Differential effects of ketamine/xyazine anesthesia on the cerebral and cerebellar cortical activities in the rat

Gokhan Ordek, Jonathan D. Groth, and Mesut Sahin

Biomedical Engineering Department, New Jersey Institute of Technology, Newark, New Jersey

Submitted 30 May 2012; accepted in final form 10 December 2012

Ordek G, Groth JD, Sahin M. Differential effects of ketamine/xyazine anesthesia on the cerebral and cerebellar cortical activities in the rat. J Neurophysiol 109: 1435–1443, 2013. First published December 12, 2012; doi:10.1152/jn.00455.2012.—Cerebellum is a highly organized structure with a crystalline morphology that has always intrigued neuroscientists. Much of the cerebellar research has been conducted in anesthetized animals, particularly using ketamine/xyazine combination in rats. It is not clear how and to what extent the cerebellar cortical circuitry is affected by this anesthesia. In this study, we recorded spontaneous and evoked potentials from the cerebellar surface with chronically implanted, flexible-substrate, multielectrode arrays in rats and compared them to the signals simultaneously recorded from the motor cortex with similar electrodes. The power spectra and the intercontact coherence plots of the spontaneous activity in the awake-quiet animals extended up to 800 Hz in the cerebellum and only up to 200 Hz in the motor cortex. Ketamine/xyazine anesthesia suppressed most of the activity in the cerebellar cortex, which was in clear contrast to the motor cortex. In the awake cerebellum, large coherence values were observed between contact pairs as far apart as ~2 mm. Otherwise, there was not a discernable relation between the coherence and the intercontact distance. These results suggest that the surface electrodes can provide much more detailed information about the state of neural circuits when they are used on the cerebellar cortex compared with the cerebral areas. This may be due to the proximity of the molecular layer cells to the pial surface in the cerebellum and anesthesia; multielectrode arrays; micro-ECoG; evoked potentials

The cerebellum is primarily considered as a center of motor coordination, although there is sufficient evidence now showing that it is also involved in a number of cognitive tasks, such as the language processing (Hart 2011), and it has also been implicated in several cognitive disorders including schizophrenia and autism (Fatemi et al. 2012; Villanueva 2012). Accordingly, much research has been put to understanding how the cerebellum processes the sensory-motor information. However, there is no consensus so far on how the cerebellum integrates information and makes its contribution to the motor control of the body, the lack of which generates symptoms that are very familiar to the clinicians.

On the hand, the cerebellum constitutes an ideal platform to study neural circuits in many respects. Many laboratories have used the cerebellar cortex as a template to understand the nervous system because of its well-defined network connectivity and relatively few types of cells involved. The cerebellar cortex has two main input pathways: the climbing fiber input, which arises from the inferior olive and terminates on the Purkinje cells, produces a complex spike with firing frequency of ~1–2 Hz (Armstrong and Rawson 1979; Thach 1968). Each Purkinje cell receives a single climbing fiber input, and the Purkinje cells innervated by the same climbing fiber form a parasagittally oriented microzone in the cortex (Andersson and Oscarsson 1978; Voogd and Glickstein 1998). The mossy fibers constitute the other input that terminates on the granular cells, which then send up axons to the surface forming parallel fibers that terminate on the Purkinje cells (Voogd and Glickstein 1998). The parallel fiber inputs are responsible for modulation of the simple spike activity in the Purkinje cells with firing frequencies from 10 to 100 Hz (Armstrong and Rawson 1979, Cerminara et al. 2009).

At a conceptual level, there are two ways in which the cerebellum may potentially encode information. The first is rate coding in which the simple spike frequency is varied. It has been shown that the Purkinje simple spike frequency is correlated with arm kinematic and dynamic variables (Albus 1971; Dean et al. 2010; Ebner et al. 2011; Hewitt et al. 2011; Lisberger 2009; McElvain et al. 2010; Pasalar et al. 2006). The second is spatiotemporal coding where the spike synchrony between multiple Purkinje cells is the mechanism for propagation of information. Spike synchrony occurs both with the complex and simple spikes (De Zeeuw et al. 2011; Wise et al. 2010).

Anesthetized animal preparations have commonly been used in electrophysiological experiments conducted in rats, although anesthesia undoubtedly affects the neuronal circuity in the cerebellar cortex. One of the most popular anesthesia regimes used for electrophysiological experiments is the ketamine/xyazine combination. Ketamine is known to affect the $\alpha$-methyl-D-aspartate (NMDA; Anis et al. 1983; Yamamura et al. 1988; Armstrong and Rawson 1979; Thach 1968). Each Purkinje cell receives a single climbing fiber input, and the Purkinje cells innervated by the same climbing fiber form a parasagittally oriented microzone in the cortex (Andersson and Oscarsson 1978; Voogd and Glickstein 1998). The mossy fibers constitute the other input that terminates on the granular cells, which then send up axons to the surface forming parallel fibers that terminate on the Purkinje cells (Voogd and Glickstein 1998). The parallel fiber inputs are responsible for modulation of the simple spike activity in the Purkinje cells with firing frequencies from 10 to 100 Hz (Armstrong and Rawson 1979, Cerminara et al. 2009).

Anesthetized animal preparations have commonly been used in electrophysiological experiments conducted in rats, although anesthesia undoubtedly affects the neuronal circuitry in the cerebellar cortex. One of the most popular anesthesia regimes used for electrophysiological experiments is the ketamine/xyazine combination. Ketamine is known to affect the $\alpha$-methyl-D-aspartate (NMDA; Anis et al. 1983; Yamamura et al. 1988; Armstrong and Rawson 1979; Thach 1968). Each Purkinje cell receives a single climbing fiber input, and the Purkinje cells innervated by the same climbing fiber form a parasagittally oriented microzone in the cortex (Andersson and Oscarsson 1978; Voogd and Glickstein 1998). The mossy fibers constitute the other input that terminates on the granular cells, which then send up axons to the surface forming parallel fibers that terminate on the Purkinje cells (Voogd and Glickstein 1998). The parallel fiber inputs are responsible for modulation of the simple spike activity in the Purkinje cells with firing frequencies from 10 to 100 Hz (Armstrong and Rawson 1979, Cerminara et al. 2009).

Anesthetized animal preparations have commonly been used in electrophysiological experiments conducted in rats, although anesthesia undoubtedly affects the neuronal circuitry in the cerebellar cortex. One of the most popular anesthesia regimes used for electrophysiological experiments is the ketamine/xyazine combination. Ketamine is known to affect the $\alpha$-methyl-D-aspartate (NMDA; Anis et al. 1983; Yamamura et al. 1988; Armstrong and Rawson 1979; Thach 1968). Each Purkinje cell receives a single climbing fiber input, and the Purkinje cells innervated by the same climbing fiber form a parasagittally oriented microzone in the cortex (Andersson and Oscarsson 1978; Voogd and Glickstein 1998). The mossy fibers constitute the other input that terminates on the granular cells, which then send up axons to the surface forming parallel fibers that terminate on the Purkinje cells (Voogd and Glickstein 1998). The parallel fiber inputs are responsible for modulation of the simple spike activity in the Purkinje cells with firing frequencies from 10 to 100 Hz (Armstrong and Rawson 1979, Cerminara et al. 2009).
Jörntell 2007). It is not well understood how the anesthesia affects the network dynamics of the cerebellum and its overall function. For this reason, we set out to look at the field potentials of the cerebellar cortex under anesthesia and in awake animals.

The large Purkinje cells and their huge dendritic trees with massive synaptic inputs are located in the layers within 300 μm from the pial surface. Thus cerebellar cortex presents a perfect paradigm to use nonpenetrating, surface electrodes with minimal disturbance to the local neurons for recordings from its molecular layer that contain very important cells of the cerebellar function. Armstrong and Drew (1980) and Eccles et al. (1968) have demonstrated that the mossy and climbing fiber activities evoked by electrical stimulation can be recorded with small ball electrodes placed on the surface and that the recorded waveforms contained the characteristic volleys that were observed with penetrating microelectrodes.

To perform field potential recordings in unanesthetized animals, we chronically implanted rats with flexible substrate multielectrode arrays (MEAs) on the paramedian lobule of the posterior cerebellum, and the motor cortex as a control, to assess the effect of anesthesia. The surface MEAs allowed us to record 32 channels of simultaneous activity from a large area of the cerebellar cortex (~1 × 2 mm) that would otherwise be very difficult to record from in awake animals using single microelectrodes. A method of multiple microelectrode recording technique was described by Sasaki et al. (1989) and later used by Lang et al. (1999) to record 32 channels of Purkinje cell activity in Crus 2b region of head restrained rats. Bosman et al. (2010) also reported up to 24 channels of simultaneous recordings in head restrained awake mouse cerebellum using a similar method developed by Mountcastle et al. (1991). These multielectrode recording techniques are very difficult to replicate and not suitable for repeated recordings in the same animal for longitudinal studies.

Spontaneous and evoked signals were recorded while the animals were under anesthesia and during wakefulness for comparison. Air puffs applied to the face and dorsal arm areas were used as a stimulus to evoke cerebellar field potentials. The results suggest that anesthesia has a very strong effect both on the spontaneous signals and the evoked potentials recorded from the cerebellum.

RESULTS

Cerebellar activity recorded in quietly resting, unanesthetized animals had frequency components up to 1 kHz, which was in clear contrast with the signals from the motor cortex under and fixed in place by gluing it to the arachnoid/pia mater using very small amounts of octyl cyanoacrylate tissue adhesive (Nexaband; WPI) over the array. A stainless steel wire that served as a reference electrode was laid down over the area on the backside of the array and fixed in position. The Omnetics microconnector at the other end of the lead wires was fixed on the skull using dental acrylic and five stainless steel screws.

Another skull opening was made over the contralateral motor cortex, and a similar MEA was implanted subdurally with the electrode corners approximately at the coordinates of anteroposterior: 2–3 mm and mediolateral: 1–3 mm to the bregma, with the array slightly turned toward the midline. The Omnetics connector for this electrode was also fixed to the skull with dental acrylic and the skin was closed with 5.0 sutures around the connectors, and the back of the neck.

Recording procedures. Spontaneous and evoked potentials were recorded while the animals were under anesthesia and in wakefulness. Anesthesia was induced in chronically implanted animals with a single intraperitoneal injection of 55 mg/kg ketamine and 12 mg/kg xylazine mixed and diluted in normal saline. The recordings were performed with two 34-channel head-stage amplifiers (gain = 100, 0.7 Hz-3 kHz; Triangular Biosystems International) inserted into the microconnectors on the head while animals were placed in a large Faraday cage. Each amplifier had its own reference electrode implanted separately nearby the array. The signals were sampled at 16 kHz and collected in 5-s episodes before the anesthesia and also as the animals were recovering from anesthesia at regular intervals. Video images were captured simultaneously with neural recordings to confirm retrospectively that the animals were not moving during data collection.

Evoked potentials were induced by single and repetitive air puffs (30 psi, duration >50 ms, where the puff onset elicits the main response) to the periphery, e.g., ipsilateral forearm, whiskers, face, and perioral areas. Multiple trials were averaged to reduce background activity against the evoked signals. During recovery from anesthesia and in awake rats, we made sure that the animal did not move immediately before the air puff thereby avoiding large spontaneous activity that would contaminate the evoked potentials. All data analysis was performed in Matlab. Preliminary results were published in the abstract form (Ordek et al. 2012).

METHODS

Surgical procedure. Flexible multielectrode arrays were chronically implanted in five Sprague-Dawley rats (350–450g) using sterile surgical techniques. All procedures were approved and performed in accordance to the guidelines of the Institutional Animal Care and Use Committee, Rutgers University, Newark, NJ. The rats were anesthetized with ketamine and xylazine (100 and 10 mg/kg, respectively, ip), and additional doses were administered as needed during the surgical procedure. The skull over the paramedian lobule of the cerebellum was removed. A custom-designed 32-channel flexible substrate electrode array (NeuroNexus) was placed subdurally on the right paramedian cortex with the medial edge of the electrode being ~1 mm away from the paravermal vein (Fig. 1). Electrode contacts were 50 μm in diameter and located 300 μm apart from each other in a 4 × 8 configuration, hence covered ~900 × 2,100 μm of the paramedian lobule in the mediolateral orientation. Before surgery, gold contacts were electrochemically coated with poly(3,4-ethylenedioxythiophene) (Venkatraman et al. 2011) to lower their impedances <50 kΩ. After an H-shaped cut was made into the dura, the electrode array was slid into position.
EFFECTS OF ANESTHESIA ON THE CEREBELLUM

Ketamine/xylazine anesthesia reduced all the frequency components in the cerebellum above the frequencies where the dip occurred (~30 Hz) both when the common-mode signal was taken out (up to 4dB) and included, with the effect being stronger in the latter case (Fig. 2B). The anesthesia had the opposite effect for components <20 Hz in the cerebellum (F = 1.99, P = 0.017, n = 20 trials). The motor cortex spectrum was virtually not affected by anesthesia >50 Hz (Fig. 2C, w/o mean). Similar to the cerebellar spectrum, the signal power was significantly higher at the lower end of the spectrum below ~50 Hz under anesthesia (P << 0.01). These results demonstrate a clear difference in the ways that the two cortices are affected by ketamine/xylazine anesthesia at high frequencies but not so much at the lower end of the spectrum.

The difference between the two cortices was evident also by the coherence analysis (Fig. 3A). In the cerebellum, the average coherence calculated between all adjacent contact pairs (300 μm center-to-center) was >0.6 (to pick an arbitrary value for comparison) for all frequencies <1 kHz before the common-mode signal was taken out. A drop in the coherence plot was observed at ~30 Hz similar to the power spectrum. The coherence values decreased <0.3 when the common-mode signal was cancelled by subtracting the spatial average of the contacts in the cerebellum. The motor cortex coherence was higher in general but extending only up to 200 Hz. The cerebellum coherence plot was reaching up to ~800 Hz, hence clearly contrasting with the motor cortex. Both cortices had large coherence at the lower end of the spectrum even after the mean was taken out (0.7 at 10 Hz). At this point, we speculated that the common-mode signal in the cerebellum recordings originated from the neurons located deep in the sulci on both sides of the paramedian lobule. Thus only the signals without mean were used in the rest of the analysis.

The cerebellar coherence was higher between adjacent contact pairs oriented in the mediolateral direction than the ones in the rostrocaudal direction (Fig. 3B). Anesthesia induced a significant decrease in the cerebellar coherence at all frequencies in both directions (red vs. yellow lines in Fig. 3B). A small bump was frequently observed in the anesthetized plots between 200 and 300 Hz as the rat was arousing from anesthesia (not shown in this plot). The motor cortex coherence slightly decreased by anesthesia across the spectrum, although the effect was not as strong as that of the cerebellum, and it was limited to the frequencies <200 Hz (Fig. 3C). In general, the impact of anesthesia was better demonstrated by the coherence analysis than the power spectra in both cortices.

Figure 4 shows the results of one-way ANOVA analysis for electrode separation on the coherence values specifically at 100 Hz, an arbitrary frequency where both cortices have high coherence between adjacent contacts. In the cerebellum, the decrease in median coherence from intercontact distance of 300–600 μm was statistically significant both in the mediolateral and rostrocaudal directions (95% confidence level is
indicated by the side notches; \( F = 16.07, P < 0.01 \). The decrease from 600 to 900 \( \mu m \) was significant only in the mediolateral direction. Increasing the intercontact distance further did not result in smaller coherence values in the cerebellum. In the motor cortex, the smallest coherence was observed at 900-\( \mu m \) distance on average; however, there was not a discernable relation between the coherence and the intercontact distance.

To better illustrate the lack of a consistent relation between contact distance and coherence in the cerebellum, we plotted the coherence spectra from a selected pairs of contacts in Fig. 5. The contact pairs as far apart as 1,825 \( \mu m \) (blue squares) has much higher coherence than the pairs with 1,530 \( \mu m \) (red pair), 1,240 \( \mu m \) (black pair), or 850 \( \mu m \) (green pair) separation. This suggests that the neural networks that are as far apart as 2 mm may be connected or driven by the same afferent pathways to the cerebellar cortex.

The difference between the two recording sites and the effect of anesthesia was further investigated with correlation analysis (Fig. 6). The signals were filtered with a wide band-pass, Pearson’s correlation coefficient was calculated between all electrode pairs in the arrays using 5-s long time signals, and correlation values were averaged from multiple acquisitions (\( N = 5 \) animals, \( n = 25 \) trials). The checker-board pattern in the matrixes indicates that the contacts that are nearby have higher correlations than the distant ones. That is because the contact numbers are arranged in rows of eight in an increasing order from left (medial) to right (lateral), as shown in Fig. 5. We can thus infer from Fig. 6B that the correlation is higher for closer contact pairs.

Fig. 3. Coherence between electrode channels from the cerebellar and the motor cortices in anesthetized and awake-quiet animals (Welch’s averaged periodogram). A: average coherence between all adjacent channel pairs (300 \( \mu m \) in mediolateral or rostrocaudal direction) in both cortices with and without the mean taken out. B and C: average coherences between all adjacent electrode contact pairs in awake-quiet and anesthetized rats in the cerebellum in the mediolateral and rostrocaudal directions separately (B), and in the motor cortex (C; in both directions). Spikes \( \approx 1 \) kHz are artifacts.

Fig. 4. One-way ANOVA analysis on the coherence values obtained with contacts separated in multiples of 300 \( \mu m \), showing the effect of contact separation on the cerebellar coherence in mediolateral and rostrocaudal directions separately (A) and on the motor cortex coherence (B). Red bar is the median; the boxes show the first and third quartiles; side notches indicate the 95% confidence interval; and wishers are the \( \pm 2.7\sigma \) (99.3% data interval).
between adjacent electrodes both in the horizontal and vertical directions in the motor cortex, and it shifts to negative numbers steadily as the separation increases. The correlations are clearly stronger, and the relation between the correlation values and the contact separation is more evident in the awake motor cortex than the awake cerebellum (compare top triangles from each matrix in Fig. 6). With anesthesia, the motor cortex signals become more correlated to each other and the effect of contact separation becomes clearer (compare top and bottom triangles in Fig. 6B). In the cerebellum, however, the correlation values shift to negatives after anesthesia and the effect of contact separation is almost lost completely. (Fig. 6A). These results seem to contradict with the coherence analysis where neither cortices showed a clear effect of contact separation. Dependency of correlation on contact separation, especially in the motor cortex, may be due to the inclusion of low frequencies in this analysis.

As the next level of analysis, we looked at the cortical potentials evoked by air puffs applied peripherally either to the face or the arm (Fig. 7). In anesthesia, a typical evoked potential from the cerebellum contained an early component with a positive deflection first arriving −6−7 ms (measured at the point where the rising edge begins) followed by a negative going edge, which agreed with the definition of mossy fiber activity in surface recordings with ball electrodes (Armstrong and Drew 1980). The second volley (P2-N2) was not always visible, but its rising edge started −12−13 ms when it was present. In some recordings, a long-lasting (up to −50 ms) negative deflection was also observed as shown in Fig. 5 after \( t = 30 \) ms.

Figure 8 shows the evoked potentials and the signal variation across the 32 electrodes of the paramedian lobule (PML) recording array while the animal is recovering from anesthesia (postinjection times are marked on the side). In deep anesthesia (top plot), evoked potentials had multiple volleys while the early arriving component was the largest. The signal amplitudes were <100 \( \mu V \) in general and had substantial variation across the electrodes. As the subject was recovering from anesthesia, the variation between recorded channels decreased due to a large component emerging in all recording channels (bottom plot). This suggests that the common-mode signal observed in the spontaneous signals from the cerebellum (Fig. 1) was most probably originated within the cerebellum, and it was not a cross talk from other brain areas or muscles.

In the anesthetized animals, the evoked potential amplitudes distributed differentially over the cerebellar array depending on the peripheral site stimulated with an air puff (Fig. 9). Multiple repetitions (\( n = 20 \)) of the stimulus consistently produced larger amplitudes on the caudal aspect of the array when the dorsal arm was stimulated, whereas the face stimulation generated larger potentials on the rostral side of the array (compare Fig. 1, A and B). These plots clearly demonstrated that evoked Fig. 6. Correlation matrixes between all 32 channels of recordings for the cerebellum (A) and the motor cortex (B). Top triangles in A and B represent the awake data, and bottom triangles represent the anesthetized data collected in 5 different days from 5 animals. Matrixes are the correlation averages from 10 epochs, where each epoch is a 5-s-long 32-channel recording. Electrode contacts are numbered sequentially as shown in Fig. 5. Cerebellar and motor cortex signals were filtered from 10–1,000 and 10–200 Hz, respectively, and spatial means are subtracted before computing the correlations in the time domain. Positive and negative correlations are indicated by different shades of red and blue, respectively.
potential amplitudes expressed a somatotopic mapping in the PML area of the cerebellum in anesthetized animals, which is a well-known phenomenon from earlier reports. In the awake animal, however, it was not possible to reproduce this map because the spontaneous cerebellar activity interfered with the evoked potentials and introduced a large variability into the evoked amplitudes (w/o mean). Therefore, it was not possible to obtain stable maps in the three animals that we tested this hypothesis.

DISCUSSION

The local field potential is the net potential of all the voltage sources in a volume conductor. In the neural tissue, field potentials arise from multiple sources. The large low frequency oscillations are mostly due to the postsynaptic potentials (Avitan et al. 2009; Mitzdorf 1985). The higher frequency components, however, have been shown to correlate better with the population spiking of the neurons (de Solages et al. 2008; Ray and Maunsell 2011). In the cerebellum, the very high-frequency oscillations arise from the synchrony of the Purkinje cells, which is produced by a network of inhibitory recurrent axon collaterals (de Solages et al. 2008).

In this study, the electrode array was implanted on the cerebellar surface subdurally. Thus the recordings must predominantly contain field potentials from the molecular layer, which primarily includes signals from ascending axons of the

![Fig. 7. Peripherally evoked cerebellar signals in anesthetized animals. A train of air puff stimuli at 1-Hz repetition rate was applied with a pressure of 30 psi to the ipsilateral dorsal arm (red trace) and the facial areas with no whiskers (blue). Multiple acquisitions of evoked potentials (n = 20) were registered at the time of stimulus (S at t = 0 ms) and averaged across both multiple stimuli and 32 channels to show characteristic waveforms generated by arm and face stimulation. Raw signals were filtered between 10 and 300 Hz with a 5th order Butterworth filter.](image)

![Fig. 8. Amplitude distribution of air-puff-evoked potentials from all 32 electrode contacts as the animal is recovering from ketamine/xylazine (55 and 12 mg/kg, respectively, ip) anesthetization in A–C. The first recording was at 20 min after anesthesia injection (A), then at 45 min later (B), and finally during wakefulness (C). Note the increase in the evoked signal amplitudes from A–C.](image)
granular cells and parallel fibers as well as the postsynaptic potentials of parallel fibers on Purkinje cell dendrites and other inhibitory cells to Purkinje cells. Secondary to the molecular layer, Purkinje cell simple and complex spike activities and the granular cells might make weaker contributions to the recorded signals due to their distance from the pial surface. Armstrong and Drew (1980) electrically stimulated the cutaneous afferents to the snout in decerebrated rats and showed that the characteristic components of the extracellular field potentials generated within the cerebellar cortex by the mossy fiber inputs were detectable with surface ball electrodes. The field potential volleys shown in Fig. 7 are in good agreement with their surface recordings (their Fig. 2), except that the arrival times are delayed by a few milliseconds in our signals. This may be attributed to the usage of air puff as a stimulus on the skin, as opposed to direct electrical stimulation of the snout sensory nerves, which may increase the delay and the variation in evoked potential arrival times and thus spread the signals out in time.

Bengtsson and Jörntell (2007) reported that mossy fiber activity (P1-N1) recorded in the granular cell layer was reduced only marginally by intravenous injection of ketamine/xylazine combination or either one of them separately. In our evoked potential recordings from the pial surface, the P1-N1 volley is much smaller than the one in the awake animal. This may be due to the difference in the strength and route of anesthesia (33/1.7 mg/kg of ketamine/xylazine iv in their case and 55/12 mg/kg ip in our case).

Bengtsson and Jörntell also reported that the N3 field potential, which was interpreted as the excitatory postsynaptic potentials of parallel fibers on the Purkinje cell dendrites, was greatly reduced as well as the climbing fiber activity at the given dose above. The N3 potential in Fig. 7 is not observable. The time duration and the shape of the long volley at the end rather fits the definition of a long-lasting negative field potential due to a reduction in simple spike activity triggered by a complex spike. This component becomes relatively insignificant compared with a much larger and shorter negative volley that emerges in the awake animal (Fig. 8). This new component is most likely the N3 potential from the parallel fiber synapses as suggested by its arrival time and duration. This confirms the report by Bengtsson and Jörntell that synaptic activity from parallel fiber-to-Purkinje cells is greatly reduced by ketamine/xylazine anesthesia. Our recordings do not show the mechanism by which this anesthesia regime effects the cerebellar cortical networks. These evoked potential results, however, confirm previous results and further show that the effects of ketamine/xylazine anesthesia on the granular and molecular layer activities of the cortex can be detected with MEAs from the cortical surface. This supports our motivation for using spontaneously generated surface potentials as a method of assessing the effects of anesthesia at the larger network level in the cerebellum.

With the spontaneous signals, the fact that the intercontact correlation is so high in the awake animal suggests that the source of the common-mode components are away from the cortical surface, most likely generated by the distant cells that are deep in the sulci on both sides of the paramedian lobule. The common-mode and the differential signal power spectra look similar and both extend into very high frequencies up to 1 kHz, including the harmonics (Fig. 2). These high frequencies are most likely to be generated either by the parallel fibers and/or the Purkinje cells of the molecular layer.

One of the defining features observed in our results is that both the spectral power and spectral coherence analysis show a large reduction in the amplitude of the high-frequency components under ketamine/xylazine. Bosman et al. (2010) reported that the ketamine caused a 20–25% decrease in the firing rate of Purkinje cell simple spikes. These numbers do not explain the marked decrease we observed in the power spectra of the signals before the common-mode signal is taken out (Fig. 2B). If it cannot be explained by the simple spike power itself, a potential explanation for the drastic change in the power and coherence plots could be that the simple spike synchrony among the Purkinje cells may have been disrupted by the anesthesia regime. Because we are recording local field potentials from the surface, desynchronized spiking of Purkinje cell networks from regions of the cortex inside the sulci can appear as no activity in the signals averaged across the medium.
The power spectrum of the signals recorded with tetrodes in the Purkinje cell layer in unanesthetized rats had a sharp peak ~254 Hz (de Solages et al. 2008). This study convincingly argued that network oscillations of simple spike activity is due to inhibitory recurrent collaterals of the Purkinje cells and that these oscillations of the Purkinje cell networks are independent of the firing frequency of the individual Purkinje neurons. The broad spectral elevation in higher frequencies in our case can be the average of signals from multiple networks of Purkinje cells oscillating at different frequencies in the awake animal (Fig. 2A). The narrower spectral peaks between 150 and 300 Hz observed sometimes in our rats recovering from anesthesia (not shown) may be the signature of a few oscillating networks that are awakening as the anesthesia wears off.

Conclusions. Our results in general agree with previous reports that ketamine/xylazine anesthesia substantially reduces the spontaneous and evoked signals in the cerebellar cortex (Bengtsson and Jörntell 2007). Despite the reports suggesting marginal effect of ketamine on the Purkinje cell activity, the studies investigating the network activity in large areas of the cerebellar cortex may better be conducted in unanesthetized animal models. There is a clear contrast between the spatial patterns of the spontaneous activity of the motor and PML cortices and the way they are influenced by the ketamine/xylazine anesthesia. On the methodology side, this clearly demonstrates the need for electrode technology that can record the cerebellar activity in behaving animals to better understand the cerebellar function at the network level. Despite decades of investigation on cerebellar function, multielectrode recordings in unanesthetized animals are very rare in the literature. Due to the proximity of the Purkinje cells and their dendrites to the cortical surface, nonpenetrating electrodes can record field potentials with large amplitudes and high-frequency components with subdural implantation. Through the use of MEAs, we were able to analyze multichannel signals from a large area of the cerebellar cortex simultaneously, which is not possible with single microelectrode implants. This can provide a powerful tool to study cerebellar function in behaving animals trained for various tasks.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: G.O. and J.D.G. performed experiments; G.O. analyzed data; G.O., J.D.G., and M.S. interpreted results of experiments; G.O. prepared figures; G.O., J.D.G., and M.S. edited and revised manuscript; M.S. conceived and designed research; M.S. drafted manuscript; M.S. approved prepared figures; G.O., J.D.G., and M.S. edited and revised manuscript; M.S. conceived and designed research; M.S. drafted manuscript; M.S. approved.

REFERENCES


J Neurophysiol • doi:10.1152/jn.00455.2012 • www.jn.org
Scheller M, Butler J, Hertle I, Schneck HJ, Franke C, Kochs E. Ketamine blocks currents through mammalian nicotinic acetylcholine receptor channels by interaction with both the open and the closed state. *Anesth Analg* 83: 830–836, 1996.


