Behavioral State Dependency of Neural Activity and Sensory (Whisker) Responses in Superior Colliculus

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Cohen JD, Castro-Alamancos MA. Behavioral state dependency of neural activity and sensory (whisker) responses in superior colliculus. J Neurophysiol 104: 1661–1672, 2010. First published July 7, 2010; doi:10.1152/jn.00340.2010. Rats use their vibrissa (whiskers) to explore and navigate the environment. These sensory signals are distributed within the brain stem by the trigeminal complex and are also relayed to the superior colliculus in the midbrain and to the thalamus (and subsequently barrel cortex) in the forebrain. In the intermediate layers of the superior colliculus, whisker-evoked responses are driven by direct inputs from the trigeminal complex (trigeminotectal) and feedback from the barrel cortex (corticotectal). But the effects of the behavioral state of the animal on the spontaneous firing and sensory responses of these neurons are unknown. By recording from freely behaving rats, we show that the spontaneous firing of whisker sensitive neurons in superior colliculus is higher, or in an activated mode, during active exploration and paradoxical sleep and much lower, or in a quiescent/deactivated mode, during awake immobility and slow-wave sleep. Sensory evoked responses in superior colliculus also depend on behavioral state. Most notably, feedback corticotectal responses are significantly larger during the quiescent/deactivated mode, which tracks the barrel cortex responses on which they depend. Finally, sensory evoked responses depend not only on the state of the animal but also on the orienting response elicited by the stimulus, which agrees with the well known role of the superior colliculus in orienting about salient stimuli.

INTRODUCTION

At the behavioral level, orienting responses are basic motor reactions to sensory stimuli by which the subject turns its sensors with respect to the source of stimulation. The superior colliculus is well known to be involved in orienting responses to stimuli from a wide range of modalities, including somatosensory, auditory, and visual (Dean et al. 1989; Meredith and Stein 1985; Schneider 1969; Sparks 1986; Sprague and Meikle 1965; Stein 1998; Stein and Meredith 1993; Wurtz and Albano 1980). Neurons in the intermediate layers of the superior colliculus of rats are highly responsive to vibrissa (whisker) stimulation (Cohen and Castro-Alamancos 2007, 2010; Cohen et al. 2008; Grunweg and Krauthamer 1990; Hemelt and Keller 2007; Mchaffie et al. 1989; Weldon and Best 1992). These whisker responses are driven by direct inputs from the trigeminal complex (trigeminotectal; peak1 at ~5 ms post-stimulus) followed by activity returning from the barrel cortex (corticotectal; peak2 at ~12 ms) (Cohen et al. 2008). However, most rodent electrophysiological studies of whisker-sensitive superior colliculus neurons have been conducted in anesthetized animals. Consequently, the neural activity of these neurons during different behavioral states is largely unknown. It is also not possible to determine the orienting response evoked by a sensory stimulus in anesthetized animals.

Here we recorded the spontaneous and whisker-evoked activity of neurons in the intermediate layers of freely behaving rats during transitions between various sleep and awake states. We found that spontaneous and sensory-evoked neural activity is highly dependent on the behavioral state of the animal and on the orienting response produced by the sensory stimulus presented.

METHODS

Adult male Spraque-Dawley rats (225–250 g) were used (n = 9). Animals were cared for in accordance with National Institutes of Health guidelines for laboratory animal welfare. All experiments were approved by the Drexel University Institutional Animal Care and Use Committee. At all times, food and water was available ad libitum. All animals were initially housed in groups of three. Once the animals underwent surgical procedures, they were individually housed for the remainder of the experimental protocol.

Surgical procedures

For all recovery surgeries, animals were anesthetized with sodium pentobarbital (50 mg/kg ip) and placed in a stereotaxic frame. All skin incisions and frame contacts with the skin were injected with lidocaine (2%). Throughout the surgery, body temperature was automatically maintained constant with a heating pad (Harvard Apparatus). During recovery from surgery, animals received a dose of buprenorphine (0.03 mg/kg im) to reduce pain. Recovery from whisker pad electrode and microelectrode implantations involved 5–7 days before re-testing.

Chronic electrophysiology

Animals were implanted with a whisker pad stimulating electrode (see following text) and two recording electrodes at the same time. The recording electrodes were aligned with the whisker pad stimulating electrode by recording evoked responses during surgery. Once in place, the electrodes attached to head connectors were fixed to the skull by screws and dental cement. In all animals, a field potential (FP) electrode was implanted in the barrel cortex (bregma: A/P: −2.7, M/L: 5.0, D/V: 0.5–1.0). The FP electrodes consisted of blunt insulated stainless steel wires (100 μm OD, ~0.5 MΩ). Concurrently, either a FP electrode or a multiunit activity (MUA) electrode was implanted in the superior colliculus (lambda: A/P: 2.2, M/L: 2.2, D/V: 4–4.5 ). The MUA electrode consisted of a higher impedance insulated tungsten electrode edged to a fine tip (100 μm outer shaft diameter, 2–7 MΩ). The reference electrode consisted of a FP electrode placed above the superior colliculus, and the ground was attached to skull screws. In some recording sessions, clear single-unit activity was recorded through the MUA electrode, but we combined these cases with MUA.
sessions. Also in some cases, FP activity was also collected in the superior colliculus through the MUA recording electrode.

Behavioral states

During recording sessions (1 per day), the head connector was attached to a headstage operational amplifier with unity gain that lead through fine tether cables to an electrical swivel that terminated in the amplifiers and recording system. The animal was allowed to freely behave in a 35 × 25 cm Plexiglas cage. During all sessions, electrophysiological activity was continuously recorded in synchrony with digital video of the behavior (30 frame/s; Cinéplex, Plexon). This allowed off-line verification of the behavioral state. For the present study, the spontaneous behavior of the animal was classified in four easily defined categories as slow wave sleep (SWS), paradoxical sleep (REM), awake immobility (AWIM), and active exploration (ACEX) by watching the behavior on video and scoring each period and by monitoring the cortical FP activity. During SWS, the animal lays with eyes closed and the cortical FP displays large amplitude synchronous oscillations (measured as a large peak in the fast Fourier transform (FFT) power spectrum <5 Hz). During REM, the animal lays with eyes closed, no overt movements, and, the cortical FP is flat (devoid of synchronous slow oscillations). Occasionally, during this period, rats can display short instances of very rapid whisker movements. During AWIM, the animal is standing or resting (not laying) with eyes open and generally fixed, and there are no active whisker movements. During ACEX, the animal moves about the cage or stands still while moving its head and whiskers to explore the environment. Periods that did not clearly fall in these categories were excluded from analysis.

Whisker pad stimulation

An insulated stainless-steel bipolar electrode was placed in the left whisker pad subcutaneously to stimulate the whisker pad (Castro-Alamancos 2004a). Electrode pole separation was ~1 mm. The wires were normally placed around whisker C2–C4. All electrodes and connectors were held in place using miniscrews and dental cement on the skull. The whisker pad stimulus consisted of a 1-ms duration electrical stimulus delivered through two wires implanted under the skin of the whisker pad (Castro-Alamancos 2004a; Cohen and Castro-Alamancos 2007). On the first day of recording, animals were placed in a recording arena, during which the appropriate intensity value was determined for each animal. A 100% value (high intensity) was set at an intensity just below that resulting in the subtle movement of a few (3–5) whiskers but no elicitation of muscle twitches (0.25–0.6 mA). The low-intensity value was set at 60% of the high intensity. Other intensities were also tested to derive intensity response curves during each behavioral state by setting the intensity value at 20, 40, 60 (low), 80, 100 (high), or 120%. Frequency response curves were also tested for the low (60%) and high (100%) intensities during each session by adjusting the frequency of a 10-stimulus train between 5, 10, 20 and 40 Hz. Single whisker pad stimuli (0.5 Hz) were delivered every 2 s, while trains of 10 stimuli at different frequencies were delivered every 5 s.

Air-puff whisker stimulation and orienting responses

A hand-held tube (1.5 mm ID) was aimed at the whiskers by the experimenter. The tube was connected to a pressure valve (15 psi) that was opened electrically so that air could be puffed onto the whiskers. The air-puff stimulus lasted 50 ms and was presented ~2 cm from above and slightly forward from the whiskers (at about a 30° angle), so that the whiskers were blown backward as in a retraction. To determine the validity of the air-puff method, the air-puff stimulus was aimed at different parts of the face and body contralateral and ipsilateral to the recording electrodes (see Results). In addition, to address reliability concerns, many trials were repeated to obtain an average response per stimulus condition. To determine the exact time the air puff reaches the whiskers, the same operation was repeated but the air puff was aimed at a sensing piezo-electric device. Thus time 0 for all electrophysiological responses corresponds to the time when the puff reaches the animal. It is worth noting that while the air-puff stimulus is not painful or harmful, it is rather salient based on the orienting responses it can elicit in animals that are awake but not moving (AWIM). Orienting responses were scored by an observer replaying video of each air-puff stimulus and determining the reaction of the animal to the air puff. The orienting scale had three levels. In one extreme was no visible reaction and in the other was an overt orienting response consisting of head/neck and/or body movements with respect to the stimulus. In between these two categories we classified small reactions that fall in a gray area between the two extremes.

Measures and statistical analyses

We measured different FP and MUA response windows, and data points correspond to recording sessions on different days (several sessions per animal). In barrel cortex, we measured the peak amplitude and time to peak (peak latency) of FP responses during a 5- to 30-ms window poststimulus. In superior colliculus, we measured the peak amplitude and time to peak of two different FP responses that encompass different time windows: peak1 (3–8 ms) and peak2 (9–20 ms). Also in superior colliculus, we measured the number of spikes evoked per stimulus of the MUA for three time windows corresponding to peak1 (3–8 ms), peak2 (9–20 ms), and peak3 (21–90 ms). The border between peak1 and peak 2 was slightly adjusted per animal (range: 1–3 ms) based on the evoked responses. Responses were the average of ≥30 stimulus trials per session. Spontaneous firing was measured for each stimulus trial during a window lasting 1 s before the stimulus. To correct the evoked responses with the spontaneous firing, the spontaneous firing value was first adjusted by the duration of the response window and then subtracted from the response. For a trial to be valid, the spontaneous firing period must be in the same behavioral state as the corresponding stimulus period. To measure responses at different frequencies (5, 10, 20, and 40 Hz), we used the last response (10th) in a 10-stimulus train. These responses were compared with the responses evoked by single stimuli at 0.5 Hz from the same state and session.

For each electrophysiological response measured during spontaneous behavior, we began by conducting two-factor ANOVAs of behavioral state (ACEX, AWIM, REM, and SWS) and stimulus intensity (20, 40, 60, 80, 100, and 120%) or stimulus frequency (0.5, 5, 10, and 40 Hz). For frequency responses, we measured the 10th response in a stimulus train. Significant main effects were decomposed by independent comparisons that were either parametric (t-test), if the two groups involved were normally distributed (according to the Shapiro-Wilk normality test), or nonparametric (Mann-Whitney) otherwise; P values were corrected by the number of comparisons.

Results

Effect of behavioral state on spontaneous neural activity

During the recording sessions, the animals displayed many behaviors and we distinguished between four clearly differentiated states (see Methods): ACEX, AWIM, REM, and SWS. Figure 1 shows typical recordings obtained as animals transition between states. Spontaneous MUA firing rate of superior colliculus (SC) neurons increased rather dramatically between SWS and ACEX (Fig. 1, A and B). Firing during AWIM was reduced compared with ACEX (Fig. 1C). Some instances of AWIM produced a complete abolishment of spontaneous firing, whereas others had significant firing that was still well below ACEX. The example traces in Fig. 1C correspond to a
transition between AWIM and ACEX for a MUA recording with two clearly separable units (see arrows). Perusal of barrel cortex FP activity and simultaneous SC MUA activity during SWS revealed that many of the large slow wave oscillations in cortex were accompanied by spiking in SC. Thus a cross-correlation between the SC MUA spikes and the barrel cortex FP revealed a much larger negative FP peak preceding each spike during SWS than during ACEX (Fig. 1D, thick traces). Moreover the correlation was not present when the same MUA spikes were shuffled by shifting them by 2 s (Fig. 1D, thin traces). Population analysis from several experiments in which animals transitioned between the two states in the same session revealed that there was a significantly larger cross-correlation (FP negative peak) during SWS than during ACEX ($n = 8$; $P < 0.01$). This indicates that much of the SC firing during SWS may have been driven by slow oscillations in cortex. Figure 1E shows typical FP and MUA responses evoked in SC, and FP responses evoked in barrel cortex, by whisker pad stimulation (100% intensity). Each response is the average of 30 stimulus trials. The arrows point to peak1 and peak2 in SC.

Figure 2A compares FFT power spectrums from spontaneous FP activity in barrel cortex (i.e., excluding periods of sensory stimulation) during the four different behavioral states for all the recording sessions ($n = 38$ sessions from 8 animals). We found that the power spectrums during awake states (ACEX and AWIM) did not differ significantly between each other except that AWIM has less delta (0–5 Hz) power. Compared with the awake states, SWS produced significantly more power at low frequencies in the delta (0–5 Hz), theta (5–10 Hz), alpha (10–20 Hz), and beta ranges (20–30 Hz) and
significantly less power at higher frequencies in the gamma range (40–50 Hz). REM produced a power spectrum similar to SWS except that during REM, low frequency (0–10 Hz; mostly delta) power was significantly suppressed and higher frequency (20–30 Hz; beta) power was significantly enhanced compared with SWS. Moreover, delta power (0–5 Hz) during REM did not differ compared with ACEX, but these two states differed considerably at higher frequencies. Thus compared with SWS, REM involves a selective suppression of delta oscillations and an increase in beta range oscillations.

Figure 2B compares FFT power spectrums from spontaneous FP activity in barrel cortex (A) and in superior colliculus (B), excluding periods of sensory stimulation, during ACEX, AWIM, paradoxical sleep (REM), and SWS. Statistically significant (\( P < 0.05 \)) differences between each pair of states for each frequency range are marked by an asterisk in the table inset. C: spontaneous firing MUA in superior colliculus, excluding periods of sensory stimulation, during ACEX, AWIM, REM, and SWS. Statistically significant (\( P < 0.05 \)) differences between the behavioral states are marked by brackets.

Figure 2B compares FFT power spectrums from spontaneous FP activity in barrel cortex during different behavioral states, the differences in SC were usually more subtle. We found that the power spectrums during waking states (ACEX and AWIM) differed slightly between each other in that AWIM produced significantly less power in the theta (5–10 Hz) and beta/gamma ranges (35–45 Hz) ranges than ACEX. Compared with the awake states, SWS produced significantly more power in the alpha-to-beta ranges (10–30 Hz) and significantly less power in the gamma range (45–50 Hz). REM differed quite significantly from all the other states. The most notable effect of REM was the much larger theta range (5–10 Hz) oscillations and the suppressed delta range oscillations compared with all other states.

Effects of behavioral state on FP responses in barrel cortex

Next we tested the effect of behavioral state on evoked responses. FP responses evoked in barrel cortex by whisker stimulation are very sensitive to the behavioral state of freely behaving rats and are generally suppressed during arousal (Castro-Alamancos 2004a,b; Castro-Alamancos and Oldford 2002). Figure 3 shows population data (\( n = 38 \) sessions from 8 animals) of FP responses evoked in barrel cortex by whisker pad stimuli of different intensities and frequencies during the
four behavioral states. The effect of stimulus intensity during the different states was tested using low frequency stimuli (0.5 Hz; Fig. 3, A and B), while the effect of stimulus frequency during the different states was tested using both low-intensity (60%; Fig. 3, C and D) and high-intensity (100%; E and F) stimuli. For each FP response evoked in barrel cortex, we measured the peak amplitude (5–30 ms poststimulus) and the time to peak (peak latency). All responses measured in the present study depress (adapt) with frequency. The effect of stimulus frequency was determined by measuring the 10th stimulus in a 10-stimulus train for 5, 10, 20, and 40 Hz, which were compared with single stimuli at 0.5 Hz (i.e., not to the 1st stimulus in the corresponding train). This is important because, in some cases, the first response in a train is affected by the previous trains. For illustrative purposes, Fig. 4 shows the responses evoked by each of the 10 stimuli in a 10-Hz train by low-intensity (60%) and high (100%)-intensity stimuli; data are shown for amplitude measurements in cortex (peak FP amplitude; Fig. 4A) and SC (peak1 and peak2 FP amplitude and MUA; B–E). For cortex FP amplitude, we noticed that during SWS responses showed a pattern with more adaptation to the 3rd stimulus in a train than to the 10th stimulus in a train (Fig. 4A). This phenomenon also occurs in urethan anesthetized animals, and we have previously shown this to be caused by subcortical inhibition because it is abolished by blocking GABA receptors in the subcortical relays (thalamic and trigeminal) leading to barrel cortex (Hirata et al. 2009).

Regarding the effects of behavioral state on responses evoked by different intensities, we found that increases in stimulus intensity significantly increased the peak amplitude (Fig. 3A) and decreased the peak latency (B) of barrel cortex FP responses during all behavioral states. However, the
magnitude of these effects was strongly dependent on behavioral state and stimulus intensity. Thus the peak amplitude of FP responses was largest during SWS and smallest during ACEX, while responses during AWIM and REM were in between these extremes and did not differ between each other (Fig. 3A). Responses evoked during SWS were significantly larger than those evoked during ACEX (for most intensities, ≥40%), AWIM (for high intensities, ≥80%), and REM (for high intensities, ≥100%). Also, responses evoked during ACEX were significantly smaller than those evoked during REM (for high intensities, ≥80%) and AWIM (for most intensities, ≥60%).

Increases in stimulus intensity significantly decreased the peak latency of FP responses during all behavioral states (Fig. 3B). The peak latencies were significantly longer during the sleep states (SWS and REM), which did not differ between each other, than during the awake states (ACEX or AWIM), which also did not differ between each other. Thus responses evoked during SWS by low-intensity stimuli had significantly longer latency peaks than those evoked during ACEX (for low intensities, between 40 and 80%) and AWIM (for low intensities, between 40 and 60%). Also responses evoked during REM had longer latency peaks than those evoked during ACEX (for all intensities, 40–120%) and AWIM (for low intensities, between 40 and 60%). Thus low-frequency FP responses evoked by different intensities have larger amplitudes and longer latencies during SWS and smaller amplitudes and shorter latencies during ACEX.

Regarding the effects of behavioral state on responses evoked by high frequencies, we found that for either low-intensity stimuli (60%; Fig. 3C and D) or high-intensity stimuli (100%; Fig. 3E and F), the peak amplitude of FP responses was...
depressed by increases in frequency (C and E), while the peak latency was generally increased by frequency (D and F). However, the magnitude of these effects was dependent on behavioral state and stimulus frequency as revealed by comparing the frequency curves among the four different behavioral states (Fig. 3, C and E). Low frequency FP responses (0.5–5 Hz) evoked in barrel cortex are significantly larger during SWS compared with ACEX for both low- and high-intensity stimuli. However, this relationship inverts as stimulus frequency increases because responses evoked during SWS adapt the most, reaching the smallest amplitudes of any state at 40 Hz. Thus high- and low-intensity responses evoked at 40 Hz during awake states (ACEX and AWIM) are significantly larger than those evoked during sleep states (REM and SWS). The peak latency generally increase as responses adapt due to increases in frequency for both low- and high-intensity stimuli (Fig. 3, D and F). In general, responses have significantly longer peak latencies during sleep (SWS and REM) compared with awake states (ACEX and AWIM), but this difference is significant only up to ~10 Hz because it is difficult to accurately determine the peak latency of very depressed (nonexistent) responses at higher frequencies (20–40 Hz).

In conclusion, intensity increases low frequency sensory FP responses in barrel cortex the most during SWS (but those responses have the longest peak latencies) and the least during ACEX (those responses have the shortest peak latencies). Low frequency responses evoked at different intensities during REM and AWIM are not significantly different in peak amplitude but differ in peak latency with REM producing significantly longer latencies. Increases in frequency depress the peak amplitude and increase the peak latency of FP responses the most during SWS (these responses are the largest at low frequencies) and the least during ACEX (these responses are already adapted at low frequencies).

Effects of behavioral state on FP responses in SC

Next we tested the effect of behavioral state on FP responses evoked by whisker pad stimulation in SC. We measured the peak amplitude of peak1 (3–8 ms poststimulus) and peak2 (9–30 ms poststimulus) responses. Figure 5 shows population data (n = 20 sessions from 6 animals) of peak1 and peak2 FP responses evoked in SC by whisker pad stimuli of different intensities and frequencies during the four distinct behavioral states, with statistically significant differences (*). The panels show the effect of intensity (A and B) and frequency (C and D) on peak1 and peak2 responses, respectively.
states. The effect of stimulus intensity during the different states was tested using low frequency stimuli (0.5 Hz; Fig. 5, A and B), while the effect of stimulus frequency during the different states was tested using both low-intensity (60%; Fig. 5, C and D) and high-intensity (100%; Fig. 5, E and F) stimuli. It is worth noting that peak2 FP responses evoked by the first stimulus in a 10 Hz train (low intensity; Fig. 4C) differed compared with 0.5 Hz single stimuli (see Fig. 5D). This was mostly because AWIM responses to the first stimulus in a train were highly sensitive and tended to depress during ongoing trains (delivered every 5 s; see METHODS).

Regarding the effects of behavioral state on low frequency responses evoked by different intensities, we found that increases in stimulus intensity significantly increased the amplitude of peak1 (Fig. 5A) and peak2 (B) SC responses during all behavioral states. However, the magnitude of these effects was dependent on behavioral state and stimulus intensity. The peak amplitude of peak1 FP responses were larger during AWIM compared with ACEX (for most intensities, 60–100%), REM (for most intensities, 60–100%), and SWS (for only 60% intensity). Peak1 FP responses were also significantly larger during SWS than during REM (for high intensities, 80–100%). The amplitude of peak2 FP responses was significantly larger during AWIM than during ACEX (for most intensities, 60–100%) and REM (for most intensities, 60–100%). Peak2 FP responses were also significantly larger during SWS than during ACEX (for most intensities, 60–100%). Thus low-frequency peak1 and peak2 FP responses evoked by different intensities were generally larger during the quiescent/deactivated mode (AWIM and SWS) than during the activated mode (REM and ACEX). Regarding peak latencies (not shown), we found some significant peak latency differences, but they were all relatively small and in the range of 0.5–1.1 ms. For instance, low frequency peak1 FP responses evoked at different intensities had significantly longer peak latencies during SWS than during ACEX and AWIM (for intensities >80%; P < 0.01). Low frequency peak2 FP responses had significantly longer peak latencies during SWS and REM than during AWIM (for intensities >40%; P < 0.01) and ACEX (for the 120% intensity only; P < 0.01).

Regarding the effects of behavioral state on responses evoked by different frequencies, we found that for either low-intensity stimuli (60%; Fig. 5, C and D) or high-intensity stimuli (100%; E and F), the peak amplitude of peak1 (C and E) and peak2 (D and F) FP responses was depressed by increases in frequency. However, comparison of the frequency curves revealed differences between the states. The amplitude of peak1 FP responses evoked at different frequencies by low- or high-intensity stimuli were larger during AWIM compared with REM but mostly for low frequencies (0.5–5 Hz for low intensity and 0.5–10 Hz for high intensity; Fig. 5, C and E). Similarly, the amplitude of peak2 FP responses evoked at different frequencies by low-intensity stimuli were larger during AWIM compared with ACEX (for frequencies between 0.5 and 20 Hz except for 10 Hz) and REM (for frequencies between 0.5 and 10 Hz; Fig. 5D). Finally, the amplitude of peak2 FP responses evoked at different frequencies by high-intensity stimuli were larger during AWIM compared with REM (for frequencies between 0.5 and 10 Hz; Fig. 5F). Regarding peak latencies (not shown), the peak1 FP responses evoked at different frequencies by low- or high-intensity stimuli had significantly longer latencies during SWS than during ACEX and AWIM (for frequencies >10 Hz; P < 0.01). Also the peak2 FP responses evoked at different frequencies by high-intensity stimuli (but not by low-intensity stimuli) had significantly longer latencies during REM and SWS than during AWIM (at 5 and 10 Hz; P < 0.01).

In conclusion, intensity increases sensory peak1 and peak2 FP responses in SC the most during the quiescent/deactivated mode (AWIM and SWS) and the least during the activated mode (ACEX and REM). Thus response amplitudes depend on the level of activation not on whether the animal is sleeping or awake. However, response latencies tend to be longer during sleep states (SWS and REM) than during waking states (ACEX and AWIM). Increases in frequency sharply depress peak1 and peak2 FP responses in SC, and responses are generally largest during AWIM and smallest during REM.

Effects of behavioral state on MUA responses in SC

Figure 6 shows population peristimulus time histograms (PSTHs) of MUA in SC (n = 25 sessions from 6 animals) evoked during four different behavioral states by low frequency (0.5 Hz) whisker pad stimuli of varying intensities (20–120%; Fig. 6A) and of varying frequencies (0.5–40 Hz) at either low intensity (60%, B) or high intensity (100%, C). Figure 7 shows response measurements taken from the individual PSTHs. Spikes per stimulus were measured (n = 25 sessions from 6 animals) for three different response windows; peak1 (3–8 ms poststimulus), peak2 (9–20 ms poststimulus), and peak3 (21–90 ms). Because there was a significant effect of behavioral state on spontaneous firing (see Fig. 2C), the evoked responses (Fig. 7) are presented corrected for the effects of spontaneous firing. This is done by subtracting from the evoked response the equivalent spontaneous firing measured during a 1 s window prior to each stimulus.

Regarding the effects of behavioral state on MUA responses evoked by different intensities (Fig. 7, A–C), we found that during all behavioral states, increases in stimulus intensity significantly increased peak1 (A), peak2 (B), and peak3 (C) MUA responses in SC. The magnitude of these effects was dependent on behavioral state and stimulus intensity. Peak1 MUA responses evoked during AWIM were significantly larger than those evoked during ACEX (for most intensities, 60–100%), while responses evoked during SWS were larger than ACEX (for high intensities, 100–120%). Peak2 MUA responses were significantly larger during SWS than during ACEX (for most intensities, 60–120%) and REM (for most intensities, 60–120%). Also peak2 MUA responses evoked during AWIM were significantly larger than those evoked during ACEX (for all intensities, 20–120%) and REM (for most intensities, 40–100%). Thus peak2 responses are larger during the quiescent/deactivated mode than during the activated mode, and these effects on peak2 (i.e., corticocortical) SC responses appear to reflect the impact of behavioral state on barrel cortex responses, which are also largest during the SC quiescent/deactivated mode. Finally, peak3 MUA responses evoked during SWS are significantly smaller than those evoked during REM (for all intensities, 20–120%), AWIM (for some intensities, 80–100%), and ACEX (for most intensities, 60–120%). Also peak3 MUA responses are larger during REM compared with AWIM (for low intensities, 20–60%) and are also larger during ACEX compared with AWIM (for low intensities, 20–60%).
Regarding the effects of behavioral state on responses evoked by different frequencies, for either low-intensity stimuli (60%; Fig. 7, D–F) or high-intensity stimuli (100%; G–I), peak1 (D and G), peak2 (E and H), and peak3 (F and I) MUA responses were depressed by increases in frequency. However, comparison of the frequency curves revealed differences between the states. Peak1 MUA responses evoked at different frequencies by low- or high-intensity stimuli were mostly not different between different states except that REM produced significantly smaller responses than AWIM (for most high frequencies, 5–20 Hz). Peak2 MUA responses evoked by low intensity (Fig. 7F) did not differ significantly for high frequencies except that 40 Hz responses during SWS were larger than during the activated mode (ACEX and REM). Peak2 MUA responses evoked by high-intensity stimuli at high frequencies (particularly 40 Hz) were generally smaller during the activated mode than during the quiescent/deactivated mode (see Fig. 7H for significant differences). Peak3 MUA responses evoked at very high frequencies (20–40 Hz) did not differ between states. However, lower frequency (0.5–10 Hz) responses during REM were generally larger than those during the quiescent/deactivated mode (AWIM and SWS).

In conclusion, peak1–peak3 evoked responses, corrected by subtracting the spontaneous firing, are increased by stimulus intensity and depress with frequency. Peak1 responses are little affected by behavioral state and are generally larger during AWIM. Low frequency peak2 responses are particularly sensitive to behavioral state, so that the quiescent/deactivated mode (AWIM and SWS) produces larger peak2 responses than the activated mode (ACEX and REM), but peak2 responses are strongly depressed during high frequencies. Interestingly, an opposite trend is present for peak3 responses evoked by different intensities, which are generally larger during the activated mode, particularly REM, and smallest during the quiescent/deactivated mode, particularly SWS.

**Sensory responses depend on behavioral reactions**

Next we tested the effects of an air puff delivered to the whiskers on sensory responses in the SC. Figure 8A shows MUA responses in SC and FP responses in barrel cortex evoked by a 50-ms air puff delivered to the whiskers (contralateral or ipsilateral to the recording electrodes) or to the trunk (body) of freely moving rats during AWIM (n = 12 sessions from 4 animals). Stimulation of the whiskers contralateral to the recording electrode evoked a clear sensory response in both SC and barrel cortex, and negligible or much smaller responses to stimulation of the ipsilateral whiskers or the body (see Fig. 8A). In some cases, we tested the effect of the air puff delivered from the same location but without impacting the animal at all. In these cases, the response was completely flat (not shown). Using this method, we tested the effect of behavioral state during AWIM and ACEX on sensory...
responses evoked by the air-puff stimulus delivered to the whiskers contralateral to the recording electrode \( (n = 8\) sessions in 3 animals). Responses were not tested during sleep because the air puff tended to wake-up animals, precluding repetition of stimulus trials. For these experiments, during ACEX the animals were never in locomotion. Consistent with the results obtained with whisker pad electrical stimuli, air-puff stimuli delivered during AWIM produced significantly larger responses than during ACEX either when responses were corrected or not corrected by spontaneous firing \( (P < 0.01; \text{Fig.} \; 8B)\). While these results reproduce those obtained with whisker pad stimuli, differences in the stimulus effects (e.g., magnitude of whisker deflection due to whisker movements) between both states may have contributed to the air-puff results.

In addition to waking-up sleeping animals, the air puff produced varying degrees of behavioral reactions in animals during AWIM. These behavioral reactions were classified off-line by analyzing video recordings, as no reaction, small reaction (a small movement), or as a clear orienting response to the stimulus. Interestingly, the MUA responses evoked in the SC and the FP responses evoked in the barrel cortex by the air-puff stimuli depended on the reaction of the animal (Fig. 8, C and D); sensory responses were largest when animals produced clear orienting responses to the stimulus. In conclusion, sensory responses evoked by air-puff stimuli are largest in both barrel cortex and SC when the animal is quiescent and orients to the stimulus.

**DISCUSSION**

The results indicate that spontaneous firing in SC is greatly dependent on behavioral state being much higher during ACEX and REM compared with AWIM and SWS. Thus according to firing rate, the SC is in an activated mode during ACEX and REM and in a quiescent/deactivated mode during AWIM and SWS. Moreover, the activated mode is associated with theta range oscillations of the FP in SC, but the origin of this activity is currently unknown; it could be volume-conducted to the SC. In barrel cortex, FP power spectrums do not vary in relation to SC activated and quiescent/deactivated modes but instead correlate better with whether the animal is sleeping (SWS and REM) or awake (ACEX and AWIM).
Sensory evoked responses are also dependent on behavioral state. FP responses in barrel cortex increase the most as a function of stimulus intensity during the SC quiescent/deactivated mode (AWIM and SWS) and the least during the activated mode (ACEX and REM), but the large responses present during the quiescent/deactivated mode have longer peak latencies. Fittingly, larger evoked responses during the quiescent/deactivated mode are also evident for corticotectal (peak2) FP and MUA responses in SC; known to depend on barrel cortex feedback (Cohen et al. 2008). Finally, whisker responses evoked by air-puff stimuli are largest in both barrel cortex and SC when the animal is quiescent and orients to the stimulus.

The present results are descriptive in nature and, as discussed in the following text, give rise to a number of imperative new questions for future work.

**SC spontaneous firing**

Based on spontaneous firing, the SC appears to come on-line during active exploratory states and rapidly goes off-line during awake immobile periods. Particularly interesting are some periods during awake immobility when SC firing ceases completely; an effect that has been previously pointed out (Weldon and Best 1992). This may indicate a tonic inhibitory input during this state. One possible source for such a tonic inhibition could be the nigrosectal pathway, which is well known to tonically inhibit the SC (Chevalier et al. 1981; Jiang et al. 2003). Future work is needed to address the functional role and the source of this inhibition of SC firing during some instances when animals are awake but immobile. The results also revealed a large increase in SC firing during active exploration. This is significant because during anesthesia, many whisker-responsive cells have nil spontaneous firing (Cohen et al. 2008; Hemelt and Keller 2007). A large number of variables may contribute to the increased firing. Among them, whisker contacts and movements during active (exploratory) whisking may be important contributors. Future work will test this possibility. Another option is that whisker responsive cells are driven by movements of other body parts during active exploration. For example, SC is known to control the direction and speed of eye (McHaffie and Stein 1982) and head (Dean et al. 1986) movements. Thus the active exploration related neural activity may have a function in sensing contact and driving movements, but it may also function as a corollary signal indicating that movement is occurring (Sommer and Wurtz 2008). Future work in the rodent vibrissa system will have to tease these options apart.

Another interesting observation is that firing in SC is not eliminated during sleep. Instead during SWS, firing seems to be driven by slow oscillations in neocortex via the corticotectal pathway. This is expected because corticotectal influence is strongest during the quiescent/deactivated mode (Cohen et al. 2008). Manipulation of corticotectal activity in sleeping animals can test this hypothesis directly in future work. A large increase in firing comparable to that observed during active exploration is also observed in sleeping animals during paradoxical sleep. The driver for this activity is not known but cannot be movement or sensory input such as during active exploration. One possibility is that SC during paradoxical sleep is driven by the actions of specific neuromodulator systems. For example, activity in cholinergic brain stem nuclei is significant during this sleep stage (el Mansari et al. 1989), and these nuclei innervate the SC (Beninato and Spencer 1986; Billet et al. 1999; Krauthamer et al. 1995).

**Sensory responses**

In addition to the effects on spontaneous firing, sensory responses in SC are also dependent on behavioral state. In particular, corticotectal responses (peak2) evoked by whisker pad stimuli...
were larger during the quiescent/deactivated mode than during the activated mode of SC. However, longer latency responses (peak3) were instead typically larger during the activated mode. Thus sensory evoked responses that are driven by different neural circuits are regulated differently by behavioral state. The stronger corticocortical responses of whisker-sensitive cells in the intermediate layers of the SC during the quiescent/deactivated mode may be useful as a powerful alerting stimulus in an animal that is sleeping, drowsy, or unattentive and an unknown moving object or animal makes contact with its whiskers. Because the target of these cells in deeper layers and in the brain stem drive orienting responses (Dean et al. 1989; Redgrave et al. 1987b; Westby et al. 1990), such a powerful alerting output makes good functional sense. Moreover, this enhanced output during the quiescent/deactivated mode of SC may also serve to trigger forebrain activation in quiescent animals by impacting on neuromodulatory systems in the midbrain and brain stem that cause cortical activation (Castro-Alamancos 2004b), which are well-known targets of SC cells (Dean et al. 1989; Redgrave et al. 1987a, 1993).

Orienting affects sensory responses

As already mentioned, the SC is well known to be involved in orienting responses to stimuli from a wide range of modalities. This has been particularly well investigated at the electrophysiological level in the visual system of primates producing saccades to visual targets (for reviews, see Sommer and Wurtz 2008; Wurtz and Albano 1980). In rats, we found that the neural activity evoked in SC by air puffs aimed at the contralateral whiskers depend on the orienting response that the animal produces to the stimulus. Whisker-responsive cells in the intermediate layers of the SC are associated with the contralaterally projecting predorsal bundle (Westby et al. 1990), which mediates approach movements toward novel stimuli (i.e., orienting responses) (Dean et al. 1989). Thus enhancement of sensory responsiveness through this pathway during orienting responses makes functional sense; the stronger neural activity during overt orienting responses may well reflect the neural drive for the orienting responses.

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