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Report

Rapid Whisker Movements in Sleeping Newborn Rats

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Summary

Spontaneous activity in the sensory periphery drives infant brain activity and is thought to contribute to the formation of retinotopic and somatotopic maps [1–3]. In infant rats during active (or REM) sleep, brainstem-generated spontaneous activity triggers hundreds of thousands of skeletal muscle twitches each day [4]; sensory feedback from the resulting limb movements is a primary activator of forebrain activity [1]. The rodent whisker system, with its precise isomorphic mapping of individual whiskers to discrete brain areas, has been a key contributor to our understanding of somatotopic maps and developmental plasticity [5–7]. But although whisker movements are controlled by dedicated skeletal muscles [8, 9], spontaneous whisker activity has not been entertained as a contributing factor to the development of this system [10]. Here we report in 3- to 6-day-old rats that whiskers twitch rapidly and asynchronously during active sleep; furthermore, neurons in whisker thalamus exhibit bursts of activity that are tightly associated with twitches but occur infrequently during waking. Finally, we observed barrel-specific cortical activity during periods of twitching. This is the first report of self-generated, sleep-related twitches in the developing whisker system, a sensorimotor system that is unique for the precision with which it can be experimentally manipulated. The discovery of whisker twitching will allow us to attain a better understanding of the contributions of peripheral sensory activity to somatosensory integration and plasticity in the developing nervous system [11–13].

Results and Discussion

Newborn Rats Exhibit Whisker Twitches during Active Sleep

Previous studies of whisker movements in infant rats have focused primarily on the emergence of synchronized movements of the whiskers (i.e., whisking) in awake animals [14–16]. Because whisking does not develop until the end of the second postnatal week, self-generated whisker movements have been overlooked as possible contributors to the perinatal development of whisker system morphology or plasticity. Here we asked whether asynchronous whisker movements (i.e., twitching) occur during active sleep in 3- to 6-day-old rats (n = 5), similar to the twitches that occur in other limbs controlled by skeletal muscles [17].

Using high-speed videography (200 frames/s), we assessed movements of the whiskers and mystacial pad as pups cycled between sleep and wakefulness (Figure 1A). During periods of active wakefulness when high-amplitude limb movements (e.g., kicking, stretching) were seen, whisker and mystacial pad movements were noisy and seemingly haphazard. All movements subsided during the transition from wakefulness to quiet sleep. Then, with the onset of active sleep as the distal limbs and tail began to twitch, contemporaneous bouts of whisker twitches were observed. We saw a diversity of whisker movements (Figure 1B; see Movie S1 available online), including independent twitches of single whiskers, simultaneous twitches of adjacent or nonadjacent whiskers, and complex movements comprising various subsets of whiskers moving in variable directions. Independent whisker twitches were easily distinguished from events involving pronounced movements of the mystacial pad in which all whiskers moved in unison. This is the first demonstration of whisker twitching in sleeping infant rats, although they have been noted anecdotally in sleeping adults [18].

Working from a catalog of 51 whisker twitches, we calculated mean maximum whisker displacements from rest of 0.13 ± 0.01 mm (range: 0.02–0.27 mm) and mean angular velocities of 121 ± 7 deg/s (range: 34–255 deg/s). The mean latency from twitch onset to maximum displacement was 65.4 ± 3.4 ms (range: 30–150 ms). Although the majority of these whisker movements were in the protraction and retraction directions, movements in a diversity of other directions were also observed (Figure S1). The patterns of movements observed here are consistent with the known anatomy of the whisker muscle system [8, 9], as well as findings in adults from whisker muscle and facial nucleus stimulation studies [19, 20] and observations of adjacent whisker movements [21].

Extrinsic Whisker Muscles Twitch during Active Sleep

Whiskers are controlled by a complex system of extrinsic and intrinsic muscles [8, 9]. To ensure that the whisker twitches observed using high-speed videography were not due to movement artifact, we recorded electromyographic (EMG) activity from two extrinsic whisker muscles in 4- to 6-day-old rats (n = 4), m. maxillolabialis and m. nasolabialis (Figure 2A). We also recorded EMG activity from the nuchal muscle, the primary elevator of the head, which provides a reliable measure of behavioral state [22]. Both extrinsic whisker muscles twitched during periods of active sleep when twitches occurred in the nuchal muscle (Figure 2B). Twitching in nuchal and extrinsic muscles exhibited strong and significant cross-correlations (Figure 2C). Similar cross-correlations were observed in the three other subjects. In general, these extrinsic whisker muscles exhibited sleep-wake profiles and patterns of twitching similar to those found in other skeletal muscles [23, 24].

Whisker Thalamus Exhibits Twitch-Dependent Activity

Sensory feedback from twitching limbs increases neural activity in the somatosensory thalamus and cortex of infant rats [1], as well as hippocampus [24]. Moreover, in the whisker system, thalamic and cortical mechanisms are responsive to mechanical whisker stimulation soon after birth [25, 26]. We found that whisker twitches result in sensory feedback to the

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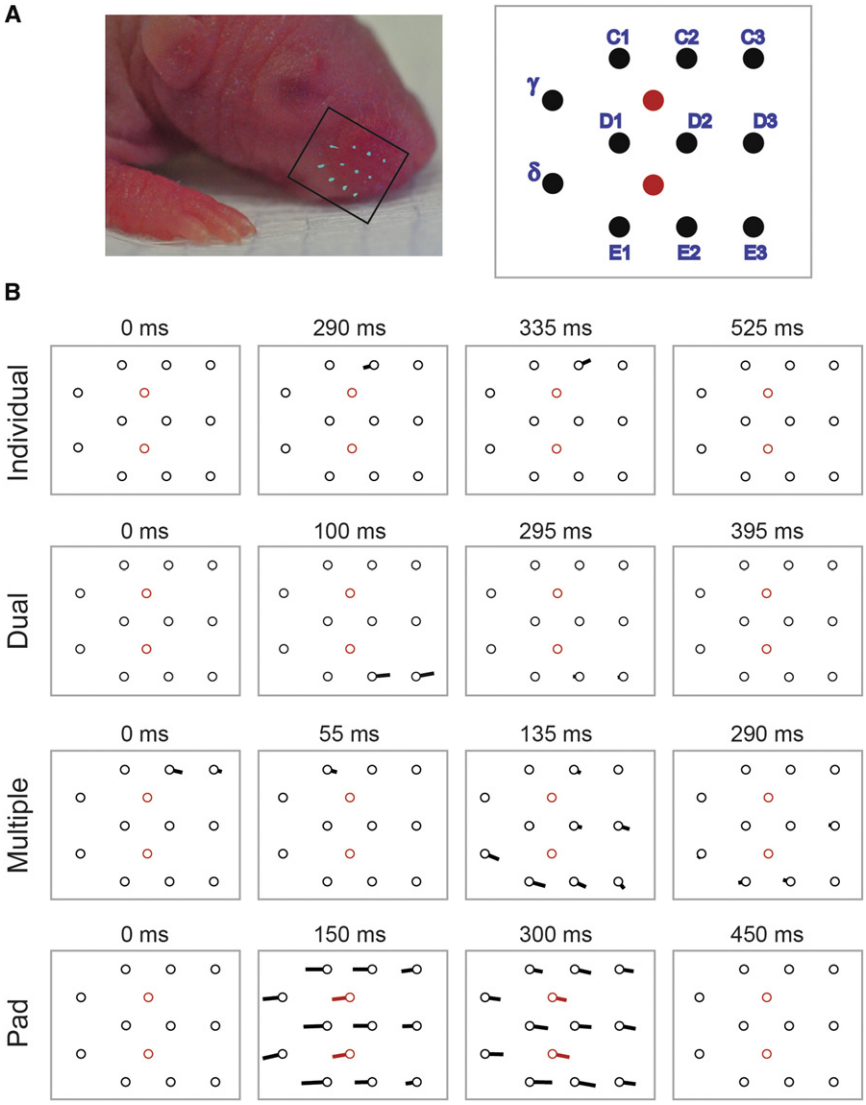

Figure 1. Diverse Patterns of Whisker Movements during Active Sleep in 4- to 6-Day-Old Rats

(A) Left shows one side of the snout, highlighting 11 marked whiskers (boxed area). Right shows labels and relative locations of the 11 marked whiskers (black circles); also shown are the two markings on the skin surface of the mystacial pad (red circles).

(B) Quiver plots depicting four types of whisker and mystacial pad movements observed using high-speed videography. The locations of the whiskers (black circles) and the mystacial pad markings (red circles) correspond to those in (A). The lines emanating from the circles are proportional to the whisker or pad displacement over the previous 25 ms (five frames); the direction of movement is also indicated. The four patterns depicted correspond to Movie S1. See Figure S1 for additional examples of the diversity of whisker trajectories.

ventral posteromedial nucleus (VPM), a primary thalamic input of the whisker system. Overall, 7 VPM units were isolated from 3- to 6-day-old rats (n = 7) (Figure 3A). For one representative unit (Figures 3B–3E), firing rate increased during periods of active sleep (Figure 3B) and exhibited a significant increase in firing rate 100 ms after a twitch, peaking at approximately 250 ms (Figure 3C). Similarly significant relationships between unit activity and twitching were documented in 5 other VPM units (one subject's EMG record was too noisy for this analysis).

All 7 VPM units exhibited bursts of neural activity. We used frequency histograms of interspike interval (ISI; Figure 3D) to define a VPM burst as the occurrence of two or more spikes with ISIs $\leq$ 150 ms. Across all subjects, the number of spikes per burst varied widely and, for some subjects, more than ten spikes per burst was not uncommon. Bursts predominated during sleep (Figure 3E). Moreover, their rate of occurrence was significantly higher during active sleep both within each subject individually (ps < 0.05) and across all subjects (t_6 = 7.4, p < 0.001; Figure 3F). The state-dependent thalamic bursting observed here is consistent with previous observations in neonatal cortex and hippocampus [1, 24] and may reflect the action of corollary discharge mechanisms [27, 28]. (This thalamic bursting should not be conflated with that observed in awake adult rats during so-called "whisker twitching," defined in that study as rhythmic 7–12 Hz whisker movements [29].)

The temporal relationship between whisker twitching and VPM activity is similar to that reported previously between limb twitching and forebrain activity, for which a causal role for twitching has been established [1, 24]. We were able to assess causality between whisker twitching and thalamic activity in one subject before, during, and after peripheral sensory blockade while recording a twitch-dependent VPM unit (Figure S2). Within 11 min of lidocaine (1%) injection into the mystacial pad, the rate of VPM bursts declined substantially as sleep-related twitching continued, consistent with peripheral sensory blockade [30]. As the effects of the lidocaine dissipated, the rate of VPM bursts returned to preinjection levels. Therefore, whisker twitches can drive VPM activity during active sleep.

Barrel Cortex Exhibits Spontaneous Activity during Active Sleep

As early as the day of birth, VPM activity is sufficient to activate barrel cortex in a precise topographic manner [26]. In light of the finding above that VPM units increase their firing rate within 100 ms of a twitch, we predicted that periods of twitching would also be accompanied by barrel-specific activation patterns. We used voltage-sensitive dye (VSD) imaging to monitor barrel cortex activity during bouts of active sleep (Figure 4A). We imaged three subjects that exhibited significant contralateral VSD responses to whisker stimulation (Figure S3A). In each subject during active sleep, we identified the first ten events in which there was EMG evidence of a twitch along with behavioral confirmation of twitching. We next examined barrel activity within a 500 ms window of each EMG-defined twitch. In total, 26/30 (86.7%) of these twitches

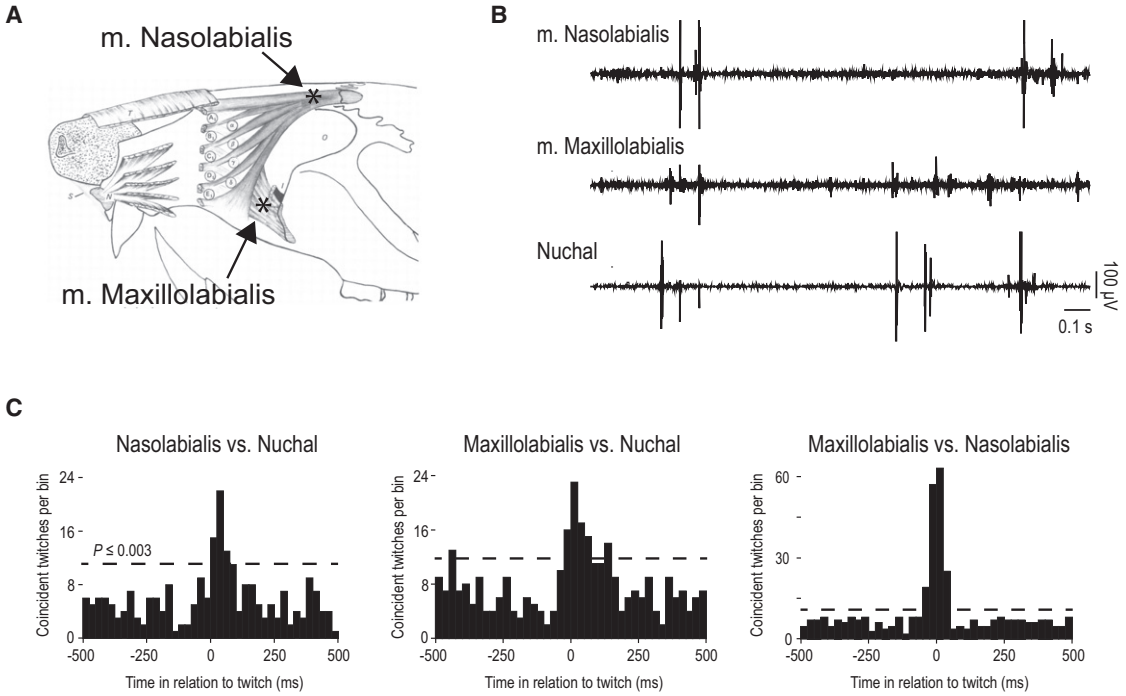

Figure 2. Extrinsic Whisker Muscles Twitch during Active Sleep

(A) Locations of EMG electrodes (black asterisks) in m. maxillolabialis and m. nasolabialis (also referred to as m. levator labii superioris in [8], from which this drawing is adapted).

(B) Raw EMG record depicting twitching in the two extrinsic muscles as well as the nuchal muscle. Sharp spikes in the EMG records indicate myoclonic twitches.

(C) Cross-correlograms showing highly correlated bouts of twitch activity for each pair of muscles. The horizontal dashed line indicates statistical significance.

were followed by clear barrel activity. Varying levels of cortical activation were found, from single to multiple barrels (Figure 4B; Movie S2), mirroring the diversity of whisker movements observed using high-speed videography.

Conclusions

The rodent whisker system affords many advantages to investigators interested in understanding the mechanisms that give rise to somatotopic maps, sensorimotor integration, and developmental plasticity [5, 11–13]. After several decades of research, however, important questions remain as to the instructive role played by the sensory periphery in early development. The present findings suggest that past assessments of the importance of the sensory periphery for the developing whisker system were handicapped by incomplete knowledge regarding the sources and timing of sensory input. Thus, contrary to the view that, in infants before the onset of whisking, whisker stimulation only arises from passive interactions with the mother and littermates [10, 12], we have shown here that self-generated, asynchronous whisker twitches drive brain activity during active sleep in a manner that is strikingly similar to the brain activation produced by twitches elsewhere in the body [1, 17, 24].

We focused here on whisker twitching in 3- to 6-day-old rats, an age when thalamocortical projections to layer 4 are established, when the sensitive period for structural cortical plasticity is ending, but when other aspects of cortical plasticity remain [5, 12, 13, 31]. Given that limb twitches occur in utero and exhibit continuity with postnatal twitching [32], it is likely that whisker twitches also commence in utero and therefore may influence subcortical and cortical development in various ways throughout early prenatal and postnatal life.

As with retinal waves [33], the developmental and spatiotemporal features of twitching may provide clues to its functions. For example, a twitch is characterized by discrete motor output, precisely timed sensory feedback, and high signal-to-noise ratio afforded by the background of muscle atonia [17]. These characteristics may make twitches better suited than other forms of motor activity (such as that produced during waking) to produce the isomorphic mapping and multilevel sensorimotor integration that characterizes the whisker system [34–36]. Also, because whisker twitches are diverse in form and direction (see Figure 1B, Figure S1, and Movie S1), they provide a range of experiences beyond that provided by whisking, which is largely limited to synchronous whisker movements along the protraction-retraction axis [19]. Accordingly, it is tempting to suggest that this diversity of whisker movements during twitching aids in establishing the "pinwheel" map of whisker directional movement [37, 38]. These and perhaps other features of whisker twitch movements, which predominate during the active-sleep-rich period of early infancy [39], may ultimately help us to better understand the functional value of that sleep state for the developing infant [17].

Experimental Procedures

All experiments were approved by the Institutional Animal Care and Use Committee of The University of Iowa. All surgeries were performed under isoflurane anesthesia and all recording was performed in unanesthetized

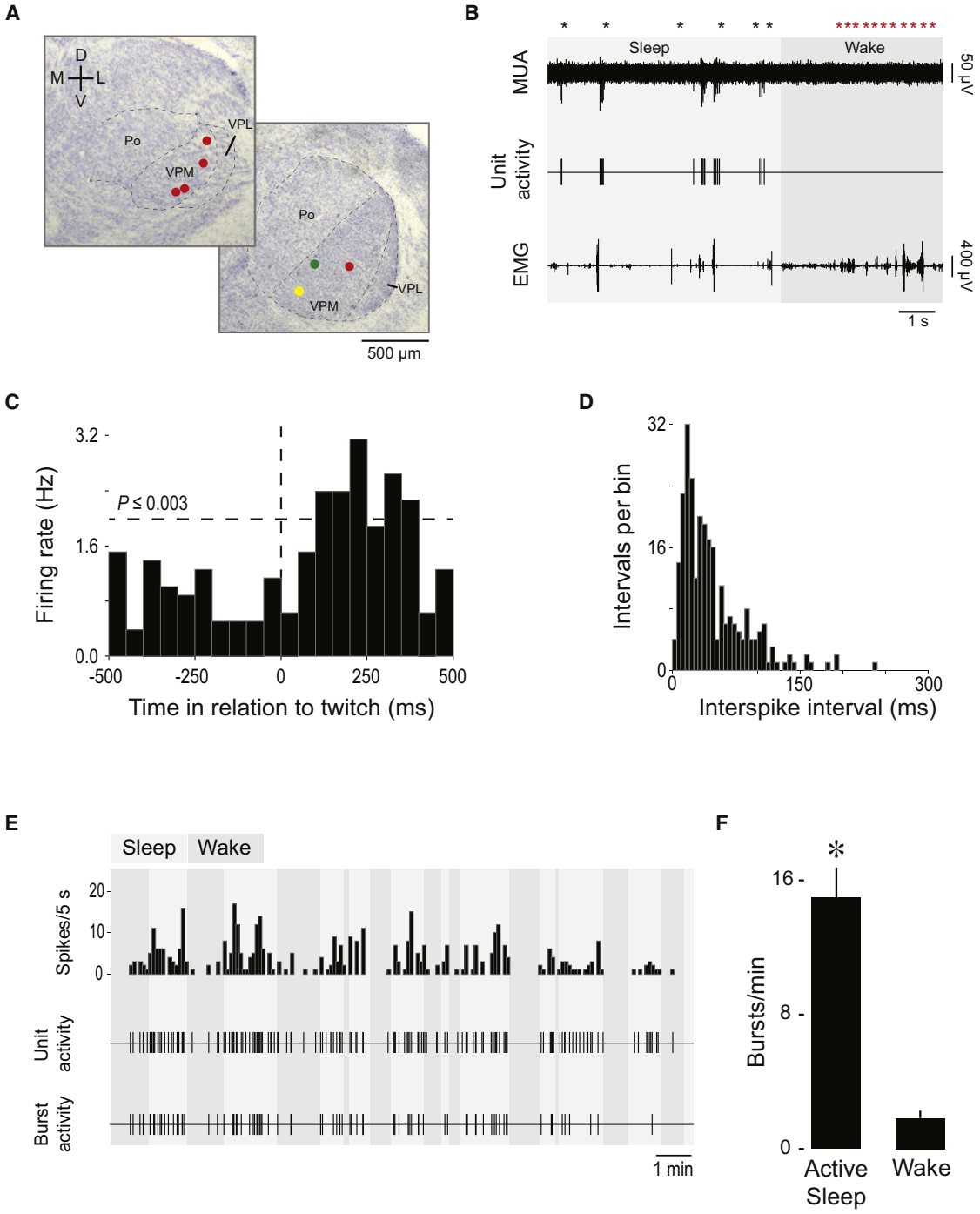

Figure 3. VPM Neurons Fire in Bursts in Close Proximity to Sleep-Related Twitching

(A) Coronal sections through the thalamus to show the recording sites. VPM, ventral posteromedial thalamus; VPL, ventral posterolateral thalamus; Po, posterior thalamus.

(B) Representative data depicting multiunit activity (MUA), single unit activity, and nuchal EMG. Twitch and wake movements (black and red asterisks, respectively) are also shown. Recording site is the yellow circle in (A).

(C) Perievent histogram for the VPM unit in (B). Vertical line at 0 ms denotes time of nuchal muscle twitch and horizontal line indicates statistical significance. The twitch-following properties of another VPM unit were further investigated by anesthetizing the whisker pad (see Figure S2).

(D) Frequency histogram of interspike intervals (ISI) for the VPM unit in (B).

(E) VPM unit firing rate (spikes/5 s), unit activity, and burst activity over a 15 min recording session.

(F) Mean (+SE) bursts/min during active sleep and wake across all seven subjects. * represents significant difference from wake, $p < 0.001$.

subjects. For analysis of whisker movements (n = 5), whiskers were trimmed to 1 mm and the tips were marked with fluorescent paint for recording under ultraviolet-light illumination. A high-speed digital video camera (IDT, Tallahassee, FL) recorded whisker movements, which were tracked and analyzed offline using ProAnalyst software (Xcitex, Cambridge, MA). EMG activity in two extrinsic whisker muscles and nuchal muscle (n = 4) was

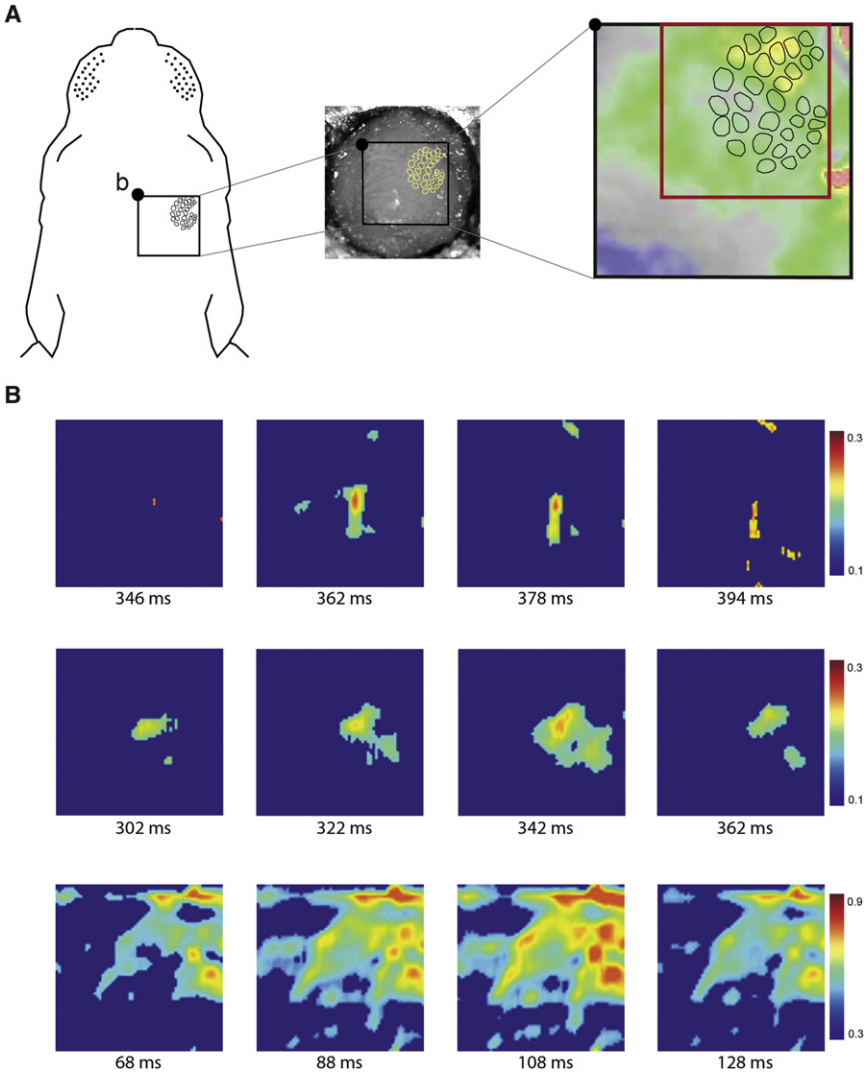

Figure 4. Voltage-Sensitive Dye (VSD) Imaging of Barrel Cortex Activity during Active Sleep in 4-Day-Old Rats

(A) The experimental setup for VSD imaging, highlighting the 4 × 4 mm cranial window (black box) to expose the right barrel cortical field. The 2.5 × 2.5 mm region of interest (ROI) is outlined by the red box. b, bregma. See Figure S3 for methods pertaining to the VSD imaging procedure.

(B) Three representative samples of barrel cortex activity, from isolated activity within 1 or 2 barrels (top two rows) to global activity across the barrel field (bottom row). Each image corresponds to the ROI in (A). The number beneath each frame denotes time from the initiation of a nuchal muscle twitch. Color bars at the right of each sequence indicate range of values of dF/F_0. For clarity of presentation, obvious instances of noise were removed manually from some VSD images.

recorded and analyzed using established methods [19, 24]. For recording of neural activity in the VPM of head-fixed pups (n = 7), the apparatus, methods, and analyses have been described previously [4, 24]. Twitch-triggered perievent histograms were constructed and a randomization procedure was used to test the relationship between VPM activity and twitching [24]. State-related differences in mean VPM burst rates were tested within subjects (Wilcoxon matched-pairs signed-ranks test) and between subjects (paired t test). For voltage-sensitive dye (VSD) imaging of barrel cortex (n = 3), pups were prepared and recorded similarly as above with the addition of a unilateral craniotomy over the right hemisphere (Figure 4A). A voltage-sensitive dye (RH1838; Optical Imaging, Inc., Rehovot, Israel) was applied topically to the dura and a window was created for imaging (MiCAM Ultima, SciMedia Costa Mesa, CA). Imaging trials consisted of contralateral and ipsilateral whisker stimulations (Figure S3A) and spontaneous behavior. dF/F_0 was calculated and analyzed offline using custom-written scripts in MATLAB (MathWorks, Natick, MA) and procedures similar to those described previously [26].

Supplemental Information

Supplemental Information includes three figures, Supplemental Experimental Procedures, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.09.009.

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