REM Sleep Twitches Rouse Nascent Cerebellar Circuits: Implications for Sensorimotor Development

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ABSTRACT: The cerebellum is critical for sensorimotor integration and undergoes extensive postnatal development. During the first postnatal week in rats, climbing fibers polyinnervate Purkinje cells and, before granule cell migration, mossy fibers make transient, direct connections with Purkinje cells. Activity-dependent processes are assumed to play a critical role in the development and refinement of these and other aspects of cerebellar circuitry. However, the sources and patterning of activity have not been described. We hypothesize that sensory feedback (i.e., reafference) from myoclonic twitches in sleeping newborn rats is a prominent driver of activity for the developing cerebellum. Here, in 6-day-old rats, we show that Purkinje cells exhibit substantial state-dependent changes in complex and simple spike activity—primarily during active sleep. In addition, this activity increases significantly during bouts of twitching. Moreover, the surprising observation of twitch-dependent increases in simple spike activity at this age suggests a functional engagement of mossy fibers before the parallel fiber system has developed. Based on these and other results, we propose that twitching comprises a unique class of self-produced movement that drives critical aspects of activity-dependent development in the cerebellum and other sensorimotor systems. © 2014 Wiley Periodicals, Inc. Develop Neurobiol 00: 000–000, 2014

Keywords: **cerebellum; myoclonic twitching; sleep; reafference; corollary discharge**

INTRODUCTION

During rapid eye movement (REM, or active) sleep, skeletal muscles throughout the body twitch, causing jerky movements of the limbs (Blumberg et al., 2013a), eyes (Seelke et al., 2005), and even whiskers (Tiriac et al., 2012). Across many species of mammals, twitching is one of the most conspicuous of all infant behavior (Gramsbergen et al., 1970; Jouvet-Mounier et al., 1970). The numbers tell the story: each day, across all skeletal muscles, infant rats produce hundreds of thousands, if not millions, of twitches (Blumberg et al., 2013b).

Similar to other forms of spontaneous activity in the developing nervous system (Katz and Shatz, 1996; O'Donovan, 1999), sensory feedback (or reafference) from twitching limbs has been implicated in the self-organization of spinal circuits and somatotopic maps (Petersson et al., 2003; Khazipov et al., 2004; Blumberg et al., 2013b). This implication rests in part on the quantity and patterning of neural activity associated with twitch-related reafference. First, using novel methods developed for in vivo recording in rat pups, Khazipov et al. (2004) discovered that

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twitches and other movements trigger oscillatory events, called spindle bursts, in primary somatosensory cortex. Second, subsequent work has demonstrated that, at least under some testing conditions, twitch-related reafference potently increases neural activity, whereas wake-related reafference does not (Mohns and Blumberg, 2010; Tiriac et al., 2012). These and other observations are building a case for considering twitching as a unique form of spontaneous activity that contributes to the development of sensorimotor systems (Blumberg et al., 2013b).

Any discussion of sensorimotor integration must include the cerebellum, a hindbrain structure that receives sensory and motor signals from spinal cord, brainstem, and cerebral cortex (Apps and Garwicz, 2005; Odeh et al., 2005; Huang et al., 2013). Inputs to the cerebellum arrive primarily through one of two pathways (Eccles et al., 1967): climbing fibers that arise exclusively from the inferior olive and mossy fibers that arise from several brainstem nuclei (e.g., pontine gray, external cuneate). Climbing fibers and mossy fibers directly or indirectly modulate the activity and plasticity of Purkinje cells (Ito, 1989), the sole output of the cerebellar cortex.

Cerebellar circuitry develops substantially over the first three postnatal weeks in rats (Altman, 1972; Shimono et al., 1976; Wang and Zoghbi, 2001). Although molecular cues play important roles in shaping cerebellar development (Wang and Zoghbi, 2001; Sillitoe and Joyner, 2007), activity-dependent mechanisms may be equally important. For example, neural activity contributes to synapse elimination and climbing fiber translocation at Purkinje cells (Kakizawa et al., 2000; Andjus et al., 2003; Kano and Hashimoto, 2011; Watanabe and Kano, 2011; Mikuni et al., 2013), as well as spinocerebellar afferent topography (Tolbert et al., 1994). It is also likely that activity-dependent processes guide the convergence of somatotopically related climbing fiber and mossy fiber inputs onto Purkinje cells (Odeh et al., 2005; Pijpers et al., 2006; Huang et al., 2013).

Little is currently known about the sources of neural activity that drive activity-dependent development of the infant cerebellum (Hashimoto and Kano, 2013). Spontaneous neural activity may contribute. Also, sensory experiences arising from interactions with the mother and littermates could drive neural activity in the cerebellum. Indeed, despite their immaturity in newborns, climbing fibers and mossy fibers transmit sensory information from the periphery to Purkinje cells (Puro and Woodward, 1977a,b).

Here we test the novel hypothesis that sensory feedback from myoclonic twitching drives activity in nascent cerebellar circuits. Recording from cerebellar cortex in 6-day-old rats, we report that Purkinje cell activity increases substantially and predominantly during periods of twitching. These results reveal for the first time a prodigious source of spatiotemporally structured activity to this critical sensorimotor structure during a period of extreme immaturity and rapid developmental change.

MATERIALS AND METHODS

Subjects

Six-day-old male and female Sprague-Dawley Norway rats (*Rattus norvegicus)* from nine litters were used. Litters were culled to eight pups within 3 days of birth. Mothers and their litters were housed and raised in standard laboratory cages (48 × 20 × 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. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) and were approved by the Institutional Animal Care and Use Committee of the University of Iowa.

Surgery

A pup with a visible milk band was removed from the home cage. Under 2–5% isoflurane anesthesia, 2–4 bipolar stainless steel hook electrodes (50 μm diameter; California Fine Wire, Grover Beach, CA) were implanted bilaterally into the nuchal muscle and in one or both of the hindlimbs and secured with collodion. A ground wire on the back was looped transdermally and secured with collodion. As described previously (Karlsson et al., 2005), a custom-built head-fix apparatus, secured to the skull with cyanoacrylate adhesive, allowed for attachment to the earbars of a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). At the end of the surgery, which lasted approximately 10 min, the pup's trunk was lightly wrapped in gauze and the pup was placed in a humidified incubator maintained at thermoneutrality (35°C) to recover for at least 1 h. Before placing the subject into the testing apparatus, the subject was reanesthetized with 2–5% isoflurane anesthesia and three small holes were drilled in the skull for insertion of the electrode, ground wire, and thermocouple (the last for measuring brain temperature). After 1–2 h of acclimation in the testing apparatus, pups typically cycle regularly between sleep and wake. Despite prolonged isolation from their mother, pups exhibit regular sleep–wake cycles in these temperature-controlled conditions for many hours (Seelke and Blumberg, 2005).

Cerebellar Neurophysiology

A total of nine 6-day-old rats were used to produce 30 units for analysis. To record from Purkinje cells, 16-channel

silicon depth electrodes (NeuroNexus, Ann Arbor, MI; Models A1x16, A1x16-Poly2), with impedances ranging from 1 to 4 MΩ, were connected to a data acquisition system (Tucker-Davis Technologies, Alachua, FL) that amplified (10,000×) and filtered (500–5000 Hz band-pass) the neural signals. Prior to insertion, the electrode surface was coated with fluorescent DiI solution (Life Technologies, Grand Island, NY) for subsequent histological verification of electrode placement. A Ag/AgCl ground electrode (Medwire, Mt. Vernon, NY, 0.25 mm diameter) was placed into the cerebral cortex. Electromyographic (EMG) electrodes were connected to a differential amplifier (A-M Systems, Carlsborg, WA) that amplified (10,000×) and filtered (300–5000 Hz band-pass; 60 Hz notch filter) the EMG signal. Neural and EMG signals were sampled at 12.5 and 1 kHz, respectively, using a digital interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK). Brain temperature, maintained at 36–37°C, was measured using a fine-wire thermocouple (Omega Engineering, Stamford, CT) placed in the cerebral cortex contralateral to the ground wire.

Using a stereotaxic apparatus, the electrode was positioned above cerebellar cortex (coordinates: 1.0–1.5 mm caudal to lambda, 1.5–2.0 mm lateral to midline, 10–12° angle) and was slowly lowered until action potentials were detected on one or more recording channels. Purkinje cell activity was identified by the occurrence of large-amplitude action potentials (signal-to-noise $\geq$ 2:1) and the presence of complex spike waveforms as described by others (Crepel, 1971; Puro and Woodward, 1977a). Using procedures similar to those described previously (Karlsson et al., 2005; Mohns and Blumberg, 2010; Tiriac et al., 2012), data acquisition began after Purkinje cell unit activity was identified and had stabilized for at least 10 min. Each recording session lasted for 15 min and comprised continuous collection of neural and EMG data as the pup cycled freely between sleep and wake. During acquisition, an experimenter monitored the subject's behavior and digitally marked the occurrence of wake and active sleep (AS) movements in synchrony with the physiological data. The experimenter was always blind to the physiological data while scoring behavior. Following the first recording session, the electrode was lowered further and additional recordings were acquired if new Purkinje cell unit activity was identified.

Histology

After the final recording session, the pup was overdosed with sodium pentobarbital (1.5 mg/g) and perfused transcardially with phosphate buffered saline followed by 4% paraformaldehyde. Recording sites were verified by visualizing the DiI tract with fluorescence illumination at 5–10× magnification using a Leica microscope (Leica Microsystems, Buffalo Grove, IL). Using a calibrated reticle, the location of the unit recordings was determined.

Data Analysis

Spike Sorting and Burst Analysis. Spike sorting was performed using template matching in Spike2 (Cambridge Electronic Design). The distributions of waveforms comprising each template were analyzed using the principal component analysis tool; waveforms greater than 3.5 standard deviations outside of each distribution were excluded. After spikes were sorted into units and converted into events, a second analysis was performed to separate complex spikes from simple spikes using a burst script for Spike2. Bursts were identified as multispike events with interspike intervals $\leq$15 ms. All unit activity comprising a burst was subsequently extracted from the raw record of unit activity, resulting in separate simple spike and complex spike records for each unit.

Identification of Behavioral State. Sleep and wake periods were defined by calculating mean EMG activity, based on methods described previously (Karlsson et al., 2005; Mohns and Blumberg, 2010; Tiriac et al., 2012). Briefly, each nuchal or hindlimb EMG record was rectified and smoothed ($\tau = 0.001$ s). The mean amplitude of high muscle tone and atonia was calculated from five representative 1-s EMG segments, and the midpoint between the two was used to establish a threshold for determining state. The EMG signal was then dichotomized into periods of high tone (indicative of wake) and atonia (indicative of sleep). Sleep bouts were further divided into periods of quiet sleep and AS: quiet sleep was characterized by hypotonia/atonia and behavioral quiescence, and AS was characterized by the appearance of myoclonic twitches against a background of muscle atonia (Seelke and Blumberg, 2008; Blumberg and Seelke, 2010). For three pups with more than one useable EMG record, nuchal and hindlimb EMG events were merged into one event channel.

Analysis of State-Dependency. Mean unit firing rates were determined across all periods of wake, quiet sleep, and AS for each pup. For any single period, firing rates that exceeded three times the standard deviation were excluded as outliers. To assess whether units were significantly more active during different behavioral states, independent pairwise comparisons of the mean firing rates during wake, quiet sleep, and AS were assessed using the Wilcoxon matched-pairs signed-ranks test (SPSS, IBM, Armonk, NY). After characterizing units as AS-On, Sleep-On, Wake-On, AS/Wake-On, or State-Independent, mean firing rates for each unit were used in a summary analysis. Mean firing rates were also assessed for all units during each state. For all tests, including those described below, alpha was set at 0.05 and a Bonferroni correction was used when appropriate.

Event Correlations. Using methods similar to those described previously (Mohns and Blumberg, 2010; Tiriac et al., 2012), twitches were identified as discrete EMG events with amplitudes exceeding three times the mean EMG baseline during atonia. For pooled data across all subjects, a twitch-triggered cross-correlogram of complex spike activity was constructed for a 1-s window (10-ms bins) around twitch onset. We tested statistical significance

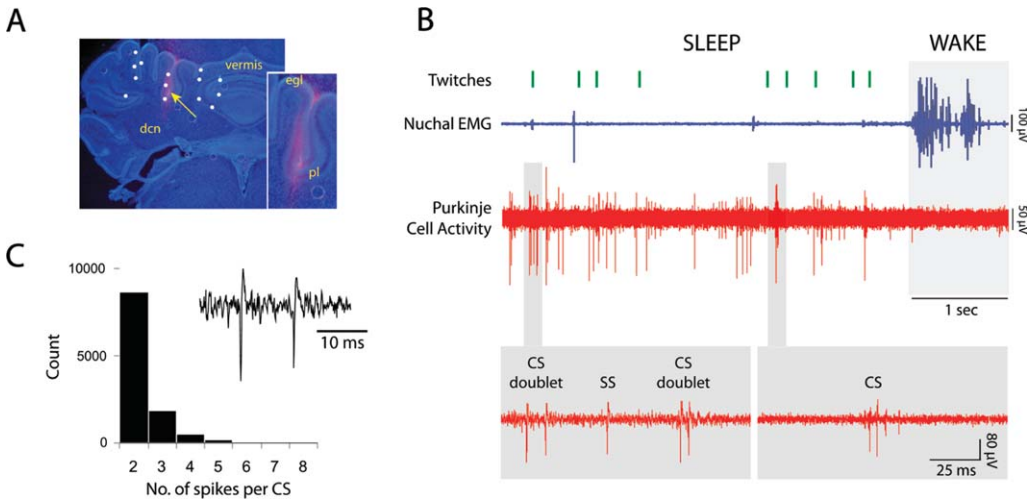

Figure 1 Purkinje cell activity in 6-day-old rats during sleep and wake. (A) The location of the recording sites (white dots). All sites were located in the Purkinje cell layer and typically yielded more than one unit. The yellow arrow indicates the track of a silicon electrode in cerebellar cortex, traversing multiple cell layers in simplex. Inset: an expanded view of the location of that track. The track was visualized by coating the electrode with fluorescent DiI. dcn, deep cerebellar nuclei; pl, Purkinje layer; egl, external granular layer. (B) Representative sample of data showing occurrences of behaviorally scored twitches (vertical green ticks), nuchal EMG, and Purkinje cell activity during sleep and wake. The neural activity (recorded from vermis) is expanded at bottom to highlight instances of complex spike (CS) and simple spike (SS) activity. (C) Frequency distribution of the number of individual spikes comprising complex spikes across all 30 Purkinje cells. Most, but not all, complex spikes were "doublets," consistent with previous reports in infant rats (Crepel, 1971; Puro and Woodward, 1977a). Inset: complex spikes were defined on the basis of action potentials exhibiting interspike intervals (ISIs) $\leq$15 ms. The example shown here has an ISI of 9 ms.

for the cross-correlogram by jittering twitch events 1000 times within a 400-ms window using PatternJitter (Harrison and Geman, 2009; Amarasingham et al., 2012). Finally, using a custom-written Matlab program, a 95% probability threshold ($p < 0.05$) was determined for the bins spanning 250 ms on either side of a twitch. For presentation purposes, cross-correlograms were smoothed using a 3-bin moving window. An identical method was used to test the relationship between simple spikes and twitching.

Window Analysis. As a population, complex spikes exhibited rhythmic activity after a twitch. For each unit, a "separation procedure" (Bloedel and Ebner, 1984; Nicholson and Freeman, 2003) was used to quantify the number of complex spikes within each of three time windows after a twitch: Window 1 at 0–50 ms, Window 2 at 100–150 ms, and Window 3 at 200–250 ms. If 45% or more of the complex spikes for that unit were associated with only one window, it was designated as either a W1 unit ($n = 16$), W2 unit ($n = 4$), or W3 unit ($n = 3$). Seven units exhibited no consistent window preference (NP). For each type of unit, cross-correlational analyses were performed to determine their relationship with twitching. Simple spike activity was assessed based on that unit's complex spike window assignment and cross-correlational analyses were performed. Again, significance was determined using the jitter procedure described above.

Complex Spike Autocorrelations. Autocorrelations of complex spikes were analyzed in 5 ms bins using the time series command in JMP (SAS, Cary, NC). For each cerebellar unit, autocorrelograms and spectral density plots were created. Using a white noise test, we tested against the null hypothesis that the production of complex spikes had no periodic component. Both Fisher's Kappa and Bartlett's Kolmogorov–Smirnov statistic were relied on to establish significant periodicity.

RESULTS

Identifying Complex and Simple Spikes

We used 16-channel silicon electrodes to record from the cerebellar cortex of head-fixed, unanesthetized 6-day-old rats ($n = 30$ units across nine subjects) as they cycled between sleep and wake. Electrodes spanned multiple cortical layers in the medial cerebellar cortex, including vermis, simplex, and crus I and II [Fig. 1(A)].

Consistent with previous studies in newborn rats (Woodward et al., 1969; Crepel, 1971; Puro and Woodward, 1977a,b), we observed multispike bursts of Purkinje cell activity with intervening periods of regularly firing single action potentials [Fig. 1(B)].

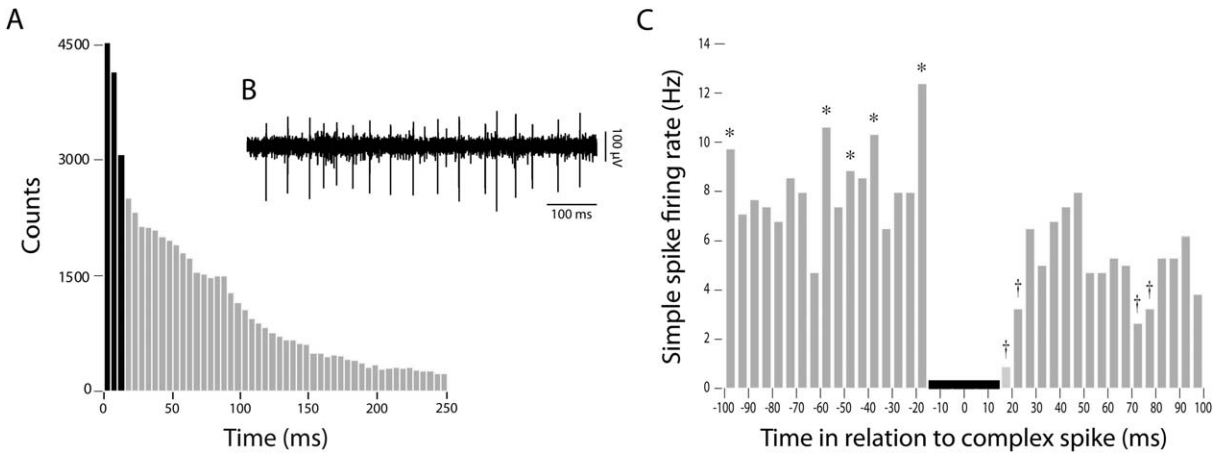

Figure 2 (A) Interspike intervals (ISIs) for all action potentials across all pups. Complex spikes were defined as two or more action potentials with ISIs $\leq$15 ms (black bars). All other unit activity was classified as simple spikes (gray bars). (B) Representative Purkinje cell recording from a 6-day-old rat to show a train of simple spikes. (C) Perievent histogram plotting simple spike firing rate per 10-ms bin in relation to complex spikes in a sleeping 6-day-old rat. The black bar designates the period when, by our definition of complex spikes, a simple spike could not occur. Note the suppression of simple spike activity after complex spikes. * significant increase in relation to jittered values, $p < 0.05$; † significant decrease in relation to jittered values, $p < 0.05$.

Importantly, stimulation of the inferior olive early in development elicits complex spikes, predominantly in the form of "doublets" comprising two individual spikes (Crepel, 1971; Puro and Woodward, 1977a). Doublets were also the predominant form of complex spikes observed here [Fig. 1(C)].

All non-complex spikes were designated as simple spikes [Fig. 2(A)]. Overall, simple spikes exhibited suppression after complex spikes, as well as higher tonic firing rates than complex spikes [Fig. 2(B,C) and Table 1]. Consistent with a previous report (Woodward et al., 1969), the firing rates reported in Table 1 are much lower than those seen in adults; preliminary analyses of Purkinje cell activity in older pups indicate that firing rates increase substantially by the end of the second postnatal week.

State-Dependency of Purkinje Cell Activity

For each Purkinje cell recording, we determined the relations among complex spikes, simple spikes, and behavioral state. For many units, it was readily apparent that complex and simple spikes occurred more often during sleep than during wake, and also more often during the bursts of twitching that characterize AS. To quantify and test these relationships, the firing rates of complex and simple spikes for each recorded unit across behavioral states was determined; for example, AS-On units exhibited significantly higher firing rates during AS than during either quiet sleep or wake [Fig. 3(A)]. On the basis of these comparisons, each unit was categorized as Sleep-On, Wake-On, AS-On, AS/Wake-On, or State-Independent. For both complex spikes (73%) and simple spikes (57%), the majority of Purkinje cells exhibited some form of state-dependent activity, and the vast majority of these state-dependent units were AS-On or AS/Wake-On [Fig. 3(B)]. Of the nine subjects, eight contributed state-dependent units. Importantly, across all units and regardless of state-dependency, complex and simple spike firing rates were significantly greater during AS than during wake or quiet sleep [Fig. 3(C)]. Although various forms of state-dependent cerebellar activity have been reported in adult rats, cats, and monkeys (Mano, 1970; Hobson and McCarley, 1974; Andre and Arrighi, 2001), this is the first demonstration of state-dependent activity in the newborn cerebellum.

Purkinje Cells Increase Activity in Close Association with Twitching

In light of evidence that neural activity in the thalamus, cerebral cortex, and hippocampus is activated in response to sensory feedback from twitching (Khazipov et al., 2004; Mohns and Blumberg, 2010; Tiriac et al., 2012), we next assessed twitch-dependent activity of all recorded Purkinje cells. Complex spikes exhibited a significant peak in

Table 1 Descriptive Statistics of Complex Spike and Simple Spike Activity Across All 30 Units in 6-Day-Old Rats

Unit Activity	Mean Interspike Interval (ms)	Mean Firing Rate (Hz)	Mean Burst Duration (ms)
Complex spikes	4.03 (0.10)[a]	0.36 (0.11)	9.15 (0.26)
Simple spikes	240.98 (14.1)	2.62 (0.49)	NA

Mean (SEM).
[a]Interspike interval within a complex spike.

activity immediately after twitching and a second peak approximately 100 ms after the first, along with a third, weak peak approximately 100 ms after the second [Fig. 4(A)]. Simple spikes also exhibited a significant peak in activity immediately after twitching; this peak was broader than that of complex spikes [Fig. 4(B)].

Next, using a "separation procedure" used previously in adult cats and weanling rats (Bloedel and Ebner, 1984; Nicholson and Freeman, 2003), we tested the possibility that the multiple peaks in complex spike activity were a population-level feature of ensembles of Purkinje cells, arising when Purkinje cells fire during the first peak or the second peak or the third, but rarely in more than one. To do this, we noted the occurrence of complex spikes within each of three 50-ms windows defined in relation to twitch-dependent activity: 0–50 ms [Fig. 4(A), red], 100–150 ms (green), and 200–250 ms (blue). Units that fired preferentially during Windows 1, 2, or 3 were designated as W1, W2, or W3 units, respectively. Cross-correlational analyses showed that

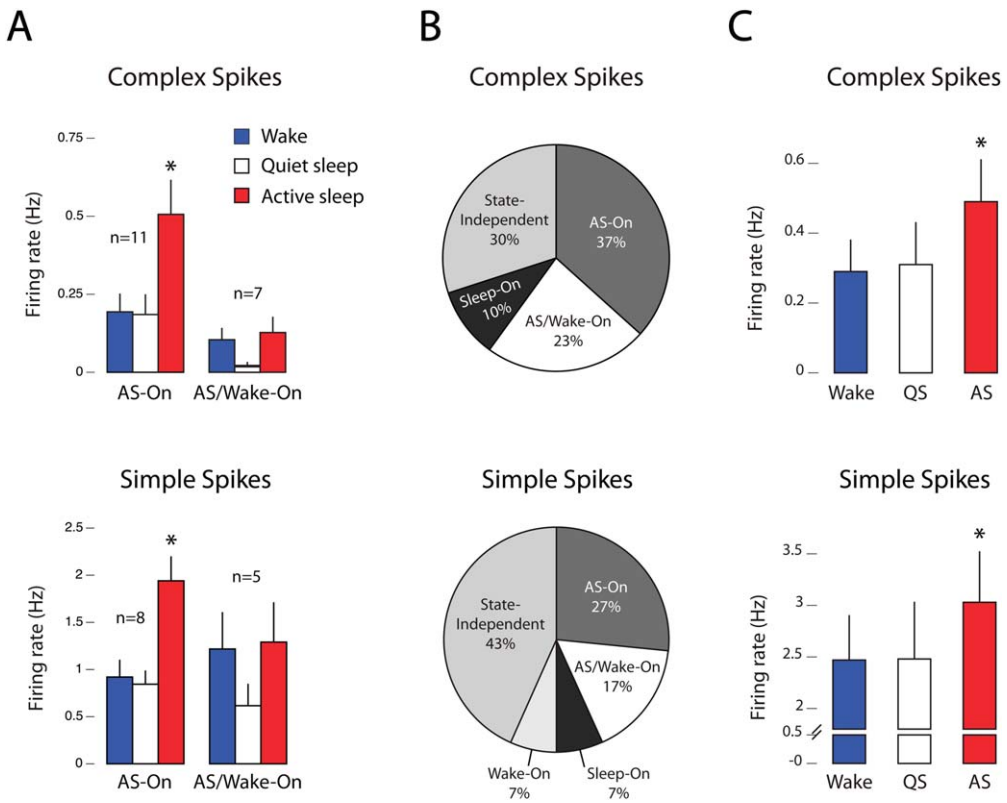

Figure 3 Complex and simple spike activity is state-dependent. (A) Firing rate (Hz) of complex spikes (top) and simple spikes (bottom) during wake, quiet sleep (QS), and active sleep (AS) in Purkinje cells characterized as AS-On and AS/Wake-On (data for Sleep-On, Wake-On, and State-Independent categories not shown). * significantly different from the other two groups ($p < 0.017$). (B) Percentage of complex and simple spikes ($n = 30$ in each group) characterized as AS-On, AS/Wake-On, Sleep-On, Wake-On, and State-Independent. (C) Firing rate (Hz) of complex and simple spikes during wake, quiet sleep, and active sleep in Purkinje cells regardless of state. * significantly different from the other two groups ($p < 0.017$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

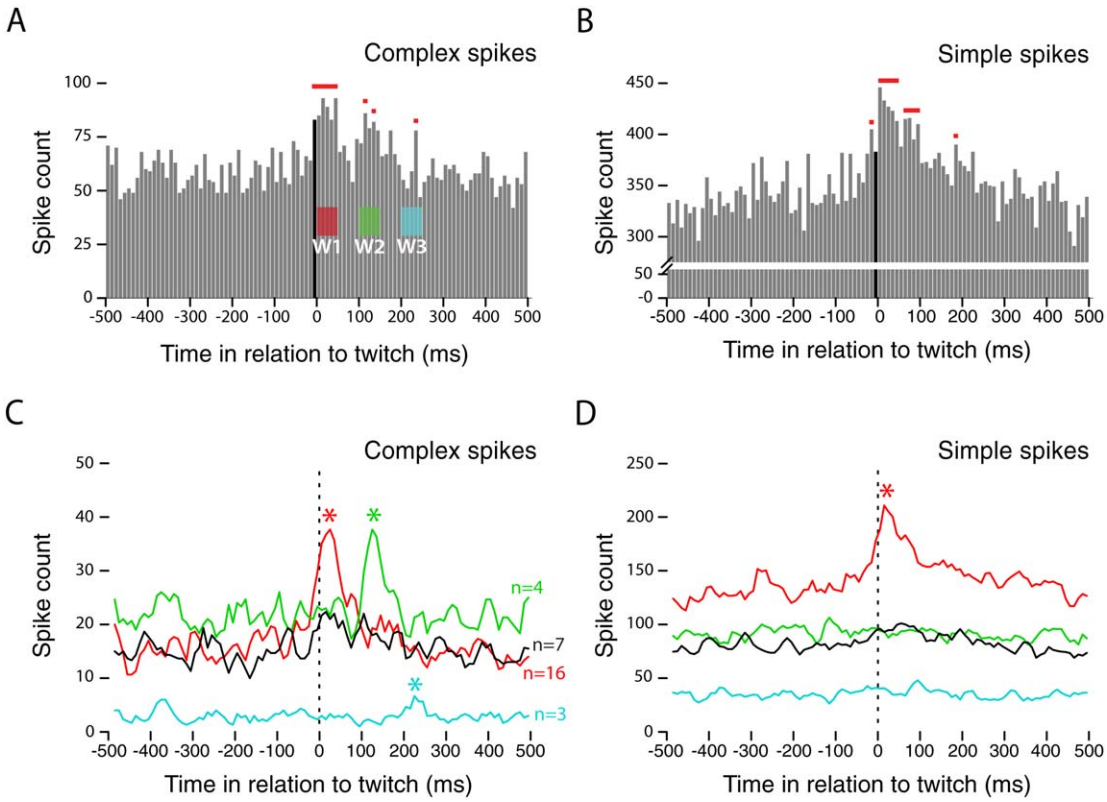

Figure 4 Complex and simple spike activity increases immediately after twitches. (A) Perievent histogram plotting the number of complex spikes per 10-ms bin in relation to myoclonic twitching in sleeping 6-day-old rats. Data were pooled across all 30 units. The vertical black bar is the last bin before a twitch and the horizontal red bars indicate statistical significance ($p < 0.05$). (B) Same as in (A) except for simple spikes ($p < 0.05$). (C) Perievent line histograms for complex spikes broken down for the W1 (red), W2 (green), and W3 (blue) units, as well as the units that showed no window preference (NP, black). For a small subset (10%) of all twitches analyzed, a unit that fired in one window fired again in one or both of the other windows. There was no clear relationship between a unit's window assignment and its state-dependency. The data were smoothed using a 3-bin moving window. * significant peak within window ($p < 0.0125$). (D) Same as in (C) except for simple spikes. * significant peak within window ($p < 0.0125$).

W1, W2, and W3 units increased their complex spike activity exclusively within their associated window [Fig. 4(C)]. Units exhibiting NP did not exhibit any twitch-related increase in complex spike activity. A similar result was obtained for simple spikes, but with only the W1 units exhibiting a significant increase in activity after a twitch [Fig. 4(D)]. Thus, twitching triggers short-latency complex and simple spike responses (W1), as well as a separate longer-latency complex spike response (W2) that may result from resetting of the intrinsic olivary rhythm (Bloedel and Ebner, 1984; Nicholson and Freeman, 2003).

Based on the complex spike data presented in Figure 4(A), we repeated the twitch-triggered cross-correlational analyses separately for the AS-On units. Complex spikes again exhibited pronounced and significant W1 and W2 peaks after twitches [Fig. 5(A)], and simple spikes again exhibited a significant W1 peak [Fig. 5(B)]. In contrast, neither AS/Wake-On nor State-Independent units exhibited pronounced peaks, thus indicating that AS-On units contributed predominantly to the overall twitch-related activity presented in Figure 4. For one representative AS-On unit recorded from crus II [Fig. 5(C)], the tight coupling between Purkinje cell activity and AS is apparent. Importantly, Purkinje cell activity decreased markedly during periods of active wake when the pup was moving its limbs.

Complex Spike Autorhythmicity

Autorhythmicity in climbing fiber activity, at a frequency of about 10 Hz, has been reported in adult rats during

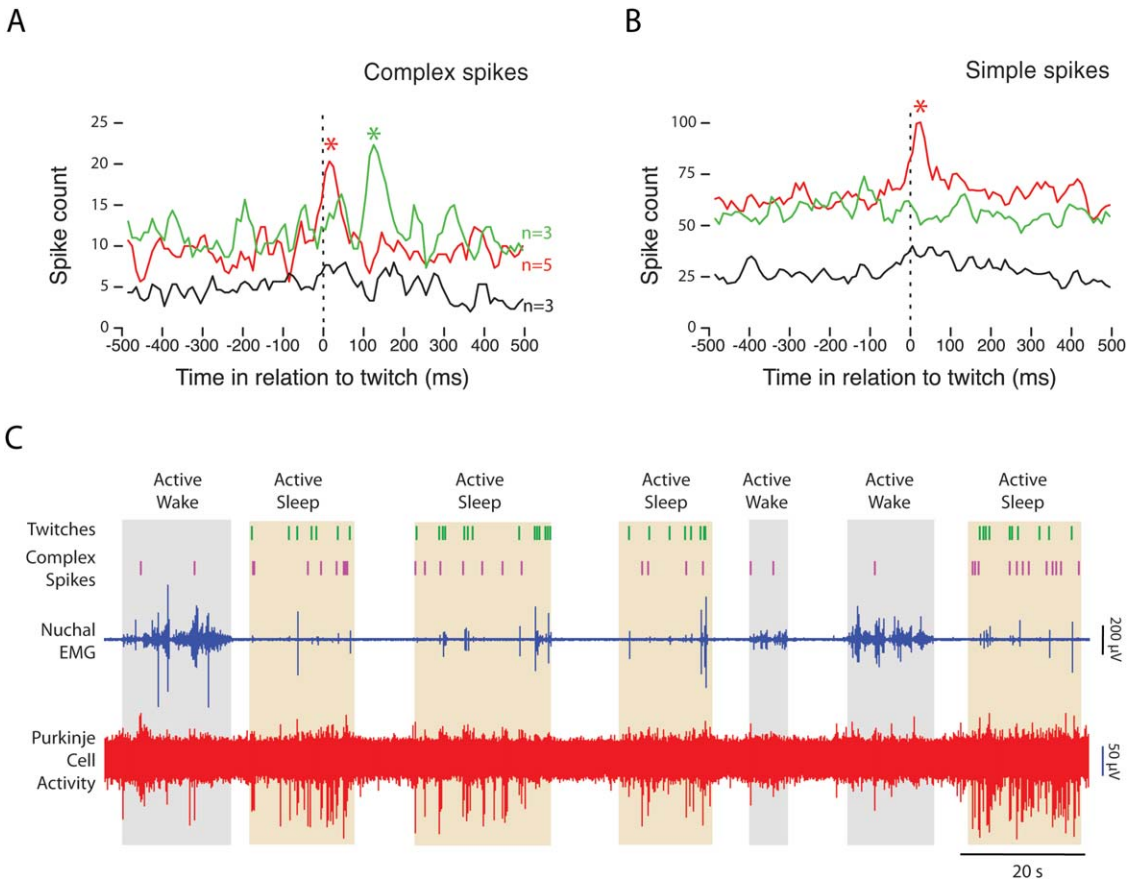

Figure 5 Twitch-related complex and simple spike activity for AS-On units. Perievent line histograms plotting complex (A) and simple spike (B) counts per 10-ms bin in relation to myoclonic twitching in sleeping 6-day-old rats. Each plot is broken down for the W1 (red), W2 (green), and units that showed no window preference (NP, black). All data were smoothed using a 3-bin moving window. * significant peak within window ($p < 0.017$). (C) Representative data showing occurrences of behaviorally scored twitches from all visible limbs and tail (vertical green ticks), complex spikes (vertical purple ticks), nuchal EMG, and Purkinje cell activity (recorded from crus I). Note the increased Purkinje cell and complex spike activity during periods of active sleep.

wakefulness, but its occurrence is variable both within and across species (Llinas and Sasaki, 1989; Kitazawa and Wolpert, 2005). To assess whether such autorhythmicity occurred in our subjects, we performed autocorrelation analyses. Of the 30 units examined, six exhibited statistically significant autorhythmicity. Of these, three exhibited strong rhythms at frequencies of 8–12 Hz (Fig. 6). Importantly, this analysis shows that the inferior olive is capable of rhythmic activity as early as 6 days of age. However, as the window analysis suggests, complex spike autorhythmicity is not responsible for the twitch-related multiple peaks in complex spike activity.

DISCUSSION

We found that the Purkinje cells of 6-day-old rats exhibit substantial state-dependent increases in complex and simple spike activity in close temporal association with twitching. These results are striking given the lack of several key cerebellar features considered integral for its mature function, including the granule cell–parallel fiber network and innervation of Purkinje cells by inhibitory interneurons and basket cells (Shimono et al., 1976). In addition, before the granule cell–parallel fiber network develops, mossy fibers form direct, transient connections onto Purkinje cell somas (Mason and Gregory, 1984; Takeda and Maekawa, 1989; Kalinovsky et al., 2011). Consequently, the observed twitch-dependent increases in simple spike activity at the age tested here indicate, for the first time in unanesthetized animals, functional engagement of these direct mossy fiber connections. All together, the current findings introduce a new and unanticipated mechanism for driving activity-dependent development of mossy and

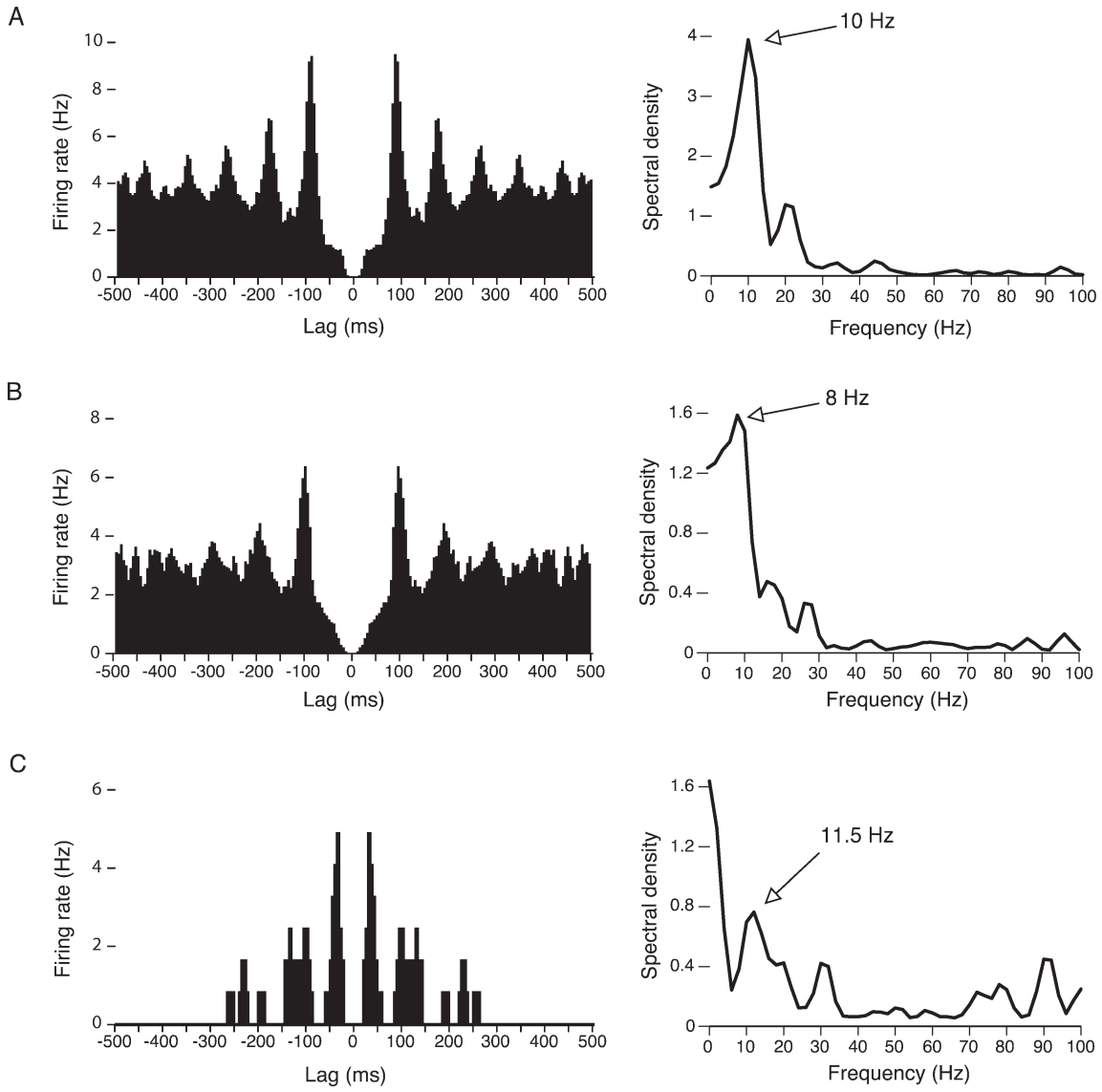

Figure 6 Complex spike autorhythmicity in sleeping 6-day-old rats. For three individual units, autocorrelograms are shown at left and spectral density plots are shown at right. (A) A unit exhibiting strong 10-Hz rhythmicity. This unit was Sleep-On and did not exhibit a window preference. (B) A unit exhibiting strong 8-Hz rhythmicity. This unit was State-Independent and exhibited a preference for Window 2 (W2). (C) A unit exhibiting weaker rhythmicity at 11.5 Hz. This unit was AS-On and exhibited a preference for Window 1 (W1 unit). These three units were recorded from two pups.

climbing fiber connectivity (Takeda and Maekawa, 1989; Kalinovsky et al., 2011; Kano and Hashimoto, 2011).

Twitching, Reafference, and Corollary Discharge

Although the function of the cerebellum continues to elude simple description, most current theories tend to focus on its role in motor timing or motor learning (Kitazawa and Wolpert, 2005). Of critical importance to these theories are the spatial and temporal details regarding the transmission of sensory and motor signals through mossy and climbing fibers. Toward that end, investigators seek to understand how the cerebellum processes the two established classes of sensory input: reafference, arising from self-produced movements, and exafference, arising from other-produced movements (Sperry, 1950; Von Holst and Mittelstaedt, 1950; Poulet and Hedwig, 2007; Crapse and Sommer, 2008). In addition to reafference, motor commands trigger corollary discharge (or efference copy), which serves the function

of helping animals distinguish between expected (i.e., reafferent) and unexpected (i.e., exafferent) signals (Crapse and Sommer, 2008). Importantly, both reafference and corollary discharge signals are conveyed to the adult cerebellum via climbing and mossy fibers (van Kan et al., 1993; Wolpert et al., 1998; Huang et al., 2013). Further investigation of the infant cerebellum may provide insight into the development of both signals in this essential sensorimotor structure.

The top-left and bottom-right cells in Figure 7 denote the two conventional classes of sensory input: reafference from self-produced movements that is modified by corollary discharge and exafference that is not. There is, however, a potential third class of input that is biologically plausible but rarely discussed: self-produced movements that are not accompanied by corollary discharge (bottom-left cell in Fig. 7). Here we propose the working hypothesis that twitches embody this novel class of self-produced movement.

We were led to this hypothesis for several reasons. Foremost among them is the observation that, similar to previous findings in hippocampus (Mohns and Blumberg, 2008, 2010) and sensory thalamus (Tiriac et al., 2012), Purkinje cell activity was prominent during AS in close temporal proximity to twitching, whereas wake-related activity was weak or absent. It should be stressed that we cannot be certain that the twitch-related Purkinje cell activity observed here reflects reafference alone. Nonetheless, the totality of evidence from cerebellum, hippocampus, and thalamus strongly suggests that—at least in rat pups under these testing conditions—reafferent signals associated with twitches are processed differently from those associated with wake movements. One possible explanation for this differential processing is that corollary discharge is absent during twitching. This notion of state-dependent modulation of corollary discharge has not, to our knowledge, been considered previously.

There is intriguing but indirect support for the hypothesis that twitches are not accompanied by corollary discharge. First, human adults awaking from REM-related dreams display deficits in their ability to distinguish self- from other-produced stimulation, suggesting suspended corollary discharge (Blagrove et al., 2006). Second, REM-dream narratives exhibit qualities suggestive of suspended corollary discharge, similar to the hallucinations of schizophrenics (Feinberg and Guazzelli, 1999; Ford et al., 2008). Accordingly, a better understanding of differential processing of corollary discharge during sleep and wakefulness could greatly aid our understanding of how we learn to distinguish self from other, and how such processes go awry.

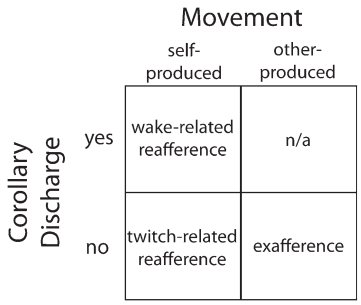

Figure 7 Hypothesized framework relating self-produced and other-produced movements with the accompaniment of corollary discharge. Conventionally, self-produced movements generate reafference that is accompanied by corollary discharge, whereas other-produced movements generate exafference that is not accompanied by corollary discharge. For obvious reasons, other-produced movements cannot be accompanied by corollary discharge (n/a: not applicable). Here we propose a third possible category whereby sleep-related twitches are self-produced movements that are not accompanied by corollary discharge.

Limitations of This Study

The present results are clear in showing that Purkinje cell activity is strongly sleep- and twitch-dependent in rats at 6 days of age. We are confident that we recorded from Purkinje cells because of the location of the electrode sites within the Purkinje layer and the detection of doublets in the neurophysiological record. Doublets are considered an immature form of complex spike based on evidence that they are produced by climbing fibers arising from the inferior olive (Crepel, 1971; Puro and Woodward, 1977a). However, a fully accurate characterization of the activity triggered by climbing and mossy fibers early in development will require selective inactivation of the inferior olive and other structures that project to the cerebellum.

As mentioned in the previous section, another concern relates to the precise timing of the Purkinje cell activity in relation to twitching. For the analyses of twitch-related neural activity reported here and elsewhere, we rely on the fact that, because of high cross-correlations in the twitch activity of skeletal muscle groups, any given muscle can act as a reasonable proxy for another one (Mohns and Blumberg, 2010; Tiriac et al., 2012). This reliance on statistical association has not presented interpretative problems when recording from forebrain sites where the latencies between twitches and reafferent-related activity are approximately 200 ms. In the cerebellum, however, the first peak in twitch-related activity was 50 ms or less, which may or may not be too short for a reafferent signal at this age (Puro and Woodward,

1977a; Takeda and Maekawa, 1989). To resolve this issue, it will be important to record from locations in the cerebellum that can be definitively related to twitching in specific muscle groups. Once that is accomplished, it may also then be possible to assess how twitch-related activity guides the development of convergent and somatotopically organized mossy and climbing fiber inputs onto Purkinje cells (Odeh et al., 2005; Pijpers et al., 2006; Huang et al., 2013).

Finally, we recorded here from 6-day-olds because this is an age when Purkinje cells are functional but granule cells have not yet migrated to the internal granular layer. It is also an age when the Purkinje cells themselves are undergoing rapid developmental change (Watanabe and Kano, 2011). Of great interest will be documenting changes in state- and twitch-dependent activity in the cerebellum at younger and older ages and relating that activity to known features of cerebellar circuitry (Shimono et al., 1976). Such a developmental study is currently underway.

A New Era in Developmental Neurophysiology

As a contribution to this special issue on "Neural Mechanisms of Behavioral Maturation," it seems worthwhile to place the present work in a broader historical context. Before 2004, there were only a handful of papers devoted to state-dependent neural activity in infant animals, including recordings at the cortical surface and multiunit recordings in the brainstem (Gramsbergen, 1976; Tamásy and Korányi, 1980; Tamásy et al., 1980; Mirmiran and Corner, 1982). For a variety of technical and methodological reasons, these studies were unable to document clear and convincing relationships between neural activity and sleep in early infancy. Moreover, with regard to the cerebral cortex, the conclusion drawn from these earlier studies was that this structure does not exhibit state-dependent activity before the emergence of slow waves at P11 (for review, see Blumberg and Seelke, 2010).

Then, beginning only a decade ago, new in vivo recording methods were introduced and refined for recording hippocampal (Leinekugel et al., 2002; Karlsson and Blumberg, 2003) and cortical (Khazipov et al., 2004) activity in freely moving and head-fixed infant rats. These methods have made it possible to investigate state-dependent neural activity in the infant brainstem (Karlsson and Blumberg, 2005; Karlsson et al., 2005) and forebrain (Mohns et al., 2006), discover previously undetected state-dependent cortical activity patterns and trace their early development (Yang et al., 2009; Seelke and Blumberg, 2010), assess the contributions to infant cortical activity of subplate neurons (Hanganu et al., 2008; Tolner et al., 2012) and the corpus callosum (Marcano-Reik and Blumberg, 2008), and investigate the consequences of twitch-related reafference for neural activity throughout the neuraxis (Khazipov et al., 2004; Mohns and Blumberg, 2008, 2010; Tiriac et al., 2012). Imaging techniques, including the use of voltage-sensitive dyes, can now capture broader features of twitch-related cortical activity (McVea et al., 2012; Tiriac et al., 2012).

Although similar in vivo recording and imaging methods are providing new insights into sensory processing in the visual system (e.g., Hanganu et al., 2006, 2007; Ackman et al., 2012), these methods can and should be expanded to the study of other behavioral contexts and the interactions among behavioral systems. For example, even in head-fixed pups, it should now be possible to investigate neural mechanisms associated with ingestion (Hall and Williams, 1983), thermoregulation (Blumberg, 2001), and learning and memory (Johanson and Hall, 1979; Campolattaro and Freeman, 2008), among other behaviors and processes. Such advances, when fully realized, hold the promise to reshape our understanding of brain–behavior relations and how they emerge across early development.

CONCLUSIONS

Spontaneous activity is a ubiquitous feature of developing nervous systems (O'Donovan, 1999). For example, spontaneous activity in the developing retina (Galli and Maffei, 1988; Wong, 1999) and cochlea (Tritsch et al., 2007) is thought to contribute to the development of the visual and auditory systems, respectively. Twitching is different from spontaneous retinal and cochlear activity in that it entails motor as well as sensory components. As such, it is ideally suited to provide information to the developing sensorimotor system about the functional properties of limbs and their neural control (Blumberg et al., 2013b). Previous findings have highlighted how reafference from twitching profoundly and selectively activates forebrain structures, including thalamus, cerebral cortex, and hippocampus (Khazipov et al., 2004; Mohns and Blumberg, 2010; Tiriac et al., 2012). Here we have shown for the first time that twitch-related processes modulate the activity of the cerebellum, a hindbrain structure that is critical for sensorimotor integration and motor learning. This finding introduces a potent source of structured activity that can plausibly account for activity-

dependent development in the cerebellar system. In doing so, these findings lend additional support to the counterintuitive notion that, contrary to the still-popular view of twitching as a functionless by-product of dreams (Blumberg, 2010), twitching is a vital source of neural activity for the developing infant.

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REFERENCES

Ackman JB, Burbridge TJ, Crair MC. 2012. Retinal waves coordinate patterned activity throughout the developing visual system. Nature 490:219–225.

Altman J. 1972. Postnatal development of the cerebellar cortex in the rat. I. The external germinal layer and the transitional molecular layer. J Comp Neurol 145:353–397.

Amarasingham A, Harrison MT, Hatsopoulos NG, Geman S. 2012. Conditional modeling and the jitter method of spike resampling. J Neurophysiol 107:517–531.

Andjus PR, Zhu L, Cesa R, Carulli D, Strata P. 2003. A change in the pattern of activity affects the developmental regression of the purkinje cell polyinnervation by climbing fibers in the rat cerebellum. Neuroscience 121:563–572.

Andre P, Arrighi P. 2001. Modulation of Purkinje cell response to glutamate during the sleep–waking cycle. Neuroscience 105:731–746.

Apps R, Garwicz M. 2005. Anatomical and physiological foundations of cerebellar information processing. Nat Rev Neurosci 6:297–311.

Blagrove M, Blakemore S, Thayer B. 2006. The ability to self-tickle following rapid eye movement sleep dreaming. Conscious Cogn 15:285–294.

Bloedel JR, Ebner TJ. 1984. Rhythmic discharge of climbing fibre afferents in response to natural peripheral stimuli in the cat. J Physiol (Lond) 352:129–146.

Blumberg MS. 2001. The developmental context of thermal homeostasis. In: Blass EM, editor. Handbook of Developmental Neurobiology, Vol. 13: Developmental Psychobiology. New York: Plenum Press, pp 199–228.

Blumberg MS. 2010. Beyond dreams: Do sleep-related movements contribute to brain development? Front Neurol 1:140.

Blumberg MS, Coleman CM, Gerth AI, McMurray B. 2013a. Spatiotemporal structure of REM sleep twitching reveals developmental origins of motor synergies. Curr Biol 23:2100–2109.

Blumberg MS, Marques HG, Iida F. 2013b. Twitching in sensorimotor development from sleeping rats to robots. Curr Biol 23:R532–R537.

Blumberg MS, Seelke AMH. 2010. The form and function of infant sleep: From muscle to neocortex. In: Blumberg MS, Freeman JH, Robinson SR, editors. The Oxford Handbook of Developmental Behavioral Neuroscience. New York: Oxford University Press, pp 391–423.

Campolattaro M, Freeman JH. 2008. Eyeblink conditioning in 12-day-old rats using pontine stimulation as the conditioned stimulus. Proc Natl Acad Sci USA 105:8120–8123.

Crapse TB, Sommer MA. 2008. Corollary discharge across the animal kingdom. Nat Rev Neurosci 9:587–600.

Crepel F. 1971. Maturation of climbing fiber responses in the rat. Brain Res 35:272–276.

Eccles JC, Ito M, Szentâgothai J. 1967. The Cerebellum as a Cognitive Machine. New York: Springer-Verlag.

Feinberg I, Guazzelli M. 1999. Schizophrenia—A disorder of the corollary discharge systems that integrate the motor systems of thought with the sensory systems of consciousness. Br J Psychiatry 174:196–204.

Ford JM, Roach BJ, Faustman WO, Mathalon DH. 2008. Out-of-synch and out-of-sorts: Dysfunction of motor-sensory communication in schizophrenia. Biol Psychiatry 63:736–743.

Galli L, Maffei L. 1988. Spontaneous impulse activity of rat retinal ganglion cells in prenatal life. Science 242:90–91.

Gramsbergen A. 1976. The development of the EEG in the rat. Dev Psychobiol 9:501–515.

Gramsbergen A, Schwartze P, Prechtl HFR. 1970. The postnatal development of behavioral states in the rat. Dev Psychobiol 3:267–280.

Hall WG, Williams CL. 1983. Suckling isn't feeding, or is it? A search for developmental continuities. Adv Study Behav 13:219–254.

Hanganu IL, Ben-Ari Y, Khazipov R. 2006. Retinal waves trigger spindle bursts in the neonatal rat visual cortex. J Neurosci 26:6728–6736.

Hanganu IL, Okabe A, Lessmann V, Luhmann HJ. 2008. Cellular mechanisms of subplate-driven and cholinergic input-dependent network activity in the neonatal rat somatosensory cortex. Cereb Cortex 19:89–105.

Hanganu IL, Staiger J, Ben-Ari Y, Khazipov R. 2007. Cholinergic modulation of spindle bursts in the neonatal rat visual cortex in vivo. J Neurosci 27:5694–5705.

Harrison MT, Geman S. 2009. A rate and history-preserving resampling algorithm for neural spike trains. Neural Comput 21:1244–1258.

Hashimoto K, Kano M. 2013. Synapse elimination in the developing cerebellum. Cell Mol Life Sci 70:4667–4680.

Hobson JA, McCarley RW. 1974. Multiple firings by cat cerebellar Purkinje cells in sleep and waking. Exp Neurol 44:41–48.

Huang CC, Sugino K, Shima Y, Guo C, Bai S. 2013. Convergence of pontine and proprioceptive streams onto multimodal cerebellar granule cells. eLife 2:e00400.

Ito M. 1989. Long-term depression. Annu Rev Neurosci 12:85–102.

Johanson I, Hall W. 1979. Appetitive learning in 1-day-old rat pups. Science 205:419–421.

Jouvet-Mounier D, Astic L, Lacote D. 1970. Ontogenesis of the states of sleep in rat, cat, and guinea pig during the first postnatal month. Dev Psychobiol 2:216–239.

Kakizawa S, Yamasaki M, Watanabe M, Kano M. 2000. Critical period for activity-dependent synapse elimination in developing cerebellum. J Neurosci 20:4954–4961.

Kalinovsky A, Boukhtouche F, Blazeski R, Bornmann C, Suzuki N, Mason CA, Scheiffele P. 2011. Development of axon-target specificity of ponto-cerebellar afferents. PLoS Biol 9:e1001013.

Kano M, Hashimoto K. 2011. Activity-dependent maturation of climbing fiber to Purkinje cell synapses during postnatal cerebellar development. Cerebellum 11:449–450.

Karlsson KÆ, Blumberg MS. 2003. Hippocampal theta in the newborn rat is revealed under conditions that promote REM sleep. J Neurosci 23:1114–1118.

Karlsson KÆ, Blumberg MS. 2005. Active medullary control of atonia in week-old rats. Neuroscience 130:275–283.

Karlsson KÆ, Gall AJ, Mohns EJ, Seelke AMH, Blumberg MS. 2005. The neural substrates of infant sleep in rats. PLoS Biol 3:e143.

Katz L, Shatz C. 1996. Synaptic activity and the construction of cortical circuits. Science 274:1133–1138.

Khazipov R, Sirota A, Leinekugel X, Holmes GL, Ben-Ari Y, Buzsáki G. 2004. Early motor activity drives spindle bursts in the developing somatosensory cortex. Nature 432:758–761.

Kitazawa S, Wolpert DM. 2005. Rhythmicity, randomness and synchrony in climbing fiber signals. Trends Neurosci 28:611–619.

Leinekugel X, Khazipov R, Cannon R, Hirase H, Ben-Ari Y, Buzsáki G. 2002. Correlated bursts of activity in neonatal hippocampus in vivo. Science 296:2049–2052.

Llinas R, Sasaki K. 1989. The functional organization of the olivo-cerebellar system as examined by multiple Purkinje cell recordings. Eur J Neurosci 1:587–602.

Mano NN. 1970. Changes of simple and complex spike activity of cerebellar Purkinje cells with sleep and waking. Science 170:1325–1327.

Marcano-Reik AJ, Blumberg MS. 2008. The corpus callosum modulates spindle-burst activity within homotopic regions of somatosensory cortex in newborn rats. Eur J Neurosci 28:1457–1466.

Mason CA, Gregory E. 1984. Postnatal maturation of cerebellar mossy and climbing fibers: Transient expression of dual features on single axons. J Neurosci 4:1715–1735.

McVea DA, Mohajerani MH, Murphy TH. 2012. Voltage-sensitive dye imaging reveals dynamic spatiotemporal properties of cortical activity after spontaneous muscle twitches in the newborn rat. J Neurosci 32:10982–10994.

Mikuni T, Uesaka N, Okuno H, Hirai H, Deisseroth K, Bito H, Kano M. 2013. Arc/Arg3.1 Is a postsynaptic mediator of activity-dependent synapse elimination in the developing cerebellum. Neuron 78:1024–1035.

Mirmiran M, Corner M. 1982. Neuronal discharge patterns in the occipital cortex of developing rats during active and quiet sleep. Brain Res 255:37–48.

Mohns EJ, Blumberg MS. 2008. Synchronous bursts of neuronal activity in the developing hippocampus: Modulation by active sleep and association with emerging gamma and theta rhythms. J Neurosci 28:10134–10144.

Mohns EJ, Blumberg MS. 2010. Neocortical activation of the hippocampus during sleep in newborn rats. J Neurosci 30:3438–3449.

Mohns EJ, Karlsson KÆ, Blumberg MS. 2006. The preoptic hypothalamus and basal forebrain play opposing roles in the descending modulation of sleep and wakefulness in infant rats. Eur J Neurosci 23:1301–1310.

Nicholson DA, Freeman JH. 2003. Developmental changes in evoked Purkinje cell complex spike responses. J Neurophysiol 90:2349–2357.

O'Donovan MJ. 1999. The origin of spontaneous activity in developing networks of the vertebrate nervous system. Curr Opin Neurobiol 9:94–104.

Odeh F, Ackerley R, Bjaalie JG, Apps R. 2005. Pontine maps linking somatosensory and cerebellar cortices are in register with climbing fiber somatotopy. J Neurosci 25: 5680–5690.

Petersson P, Waldenström A, Fåhraeus C, Schouenborg J. 2003. Spontaneous muscle twitches during sleep guide spinal self-organization. Nature 424:72–75.

Pijpers A, Apps R, Pardoe J, Voogd J, Ruigrok TJH. 2006. Precise spatial relationships between mossy fibers and climbing fibers in rat cerebellar cortical zones. J Neurosci 26:12067–12080.

Poulet JFA, Hedwig B. 2007. New insights into corollary discharges mediated by identified neural pathways. Trends Neurosci 30:14–21.

Puro DG, Woodward DJ. 1977a. Maturation of evoked climbing fiber input to rat cerebellar Purkinje cells (I.). Exp Brain Res 28:85–100.

Puro DG, Woodward DJ. 1977b. Maturation of evoked mossy fiber input to rat cerebellar Purkinje cells (II.). Exp Brain Res 28:427–441.

Seelke AMH, Blumberg MS. 2005. Thermal and nutritional modulation of sleep in infant rats. Behav Neurosci 19: 603–611.

Seelke AMH, Blumberg MS. 2008. The microstructure of active and quiet sleep as cortical delta activity emerges in infant rats. Sleep 31:691–699.

Seelke AMH, Blumberg MS. 2010. Developmental appearance and disappearance of cortical events and oscillations in infant rats. Brain Res 1324:34–42.

Seelke AMH, Karlsson KÆ, Gall AJ, Blumberg MS. 2005. Extraocular muscle activity, rapid eye movements and the development of active and quiet sleep. Eur J Neurosci 22:911–920.

Shimono TT, Nosaka SS, Sasaki KK. 1976. Electrophysiological study on the postnatal development of neuronal mechanisms in the rat cerebellar cortex. Brain Res 108: 279–294.

Sillitoe RV, Joyner AL. 2007. Morphology, molecular codes, and circuitry produce the three-dimensional complexity of the cerebellum. Annu Rev Cell Dev Biol 23:549–577.

Sperry R. 1950. Neural basis of the spontaneous optokinetic response produced by visual inversion. J Comp Physiol Psychol 43:482–489.

Takeda TT, Maekawa KK. 1989. Transient direct connection of vestibular mossy fibers to the vestibulocerebellar Purkinje cells in early postnatal development of kittens. Neuroscience 32:99–111.

Tamásy V, Korányi LL. 1980. Multiunits in the mesencephalic reticular formation: Ontogenetic development of wakefulness and sleep cycle in the rat. Neurosci Lett 17: 143–147.

Tamásy V, Korányi L, Lissák K. 1980. Early postnatal development of wakefulness-sleep cycle and neuronal responsiveness: A multiunit activity study on freely moving newborn rat. Electroencephalogr Clin Neurophysiol 49:102–111.

Tiriac A, Uitermarkt BD, Fanning AS, Sokoloff G, Blumberg MS. 2012. Rapid whisker movements in sleeping newborn rats. Curr Biol 22:2075–2080.

Tolbert DLD, Pittman TT, Alisky JMJ, Clark BRB. 1994. Chronic NMDA receptor blockade or muscimol inhibition of cerebellar cortical neuronal activity alters the development of spinocerebellar afferent topography. Dev Brain Res 80:268–274.

Tolner EA, Sheikh A, Yukin AY, Kaila K, Kanold PO. 2012. Subplate neurons promote spindle bursts and thalamocortical patterning in the neonatal rat somatosensory cortex. J Neurosci 32:692–702.

Tritsch N, Yi E, Gale J, Glowatzki E, Bergles D. 2007. The origin of spontaneous activity in the developing auditory system. Nature 450:50–55.

van Kan P, Gibson A, Houk J. 1993. Movement-related inputs to intermediate cerebellum of the monkey. J Neurophysiol 69:74–94.

Von Holst E, Mittelstaedt H. 1950. The principle of reafference: Interactions between the central nervous system and the peripheral organs. Naturwissenschaften 37:464–476.

Wang VYV, Zoghbi HYH. 2001. Genetic regulation of cerebellar development. Nat Rev Neurosci 2:484–491.

Watanabe M, Kano M. 2011. Climbing fiber synapse elimination in cerebellar Purkinje cells. Eur J Neurosci 34: 1697–1710.

Wolpert DM, Miall CR, Kawato M. 1998. Internal models in the cerebellum. Trends Cogn Sci 2:338–347.

Wong R. 1999. Retinal waves and visual system development. Annu Rev Neurosci 22:29–47.

Woodward DJ, Hoffer BJ, Lapham LW. 1969. Postnatal development of electrical and enzyme histochemical activity in Purkinje cells. Exp Neurol 23:120–139.

Yang J-W, Hanganu-Opatz IL, Sun J-J, Luhmann HJ. 2009. Three patterns of oscillatory activity differentially synchronize developing neocortical networks in vivo. J Neurosci 29:9011–9025.