D; see Supplemental Fig. S2). We assessed the goodness of fit using the time rescaling theorem. We rescaled the spike train according to

\[ z_k = \int_{u_k}^{u_{k+1}} \lambda(\theta_k H_t) \, du \]

In the preceding equation, \( s_k \) is the time of the \( k \)th spike. If \( \lambda(\theta_k H_t) \) correctly describes the conditional intensity function underlying the spike train, the \( z_k \) will be independent and exhibit an exponential distribution with rate parameter \( \lambda(\theta_k H_t) \).

Therefore \( u_k \) in the equation below will have a uniform distribution on the interval from 0 to 1

\[ u_k = 1 - \exp(-z_k) \]

We can assess the goodness of fit using the Kolmogorov-Smirnov (K-S) plot, which allows us to assess how similar the distribution of the \( u_k \)s is to that of a uniform distribution. If the model accurately captures the spiking of the neuron modeled, the ordered \( u_k \)s should lie along the identity line (Supplemental Fig. S2).

Results

Unit isolation and classification

We studied barrel cortex activity during several states, including active maze exploration and sleep. Electrophysiological recordings were taken from the vibrissa representation of primary somatosensory cortex (SI) while a rat ran a circular maze for a food reward. The circular maze contained regions in which tactile cues were placed on the walls (Fig. 1A; see Methods). Recordings were also made during sleep before and after maze running.

Single units were identified by their activity during the sleep session before maze running (Supplemental Fig. S1; see Methods). This method conferred two main advantages. First, isolated units were identified independent of the run session, avoiding the selective discovery of sensory-driven neurons. Second, because the sleep sessions were on average ~2 h [113.8 ± 34.3 (SD) min] in length, cells that had low firing rates would be readily identified if present. For example, a neuron firing at 0.1 Hz would be expected to spike 683 times in a 113.8 min period, which provides more than an adequate number of spikes for unit identification under our clustering procedure (Supplemental Fig. S1). To further avoid bias in the selection of single units, tetrodes were adjusted only at the end of the recording day, while the rat was in the sleep box, and data were collected the following day on all tetrodes regardless of experimenter identification of activity on a given channel. Furthermore, adjustment consisted of blindly advancing the tetrode 40–80 μm. These steps helped to prevent biasing the selection of cells toward those that were more active on the track (see Methods).

We classified cells as either RSUs or FSUs based on the peak-to-trough width of the average action potential waveform of each cell and the average firing rate of each cell during maze running (Fig. 2A) (Agmon and Connors 1989; Andermann and Moore 2006; Andermann et al. 2004; Bruno and Simons 2002; McCormick et al. 1985). This identification is particularly important because interneurons (FSUs) are known to have higher firing rates (Simons and Carvell 1989).

1 The online version of this article contains supplemental data.
Firing rates during stimulus- and non–stimulus-related activity

Figure 1 provides an example of the activity of a single RSU. This neuron had a baseline firing rate of 8.3 Hz outside the texture and reached a peak firing rate within the texture above 35 Hz (Fig. 1, B and C). When the firing rate of this cell was modeled as a function of position and spiking history using the generalized linear model (Truccolo et al. 2005), the positional component of the model showed that this cell fired between 5 and 10 Hz outside of the texture and reached a peak firing rate within the texture above 30 Hz (Fig. 1 D; firing rate of the cell relative to position when the spiking of the cell has been modeled as a function of position and spiking history).

Firing rates during SWS

We also examined the firing rates of the RSUs during SWS, which has the lowest mean (3.8 Hz) and median (2.9 Hz) firing rates of any of the states from which we recorded (Supplemental Fig. S3). The rates were significantly lower during SWS than during maze running outside or within the textured region (K-S test, 1-tail test, \( P = 0.0031 \) and \( P = 0.0050, n = 95 \)). However, even during SWS, 80.0% of cells fired above 1 Hz (Fig. 3, A and B).

Even during SWS, all the FSUs fired above 1 Hz, with the lowest firing rate being 6.5 Hz. The average and median firing rates of the FSUs during SWS were 14.2 and 11.8 Hz (\( n = 10 \)), respectively (Fig. 2, B and E). The lowest firing rate of any FSU within the textured region of the track was 17.8 Hz, and the average and median rates were 30.9 and 27.4 Hz (\( n = 10 \)), respectively (Fig. 2, C and E). Like the RSUs, the difference in the firing rates of the FSUs in these two regions of the track was not significantly different (K-S test, 1-tail test, \( P = 0.3530, n = 10 \)).

Firing rates during stimulus- and non–stimulus-related activity

Analysis of the firing rates of individual cells showed that, while the animal was running around the track in nontextured and nonfood well regions, 78.9% of the RSUs fired above 1 Hz, with average and median rates of 6.1 and 4.6 Hz (\( n = 95 \)), respectively (Fig. 2, B and D). While the rat was running within the textured region, 84.2% of RSUs fired above 1 Hz, with average and median rates of 7.8 and 4.0 Hz (\( n = 95 \)), respectively (Fig. 2, C and D). The difference in the firing rates of the RSUs in these two regions of the track was not significantly different (K-S test, 1-tail test, \( P = 0.2660, n = 95 \)).

The lowest firing rate of any FSU outside of the textured region was 9.9 Hz, and the average and median rates were 31.1 and 34.8 Hz (\( n = 10 \)), respectively (Fig. 2, B and E). The lowest firing rate of any FSU within the textured region of the track was 17.8 Hz, and the average and median rates were 30.9 and 27.4 Hz (\( n = 10 \)), respectively (Fig. 2, C and E). Like the RSUs, the difference in the firing rates of the FSUs in these two regions of the track was not significantly different (K-S test, 1-tail test, \( P = 0.3530, n = 10 \)).
suggest that activity during SWS, like baseline awake activity and stimulus-related activity, is not sparse at the individual cell or population level. Similar activity patterns were also observed in nonvibrissa SI regions (Supplemental Fig. S4).

**Firing rates during the administration of isoflurane**

We also examined how isoflurane administration affected firing rates. To do this, we made recordings from two rats, during maze running, before and after maze running, as well as during the administration of isoflurane immediately after the behavioral session. We found that the firing rates of the RSUs declined markedly during the administration of isoflurane, when considered collectively or individually (Supplemental Figs. S5–S9). To quantify the effect of isoflurane on firing rate, we compared the firing rate during maze running to the rate at the point when the rats were at a depth of anesthesia used in standard in vivo electrophysiological preparations, as assessed by breathing rate and the hindpaw withdrawal reflex. When this depth of anesthesia was reached, the delivery of isoflurane was stopped. The average and median firing rates during maze running were 3.88 and 2.80 Hz, respectively. The average and median firing rates for the isoflurane condition were 0.15 and 0.06 Hz, respectively (Fig. 4). The firing rates of the RSUs were significantly lower during the isoflurane condition than during maze running (K-S test, 1-tail test, $P = 0.000000015$, ...
n = 40). Whereas 62.5% of the RSUs fired above 1 Hz during maze running, in the isoflurane condition, all of the RSUs fired below 1 Hz.

Modulation during stimulus-related activity

In examining the modulation of these cells in the textured region, we first characterized their collective firing rates within and outside of the texture. When RSUs were considered collectively, the average firing rate per cell for the 200 ms period before entry into the texture was 5.7 Hz (n = 95 neurons) and the firing rate peaked at 9.2 Hz (n = 95 neurons) within the texture (Fig. 5A). We also considered separately those cells that were upmodulated and those that were downmodulated within the texture. For our purposes, we defined a cell as upmodulated or downmodulated if it increased or decreased its baseline firing rate by 20%. The upmodulated cells peaked at 15.3 Hz (n = 49 neurons) within the texture and the downmodulated cells reached a minimum of 1.4 Hz (1.7 Hz, n = 36 neurons). These rates were also calculated as a function of the rat’s position on the maze. In this analysis, the

![Fig. 3](file://localhost/mnt/www/jn.org/2010/04/issue/03.png)

**FIG. 3.** Distribution of slow wave sleep (SWS) firing rates. A: distribution of both RSU (n = 95) and FSU (n = 10) firing rates during SWS. B: each point on the graph represents the fraction of RSUs that fire at or below a given firing rate. The black horizontal line (gray line, gray dotted line) represents the fraction of cells that fire below 1 Hz during SWS (during awake activity outside of texture, during awake activity inside of texture). The firing rate during SWS is significantly less than the rate outside of the texture or within the texture. (K-S test, 1-tail test \( P = 0.0031 \) and \( P = 0.0050, n = 95 \)). C: each point on the graph represents the fraction of FSUs that fire at or below a given firing rate (n = 10). The firing rate during SWS is significantly less than the rate outside of the texture or within the texture (K-S test, 1-tail test \( P = 0.0155 \) and \( P = 0.0006, n = 10 \)).

![Fig. 4](file://localhost/mnt/www/jn.org/2010/04/issue/03.png)

**FIG. 4.** Distribution of firing rates of RSUs (n = 40) during maze running and during the isoflurane condition. A: each point on the graph represents the fraction of RSUs that fire at or below a given firing rate. The gray horizontal line represents the fraction of cells that fire below 1 Hz during maze running. The firing rates of the RSUs were significantly lower during the isoflurane condition than during maze running (K-S test, 1-tail test \( P = 0.000000015, n = 40 \)). Inset: average firing rates during maze running and during the isoflurane condition.
upmodulated cells peaked at 15.0 Hz (n = 49 neurons) within the texture and the downmodulated cells reached a minimum of 1.7 Hz (n = 36 neurons; Fig. 5C). Although our N was small, the same analysis on FSUs showed that when the FSUs were considered collectively, the average firing rate per cell for the 200 ms period before entry into the textured region of the track was 28.9 Hz (n = 10 neurons), whereas the peak firing rate reached at 43.6 Hz within the texture (Fig. 5B). The upmodulated cells (n = 2 neurons) peaked at 54.2 Hz within the texture, and the downmodulated cells reached a minimum at 4.7 Hz (n = 2 neurons). When the rates for the FSUs were calculated as a function of the rat’s position on the maze, the upmodulated cells peaked at 59.7 Hz (n = 2 neurons) within the texture and the downmodulated cells reached a minimum of 6.2 Hz (n = 2 neurons; Fig. 5D).

To assess the population response during cue interaction, we calculated the fraction of cells that were modulated within the texture. We found that 89.5% (85/95) of cells were either upmodulated (49/95, 51.6%) or downmodulated (36/95, 37.9%) throughout the length of the texture (Fig. 5E). When the texture was divided into beginning, middle, and end sections, each of equal length, more cells were upmodulated than downmodulated at the beginning [51/95 (53.7%) vs. 31/95 (32.6%)] and end of the texture [44/95
lated and 20% (n = 10 neurons) of the cells were modulated in the beginning and end sections of the texture, with 20% (n = 2 neurons) of the cells being upmodulated and 20% (n = 2 neurons) of the cells being downmodulated in both sections. In the middle section of the texture, 70% of the cells were modulated, with 40% (n = 3 neurons) of the cells being upmodulated and 30% (n = 4 neurons) of the cells being downmodulated.

**DISCUSSION**

Our results showed that firing rates varied as a function of behavioral state, with reduced rates during SWS relative to other sleep states (Supplemental Fig. S4) and active behavior. Firing rates were also modulated by the textured region of the track compared with other regions of the track. We also observed that firing rates in the barrel cortex were typically >1 Hz, across all recorded nonanesthetized brain states and across both cell types identified: RSUs and FSUs. These findings provide initial quantitative measures of firing rates across cell types in a key model system, the barrel cortex, under several regimens of behavior.

The reported activity level and responsiveness of barrel cortex neurons tends to depend on the recording method used and the conditions under which the recording method was applied. In vivo experiments in anesthetized animals in which whole cell recordings were made suggest that activity in the barrel cortex is sparse (Brecht and Sakmann 2002; Brecht et al. 2003, 2005; de Kock et al. 2007; Moore and Nelson 1998; Petersen et al. 2003). For example, Brecht and Sakmann (2002) found in anesthetized rats that in layer IV the spontaneous firing rate averaged 0.053 ± 0.12 Hz and that the deflection of the principal whisker elicited on average 0.14 ± 0.29 action potentials. In vivo experiments in anesthetized animals using two-photon imaging showed similar spontaneous and stimulus-evoked activity (Kerr et al. 2005, 2007; Sato et al. 2007). For example, Kerr et al. (2007) found that the spontaneous firing rate of layer II/III neurons interpreted from calcium imaging was 0.048 ± 0.002 Hz.

Somewhat higher rates have been observed in in vivo experiments using sharp electrodes in anesthetized rats. These experiments suggest that spontaneous rates in barrel cortex are above 1 Hz (Hasenstaub et al. 2007; Higley and Contreras 2003; Sachdev et al. 2004), with the number of spikes generated per principal vibrissa deflection varying from less than one spike per stimulus (Higley and Contreras 2003) to greater than one spike per stimulus (Hasenstaub et al. 2007; Sachdev et al. 2004). Many in vivo experiments using extracellular recordings in anesthetized rats suggest that spontaneous rates are nonsparse as well (Ahissar et al. 2000; Armstrong-James et al. 1992; Simons and Carvell 1989). Most of these studies provide a qualitative description. Armstrong-James et al. (1992) found that, in urethane-anesthetized rats, “more than 80% of cells had spontaneous firing rates between <1 and 4 Hz,” but that rates above 8 Hz were extremely rare. Simons and Carvell (1989) found that spontaneous firing rates of RSUs were 1.09 ± 1.53 (SD) spikes/s, although later recordings by the same group suggest lower values (Lee and Simons 2004). Most of these studies suggest that the number of action potentials elicited by a principal whisker deflection is less than one or slightly greater than one (Armstrong-James et al. 1992; Diamond et al. 1994; Krupa et al. 2004; Lee and Simons 2004; Simons and Carvell 1989).

In head-fixed, awake animals, similar trends are observed. The few studies that have conducted whole cell recordings from awake head-fixed animals have shown lower spontaneous rates and stimulus-elicited responses (Crochet and Petersen 2006; Poulet and Petersen 2008) than similar studies using extracellular electrodes. For example, Crochet and Petersen (2006) examined the activity of barrel cortex neurons in head-fixed rats, in which all the vibrissae but one were trimmed, during quiet periods of awake activity and during periods of whisking. They found that the spontaneous firing rate was 0.87 ± 1.38 Hz during quiet periods and that during whisking periods, it was 1.31 ± 2.02 Hz. Although low, this spontaneous rate is higher than those rates observed in whole cell anesthetized preparations. Passive stimulation of the whisker during whisking elicited 0.014 ± 0.094 action potentials, after taking into account the baseline rate. Passive stimulation of the whisker during quiet awake periods elicited 0.119 ± 0.24 action potentials, after taking into account the baseline rate. In contrast, head-fixed experiments using extracellular recording methods show examples of spontaneous rates >1 Hz and that can extend up to tens of hertz and show elicited responses of greater magnitudes (Melzer et al. 2006; Nicolelis et al. 1995; Sachdev et al. 2000; Wiest et al. 2005).

In summary, anesthetized and head-fixed preparations show variation in their reported firing rates; however, in general, extracellular recordings from freely behaving animals typically show higher spontaneous rates and elicited responses (Fee et al. 1997; Kleinfeld et al. 2002; Krupa et al. 2004; Prigg et al. 2002). In one example, during free behavioral exploration of a screen by the vibrissae, Kelly et al. (1999) found mean firing rates of 27.6 ± 21.0 Hz in barrel cortex neurons. More recently, Jadhav et al. (2009) reported “sparse” responses in the barrel cortex during surface exploration. This term, however, was applied to the probability of spiking for a given neuron in response to a single vibrissal slip during surface contact. When firing rate was analyzed across time, as in this study, neurons showed mean baseline firing rates of ~7.5 Hz and a range from 0.3 to 48 Hz, and examples shown during surface contact indicate firing of ~15 Hz, similar to our findings. These rates are consistent with recent studies showing that multiple slips occur in tens of milliseconds of surface contact (Jadhav et al.
2009; Ritt et al. 2008; Wolfe et al. 2008). As such, the mean firing rates over time in Jadhav et al. (2009) are likely similar to those reported here and may even exceed those observed here when calculated based on time of surface contact. Very few studies have made whole cell recordings from freely behaving animals. Lee et al. (2006) did so, recording from six cells in the hindlimb area of the primary motor cortex. Five of the six cells displayed rates below 1 Hz, but the recording procedure required administration of an anesthetic and an antagonist to the anesthetic just before recording, and this procedure may have had an effect on the physiology as discussed by the authors (Lee et al. 2006).

All of the recording methods used may not accurately reflect firing rates, because of biases of the methods themselves. It has been argued that high firing rates reported across various sensory systems using extracellular recordings, the method used in this study, may result from the overestimation of firing rates caused by sampling bias (Olshausen and Field 2005). In this study, we took measures to avoid this potential bias. First, we blindly adjusted tetrodes at the end of each recording day and took recordings from any cell that was present the following day. This step helped to reduce bias toward recording neurons with high firing rates. Second, we clustered our units during sleep before maze running. This helped to avoid the selective discovery of sensory-driven neurons. Third, we recorded on average ~2 h (113.8 ± 34.3 min) of sleep before maze running, so we were able to detect units that had low spiking rates. Fourth, we classified our units into FSUs and RSUs. This measure prevented our rate estimates from being biased by FSUs, which are known to have higher rates (Simons and Carvell 1989) and may dominate multiunit recording postings.

Recent studies using extracellular recordings in awake head-fixed animals suggest that rates may vary across cortical layers in both the auditory cortex (Sakata and Harris 2009) and barrel cortex (de Kock and Sakmann 2009). Sakata and Harris (2009) reported layer specific differences in evoked and spontaneous responses in the auditory cortex, using juxtacellular recordings; however, Hromádka et al. (2008), using cell-attached recordings in awake head-fixed rats, found that all layers of the auditory cortex had similar spontaneous and evoked rates, with the exception of layer II, which had lower rates. De Kock and Sakmann (2009) found that the average spontaneous rates and average rates during whisking of the pyramidal cells in layers II/III and VI of the barrel cortex were below 1 Hz, and the average spontaneous rates and average rates during whishing of pyramidal cells in layers IV and V were above 1 Hz. The finding that layers II/III and VI displayed lower rates than layer V is consistent with whole cell anesthetized recordings (Brecht et al. 2003; de Kock et al. 2007), but the finding with respect to layer IV cells is inconsistent in that layer IV cells in whole cell anesthetized recordings have been reported to have even lower spontaneous rates than layers II/III and V (Brecht and Sakmann 2002). Not all studies using extracellular recordings report this general pattern seen by de Kock and Sakmann (2009). Lee et al. (2007), using extracellular recordings in anesthetized rats, found the average spontaneous rates of layer II/III RSUs to be well above 1 Hz (3.09 ± 2.74 Hz), and the average spontaneous rates of layer IV RSUs (0.92 ± 0.72 Hz) to be less than those of layer II/III. Because in this study we cannot definitively determine the layer of the cells used in our recordings, it is possible that our results are influenced by laminar differences. Specifically, if we were biased in our recordings toward layer V neurons, a population that studies indicate has relatively higher firing rates, it would influence our estimates of the firing rate distributions.

Previous studies that report “sparse” coding in the barrel cortex—rates <1 Hz, even during sensory stimulation—may differ in their conclusions from this study because of several factors. One possibility as detailed above may be the recording method itself. Another possibility is that differences in the conditions under which recordings were made may affect the rates as well. Many studies were conducted in anesthetized animals. This altered state may cause reduced rates; studies investigating neural activity in barrel cortex suggest that spontaneous and stimulus-evoked rates are significantly lower under anesthesia even within a given recording paradigm (Armstrong-James and George 1988). Our own findings suggest that when the rats were administered isoflurane and at a depth of anesthesia used in standard in vivo electrophysiological preparations, all RSUs spontaneously fired below 1 Hz. Our findings suggest that the different rates observed in anesthetized and awake recordings are not the result of identification of different neurons—as in our work, the same cells were tracked through the onset of anesthesia—but rather a shift in activity level with anesthetic induction.

In studies of head-fixed animals, animals do not engage the vibrissae to guide body navigation (an essential background element of all free behavioral studies), and even when whisking, animals are restrained in their active sensing behaviors relative to object exploration. For example, changes in head orientation and body motion are potentially key to moving the vibrissae to obtain sensory information (Berg and Kleinfeld 2003; Ritt et al. 2008; von Heimendahl et al. 2007). Loss of such control could decrease firing rates systemically in this preparation, even though animals are awake. These considerations do not explain the discrepancy between the nonsparse firing rates we found during SWS and the sparse rates found in head-fixed animals in other studies, and reconciliation of these different data sets will require further study. The differences could reflect more subtle methodological variations across laboratories or an active suppressive process recruited by head posturing.

In summary, these observations are consistent with the majority of prior studies using free behavior that suggest single unit activity is nonsparse in the barrel cortex, and we add to these studies by taking measures to eliminate possible sampling biases, quantifying the firing rate changes observed and identifying specific single unit types.

Nonsparse firing has several implications for neural coding in the barrel cortex. One advantage to a greater range of firing rates is that the relative amount of modulation in activity might provide information about the magnitude of some characteristic of the vibrissa-stimulus interaction, such as the velocity of vibrissa deflection (Arabzadeh et al. 2003, 2005; Simons and Carvell 1989). Modulation across a large population of neurons in a spatially organized cortical map could allow for the differential representation of distinct features, such as the direction (Andermann and Moore 2006) or frequency (Andermann et al. 2004; Moore and Andermann 2005) of sensory input. In addition, high rates in the barrel cortex might enable a single neuron to convey multiple pieces of information within
a whisk cycle (4–12 Hz), either through the relative timing between spikes (Jones et al. 2004) or through spike timing relative to ongoing state properties reflected in the local field potential (Haslinger et al. 2006).

In summary, we quantified the activity of RS and FS single units in barrel cortex across a variety of states, including free behavioral contact with texture and sleep. We observed spontaneous firing rates and rates of neural modulation by stimulus presence that exceed those previously reported. Our data indicate that sensory coding at both the individual and population levels could take advantage of these relatively higher firing rates for a variety of forms of information representation.

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