Rapid Whisker Movements in Sleeping Newborn Rats

Alexander Tiriac,1 Brandt D. Uitermark,1 Alexander S. Fanning,1 Greta Sokoloff,1 and Mark S. Blumberg1,∗
1Department of Psychology, Program in Behavioral and Cognitive Neuroscience, The University of Iowa, Iowa City, IA 52242, USA

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

*Correspondence: mark-blumberg@uiowa.edu
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\(_0\) = 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.

**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.

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
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
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 (Xcitech, Cambridge, MA). EMG activity in two extrinsic whisker muscles and nuchal muscle (n = 4) was

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.
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 peri-event 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/F0 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.

Acknowledgments

For technical support and guidance we thank Bob McMurray, Cassie Coleman, Ashlynn Gerth, William Todd, Josh Weiner, Jian-Young Wu, and Weifeng Xu. Jenq-Wei Yang and Heiko Luhmann generously provided their custom-written VSD imaging analysis software. This work was supported by grants from the National Institutes of Health (HD63071, MH66424) to M.S.B.

Received: July 29, 2012
Revised: August 28, 2012
Accepted: September 6, 2012
Published online: October 18, 2012

References

    Minlebaev, M., Ben-Ari, Y., and Khazipov, R. (2007). Network mecha-
25. nisms of spindle-burst oscillations in the neonatal rat barrel cortex.
24. Diamond, M.E., von Heimendahl, M., Knutsen, P.M., Kleinfeld, D., and
    and disappearance of cortical events and oscillations in infant rats.
    Brain Res. 1324, 34–42.
    Extracellular muscle activity, rapid eye movements and the development
18. Seelke, A.M.H., and Blumberg, M.S. (2010). The development of
    52, 328–336.
17. Seelke, A.M.H., and Blumberg, M.S. (2010). The development of
    52, 328–336.
    and disappearance of cortical events and oscillations in infant rats.
    Brain Res. 1324, 34–42.
    movements contribute to brain development? Front Neurol 1, 140.
    and disappearance of cortical events and oscillations in infant rats.
    Brain Res. 1324, 34–42.
    Extracellular muscle activity, rapid eye movements and the development
    and disappearance of cortical events and oscillations in infant rats.
    Brain Res. 1324, 34–42.
    movements contribute to brain development? Front Neurol 1, 140.
    movements contribute to brain development? Front Neurol 1, 140.
    movements contribute to brain development? Front Neurol 1, 140.
    movements contribute to brain development? Front Neurol 1, 140.
7. Seelke, A.M.H., and Blumberg, M.S. (2010). Beyond dreams: do sleep-related
    movements contribute to brain development? Front Neurol 1, 140.
    movements contribute to brain development? Front Neurol 1, 140.
Rapid Whisker Movements
in Sleeping Newborn Rats
Alexandre Tiriac, Brandt D. Uitermarkt, Alexander S. Fanning, Greta Sokoloff, and Mark S. Blumberg

1. Supplemental Figures and Tables
   - Figure S1, related to Figure 1
   - Figure S2, related to Figure 3
   - Figure S3, related to Figure 4

2. Supplemental Experimental Procedures

3. Supplemental References
Figure S1. Representative whisker-twitch events in 4-6-day-old rats to show the diversity of whisker trajectories

Six patterns are shown, each color-coded to match the whisker labels in the upper left-hand corner of each plot. The origin of each plot represents the resting position for each whisker. Positive values on the x-axis represent protraction (P) and negative values represent retraction (R). Positive values on the y-axis represent the dorsal direction (D) and negative values represent the ventral direction (V). Values on the x and y axes are in mm.
Figure S2. Sensory Blockade of Whiskers Decreases Peripheral Sensory Feedback to VPM in a 4-Day-Old Rat

(A) Twenty min before lidocaine (1%) injection into the contralateral mystacial pad, the VPM unit exhibits strong activation in response to contralateral whisker stimulation using a fine brush (denoted by horizontal bars). Ipsilateral whisker stimulation does not evoke a response. Both multiunit activity (MUA) and single unit activity are shown.

(B) Perievent histogram for the same VPM unit in (A). The firing rate increases significantly 100 ms after a nuchal muscle twitch, denoted by the vertical line at 0 ms. The horizontal line indicates statistical significance.

(C) The activity of the VPM unit is depicted before and after lidocaine injection. Behavioral state is indicated on the top row, with sleep denoted by light gray and wake denoted by dark gray. Nuchal muscle twitch activity, VPM unit activity, burst activity, and bursts/min are also shown. The number of bursts/min decreases steadily between 11 and 25 min post-injection, before returning to pre-injection levels as the lidocaine wears off. At the end of the recording session, we confirmed that stimulation of the contralateral and ipsilateral whiskers yielded results identical to those in (A) (data not shown). The location of the recording site is indicated by the green circle in Figure 3A.
Figure S3. Barrel Cortex Responses to Evoked Whisker Stimulation in 4-Day-Old Rats

(A) Left: Voltage-sensitive dye (VSD) optical signals to single trials of contralateral (black) and ipsilateral (red) whisker stimulation for one subject with all whiskers intact. Right: Mean (+SE) VSD responses over the entire ROI across 7 pairs of contralateral and ipsilateral trials for the same subject. * significant difference from ipsilateral, $P<0.05$.

(B) VSD images for an additional subject in which all contralateral whiskers except C2 and E2 were plucked and C2 was stimulated. As expected, the C2 barrel (dashed circle) is differentially activated by the whisker stimulation. With this procedure, we were able to orient a map of barrel cortex within the ROI. Color bar at right indicates the range of values for $dF/F_0$. In the optical signal presented below, red arrows indicate acquisition times of VSD images.
Supplemental Experimental Procedures

All experiments were approved by the Institutional Animal Care and Use Committee of The University of Iowa.

Subjects

Sprague-Dawley Norway rats (*Rattus norvegicus*) at 3-6 days of age were used in all experiments. Males and females were used and littermates were always assigned to different experimental groups. Litters were culled to 8 pups within 3 days of birth. Mothers and their litters were housed and raised in standard laboratory cages (48 x 20 x 26 cm). Food and water were available ad libitum. All animals were maintained on a 12-h light-dark schedule with lights on at 0700 h.

High-Speed Videography of Whisker Movements

Five 4- to 6-day-old rats were used. Under isoflurane anesthesia, the pup’s whiskers were trimmed to approximately 1 mm from the skin surface so that all whisker tips were in the same focal plane. The tips were marked with paint that fluoresces under ultraviolet-light illumination (Figure 1A). Several additional marks were placed on the mystacial pad. The skin overlying the skull was removed and a custom-built head-fix device was attached to the skull with cyanoacrylate adhesive [4]. The pup was then transferred to a humidified incubator maintained at thermoneutrality (35 °C). The head-fixed pup was supported on a narrow platform and its limbs dangled freely on both sides of the platform. A high-speed digital video camera (IDT, Tallahassee, FL) with a 105 mm micro lens (Nikon, Tokyo, Japan) recorded whisker movements at 200 frames/s. Two LEDs were positioned on either side of the whiskers to allow simultaneous documentation of sleep and wake limb movements. Motion Studio software (IDT, Tallahassee, FL) was used to record videos. A calibration image was acquired by placing a ruler next to the whiskers. When the pup was cycling between sleep and wakefulness, multiple 10-s recordings of whisker activity were obtained. An experimenter observed the subject and marked the occurrence of myoclonic twitching of the distal limbs and tail (indicative of active sleep) and high-amplitude limb movements (indicative of wakefulness) [1, 2].

In total, 70 videos were recorded. Two independent and blind behavioral scorers reviewed a subset of video clips and produced a catalogue of 29 events comprising 51 whisker movements. These 51 movements were tracked using ProAnalyst. Tracked data for each whisker movement were exported to Excel (Microsoft, Redmond, WA) and mean maximum displacement and angular velocity were calculated [3].

Electromyographic Analysis of Extrinsic Whisker Muscle Activity

Four 4- to 6-day-old rats were used. Under isoflurane anesthesia, pups were prepared for EMG recording from two extrinsic whisker muscles [3] (see Figure 2A) and nuchal muscle (the elevator muscle of the head). After surgery and recovery, the pup was secured in a stereotaxic apparatus. The 3 sets of EMG electrodes were connected to a differential amplifier (A-M Systems, Carlsborg, WA; amplification: 10,000x; filter setting: 300-5000 Hz). EMG signals were sampled at 1 kHz using a digital interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK). Recordings were 15 min in duration and consisted of continuous EMG monitoring of the pup as it cycled freely between sleep and wakefulness. The experimenter, blind
to the EMG signals, marked the occurrence of sleep and wake movements. After the recording session, pups were overdosed with sodium pentobarbital (1.5 mg/g) and the region around the mystacial pad was dissected to confirm EMG placements.

Sleep and wake periods were defined by calculating mean EMG activity, as described previously [4]. Briefly, each nuchal EMG record was rectified and smoothed (τ = 0.05 s). The mean amplitude of high muscle tone and atonia was calculated from five 1-s representative EMG segments, and the midpoint between the 2 means was determined. The EMG signal was then dichotomized into periods of high tone (indicative of wake) and atonia (indicative of sleep). To identify and quantify twitch events [5], wake periods were removed from the EMG records. Next, using a threshold set to 2x the baseline mean EMG value for periods of atonia, twitch events were marked. The temporal correlations among twitches occurring in the nuchal and whisker muscles were analyzed using cross-correlograms [5]. We considered the correlation between twitches in any pair of muscle groups to be significant when at least 1 of the 40 bins in the cross-correlogram had a value greater than the mean of 5 randomized cross-correlograms plus 3 standard deviations (P < 0.003, two-tailed).

**Thalamic Neurophysiology**

A total of 358 3- to 6-day-old rats were used. We identified clear units in 88 subjects and, of these, there were 7 subjects with 7 units for which electrode placement within VPM was confirmed and nuchal EMG records were adequate for analysis. The apparatus and methods used here for recording state-dependent thalamic activity in head-fixed infant rats are similar to those used previously to examine activity in other brain areas [5-8]. Platinum/iridium tetrodes and single-ended electrodes (Thomas Recording, Giessen, Germany), with impedances ranging from 1-2 MΩ, were connected to a data acquisition system (Tucker-Davis Technologies, Alachua, FL; amplification: 10,000x; filter setting: 1-3000 Hz). A chlorinated silver (Ag/AgCl) electrode (MedWire, Mt. Vernon, NY, 0.25 mm diameter), placed into the cerebellum, served as ground. EMG electrodes were connected to a differential amplifier (amplification: 10,000x; filter setting: 300-5000 Hz). Neural and nuchal EMG signals were sampled at 12.5 kHz and 1 kHz, respectively, using a digital interface and Spike2. Brain temperature (36-37 ºC) was measured using a fine-wire thermocouple (Omega Engineering, Stamford, CT) placed in the contralateral cortex. After the electrode was lowered into the thalamus (coordinates: 1.8-2.0 mm caudal to bregma, 1.5-1.6 mm lateral to midline) and state-dependent units were identified, neural, EMG, and behavioral sleep-wake data were recorded for 15 min at a time. As sleep-wake behavior was scored, the experimenter was blind to the EMG and neural signals. After the last recording session, a marking lesion was produced by passing a 50 µA anodal current through the recording electrode for 5 s using a linear stimulus isolator (World Precision Instruments, Sarasota, FL). The pup was then overdosed with sodium pentobarbital (1.5 mg/g) and prepared for histology. Electrode position was verified at 4X magnification using a Leica microscope (Leica Microsystems, Buffalo Grove, IL).

Spike sorting was performed using principal component analysis in Spike2. Signal artifacts were identified and removed manually. A cluster was judged to contain a single unit only if the autocorrelation analysis indicated a refractory period of at least 2 ms. To quantitatively determine the relationship between thalamic activity and twitching, twitch events were first identified and marked as described above. Then, twitch-triggered perievent histograms of thalamic unit activity were constructed and a randomization procedure was used for statistical
testing [5]. Based on the frequency histograms of interspike interval (ISI) for each VPM unit, a burst was defined as two or more action potentials with ISIs ≤ 150 ms. State-related differences in mean VPM burst rates were tested using a Wilcoxon matched-pairs signed-ranks test for within-subjects comparisons and a paired t test for the between-subjects comparison with alpha set at 0.05.

**Voltage-Sensitive Dye (VSD) Imaging of Barrel Cortex Activity**

Three 4-day-old rats were used and, under isoflurane anesthesia, were prepared similarly to those described above for thalamic recording. In addition, a unilateral craniotomy was performed over the right hemisphere (Figure 4A). The skull was scored with a handheld drill along midline from lambda to bregma and approximately 5 mm lateral to midline and was removed in one piece while leaving the dura intact. A voltage-sensitive dye (RH1838; Optical Imaging, Inc., Rehovot, Israel), dissolved at 2 mg/ml in ACSF (pH 7.4), was applied topically to the dura for 90 min while the pup was maintained at thermoneutrality. Every 20-30 min the dye was removed and reapplied to ensure even coverage. After 90 min, the cortex was washed with ACSF for 10 min to remove unbound dye. The craniotomy was then covered in 1% agarose dissolved in ACSF. A coverslip was placed over the agarose to stabilize the cortex and provide a window for VSD imaging (MiCAM Ultima, Scimeda, Costa Mesa, CA).

Each pup was imaged while head-fixed as described above. EMG signals and sleep-wake behaviors were acquired using a differential amplifier (amplification: 10,000x; filter setting: 300-5000 Hz) and digital acquisition system (MP150, Biopac Systems, Goleta, CA). The voltage-sensitive dye was excited with a 150 W halogen light, band-pass filtered through a 650 nm dichroic mirror and focused onto the cortex through a Leica Planapo 2X lens (Leica Microsystems, Wetzlar, Germany). The emitted fluorescence was captured, long-pass filtered at >665 nm, and focused using a Leica Planapo 1X lens onto the detector chip of the camera. The detector chip was 100x100 pixels and received optical signals from a 4x4 mm region of cortex. Placement of the camera over barrel cortex was standardized using previously reported coordinates [9]. The camera was focused on the surface of the cortex and then adjusted to focus no more than 250 μm below the surface. Mechanical stimulation of contralateral whiskers was used to confirm barrel cortex location within the imaging window (Figure S3A).

Stimulation trials consisted of mechanical stimulation of the contralateral or ipsilateral whiskers. Stimulation trials were 4 s in duration and the onset of stimulation was marked with a button press. Behavioral trials were 16 s in duration, during which time the experimenter scored sleep and wake behaviors as VSD images were collected at 500 frames/s. \(dF/F_0\) was calculated and analyzed offline using custom-written procedures in MATLAB (MathWorks, Natick, MA). For offline analysis, a 2.5 x 2.5 mm region-of-interest (ROI) was constructed. For stimulation trials, \(dF/F_0\) values exceeding 1.5% were excluded from the analysis to minimize noise. The \(dF/F_0\) signal of the ROI was considered significant if it exceeded 7 times the standard deviation of the first 8 reference frames [9]. Only if activity in the ROI was significantly higher for contralateral stimulation trials (one-tailed t test; \(P < 0.05\)) were spontaneous behavior trials also analyzed.

For one additional 4-day-old rat, all whiskers except C2 and E2 were plucked on the side contralateral to the craniotomy. When the C2 whisker was stimulated, we observed evoked activity isolated to the C2 barrel (Figure S3B). Single whisker stimulation allowed us to orient a map of barrel cortex within the ROI.
Supplemental References


