DIFFERENTIAL EFFECTS OF KETAMINE/XYLAZINE ANESTHESIA
ON THE CEREBRAL AND CEREBELLAR CORTICAL ACTIVITIES IN THE RAT

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ABSTRACT

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/xylazine 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, multi-electrode arrays (MEA) in rats and compared them to the signals simultaneously recorded from the motor cortex with similar electrodes. The power spectra and the inter-contact 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/xylazine 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 inter-contact 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 as compared to the cerebral areas. This may be due to the proximity of the molecular layer cells to the pial surface in the cerebellum.

INTRODUCTION

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 around 1-2 Hz (Armstrong & Rawson 1979, Thach 1968). Each Purkinje cell receives a single climbing fiber input and the Purkinje cells innervated by the same climbing fiber forms a parasagitally-oriented microzone in the cortex (Andersson & Oscarsson 1978, Voogd & Glickstein 1998). The mossy fibers constitute the other input that terminate on the granular cells which then send up axons to the surface forming parallel fibers that terminate on the Purkinje cells (Voogd & 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 & 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 circuitry in the cerebellar cortex. One of the most popular anesthesia regimes used for electrophysiological experiments is the ketamine/xylazine combination. Ketamine is known to affect the N-methyl-D-aspartate (NMDA) (Anis et al 1983, Yamamura et al 1990), GABAergic (Hevers et al 2008), nicotinic (Scheller et al 1996), muscarinic (Hustveit et al 1995), and opioid receptors (Smith et al 1987). It has also been shown to affect the voltage gated sodium and potassium channels (Schnoebel et al 2005). Xylazine is an alpha-2-andrenergic receptor agonist (Hsu 1981). Hirona and Obata, using the alpha-2 agonist clonidine, were able to show that activation of the alpha-2 receptor causes a reduction in the firing of presynaptic interneurons and a reduction in the release of GABA (Hirono & Obata 2006).

Ketamine/xylazine anesthesia reduces the N3 component of the extracellular field potentials according to Bengtsson and Jörntell (Bengtsson & Jörntell 2007), which represents the activity by the rising axons of the granular cells and their parallel fiber collaterals (Armstrong & Drew 1980, Eccles et al 1968). The same group also demonstrated that ketamine/xylazine anesthesia reduces the climbing fiber evoked response (Bengtsson & 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 300µm from the pial surface. Thus, cerebellar cortex presents a perfect paradigm to use non-penetrating, 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, and Eccles et al. 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 (Armstrong & Drew 1980, Eccles et al 1968).

In order to perform field potential recordings in unanesthetized animals, we chronically implanted rats with flexible substrate multi-electrode 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 (about 1x2mm) 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. (Sasaki et al 1989), and later used by Lang et al. to record 32 channels of Purkinje cell activity in Crus
2b region of head restrained rats (Lang et al 1999). Bosman et al. also reported up to 24 channels of simultaneous recordings in head restrained awake mouse cerebellum (Bosman et al 2010) using a similar method developed in (Mountcastle et al 1991). These multi-electrode 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 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.

METHODS

A. Surgical Procedure

Flexible multi-electrode 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 (100mg/kg and 10mg/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, MI) was placed subdurally on the right paramedian cortex with the medial edge of the electrode being about 1mm away from the paravermal vein (Fig. 1). Electrode contacts were 50µm in diameter and located 300µm apart from each other in a 4x8 configuration, hence covered approximately 900 x 2100µm of the paramedian lobule in the mediolateral orientation. Prior to surgery, gold contacts were electrochemically coated with poly(3,4-ethylenedioxythiophene) (Venkatraman et al 2011) to lower their impedances below 50kΩ. After making an H shaped cut into the dura, the electrode array was slid under and fixed in place by gluing it to the arachnoid/pia mater using very small amounts of octyl cyanoacrylate tissue adhesive (Nexaband, WPI, Inc., FL) at four corners. The dura was sealed with a fast curing silicone elastomer (Kwik-Cast, WPI Inc.) 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 micro connector at the other end of the lead wires was fixed on the skull using dental acrylic and five stainless steel screws.

Place holder for Figure 1

Another skull opening was made over the contralateral motor cortex and a similar multi-electrode array (MEA) was implanted subdurally with the electrode corners approximately at the coordinates of AP: 2-3mm and ML: 1-3mm to the bregma, with the array slightly turned toward the midline. The Omnetic 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.
B. Recording Procedures

Spontaneous and evoked potentials were recorded under anesthesia and in wakefulness. Anesthesia was induced in chronically implanted animals with a single intraperitoneal injection of 55mg/kg ketamine and 12mg/kg xylazine mixed and diluted in normal saline. The recordings were performed with two 34-channel head-stage amplifiers (Gain=100, 0.7Hz-3kHz, Triangular Biosystems International, NC) inserted into the micro connectors 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 16kHz and collected in 5s 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 (30psi, duration > 50ms, 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 G. 2012).

RESULTS

Cerebellar activity recorded in quietly resting, unanesthetized animals had frequency components up to 1kHz, which was in clear contrast with the signals from the motor cortex (solid red and blue in Fig. 2A). Interestingly, the cerebellar spectrum had a dip around 30Hz, roughly in the high beta and low gamma bands. The common-mode component was taken out by subtracting the spatial average of all electrodes from individual channels. The large bump at high frequencies in the spectrum was gone when the mean was taken out, although the signal levels were still above the noise ground. In contrast, the signal power in the motor cortex sharply declined above 60Hz with or without the average is taken out. When the two cortices were compared (w/o mean), motor cortex had almost 10dB higher signal levels than the cerebellum below 60Hz. Above 200Hz, however, the two spectra were comparable in amplitude. We conclude from the relative amplitude changes vs. frequency that the cerebellar spectrum extends up to hundreds of Hz while the signal power in the motor cortex was concentrated below 100Hz.

Ketamine/xylazine anesthesia reduced all the frequency components in the cerebellum above the frequencies where the dip occurred (~30Hz) 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 below 20Hz in the cerebellum (F = 1.99, p= 0.017, n=20 trials). The motor cortex spectrum was virtually not affected by anesthesia above 50Hz (Fig. 2C, w/o mean). Similar to the cerebellar spectrum, the signal power was significantly higher at the lower end of the spectrum
below ~50Hz 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.

Place holder for Figure 2

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 (300µm center-to-
center) was above 0.6 (to pick an arbitrary value for comparison) for all frequencies below 1kHz before
the common-mode signal was taken out. A drop in the coherence plot was observed around ~30Hz
similar to the power spectrum. The coherence values decreased below 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 200Hz. The cerebellum coherence plot
was reaching up to ~800Hz, 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 10Hz). At this
point, we speculated that the common-mode signal in the cerebellar 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 200-300Hz 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 below 200Hz (Fig. 3C). In general, the impact of anesthesia was better demonstrated
by the coherence analysis than the power spectra in both cortices.

Place holder for Figure 3

Figures 4 show the results of one-way ANOVA analysis for electrode separation on the coherence values
specifically at 100Hz, an arbitrary frequency where both cortices have high coherence between adjacent
contacts. In the cerebellum, the decrease in median coherence from inter-contact distance of 300µm to
600µm 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 600µm to 900µm was
significant only in the mediolateral direction. Increasing the inter-contact distance further did not result
in smaller coherence values in the cerebellum. In the motor cortex, the smallest coherence was
observed at 900µm distance on average, however, there was not a discernable relation between the
coherence and the inter-contact distance.

Place holder for Figure 4

In order 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 1825µm (blue squares) has much higher coherence than the pairs with 1530µm (red pair), 1240µm (black pair), or 850µm (green pair) separation. This suggests that the neural networks that are as far apart as 2mm 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 checkerboard pattern in the matrices 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 8 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 (dark red) 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 upper 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 upper and lower 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 around 6-7ms (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 & Drew 1980). The second volley (P2-N2) was not always visible, but its rising edge started around 12-13ms when it was present. In some recordings, a long lasting (up to ~50ms) negative deflection was also observed as shown in Fig. 5 after t=30ms.

Figure 8 shows the evoked potentials and the signal variation across the 32 electrodes of the PML recording array while the animal is recovering from anesthesia (post injection 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 less than 100µV 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. 1A) was most probably originated within the cerebellum, and it was not a crosstalk from other brain areas or muscles.

**Place holder for Figure 8**

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 A and B). These plots clearly demonstrated that evoked 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.

**Place holder for Figure 9**

**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 & Maunsell 2011). In the cerebellum, the very high frequency oscillations (VFHO) 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 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 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 (Armstrong & Drew 1980). 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 millisecond 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 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 (Bengtsson & Jörntell 2007). 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 post synaptic 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 to 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-PCs 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 micro electrode arrays 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 inter-contact 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 1kHz, including the harmonics (Fig. 2). These high frequencies are most likely to be generated either by the parallel fibers and/or the PCs 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. reported that the ketamine caused a 20-25% decrease in the firing rate of Purkinje cell simple spikes (Bosman et al 2010). 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 can not 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 depths of 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 around 254Hz (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 PC 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 150-300Hz 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 agreed with previous reports that ketamine/xylazine anesthesia substantially reduces the spontaneous and evoked signals in the cerebellar cortex (Bengtsson & Jörntell 2007). Despite the reports suggesting marginal effect of ketamine on the PC 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 in order to better understand the cerebellar function at the network level. Despite decades of investigation on cerebellar function, multi-electrode 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, non-penetrating 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 multi-channel 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.
FIGURE LEGENDS

Fig. 1. Drawing of the posterior cerebellum with the MEA positioned on the right paramedian lobule (PML). CS: copula pyramidis.

Fig. 2. Power spectra of micro-ECoG recordings from the cerebellar and the motor cortices in anesthetized and awake-quiet animals. Multiple epochs of 5 sec long signals were collected on different days in 4 rats. First, all 32 channels of recording from multiple epochs (N=20) were averaged (Welch’s method) for a robust measure of frequency content. A. Comparison of motor cortex with cerebellum before and after the mean signals are taken out, B: Effect of anesthesia on the cerebellum, C: Effect of anesthesia on the motor cortex. The effect of anesthesia is much more prominent on the cerebellum than it is on the motor cortex.

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 (300µm 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 B: in the cerebellum in the mediolateral and rostrocaudal directions separately, and C. in the motor cortex (in both directions). The spikes around 1kHz are artifacts.

Fig. 4. One-way ANOVA analysis on the coherence values obtained with contacts separated in multiples of 300µm, showing the effect of contact separation on the cerebellar coherence in mediolateral and rostrocaudal directions separately (A) and on the motor cortex coherence (B). The red bar is the median; the boxes show the first and third quartiles; the side notches indicate the 95% confidence interval; and the wishers are the ±2.7σ (99.3% data interval).

Fig. 5. Sample coherence spectra for four different contact pairs on the cerebellum in an awake-quiet rat. The bottom figure shows the positions of these pairs on the micro ECoG array using color coded squares as in the corresponding plots. The contacts are numbered in groups of 8 in each row, increasing from left to right.

Fig. 6. Correlation matrices between all 32 channels of recordings for the cerebellum (A) and the motor cortex (B). The top triangles in each figure represents the awake data and the bottom triangle represents the anesthetized data collected in five different days from 5 animals. The matrices are the correlation averages from 10 epochs, where each epoch is a 5 second long 32 channel recording. Electrode contacts are numbered sequentially as shown in Fig. 5. The cerebellar and motor cortex signals were filtered from 10-1000Hz and 10-200Hz respectively and the 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.

Fig. 7. Peripherally evoked cerebellar signals in anesthetized animals. A train of air-puff stimuli at 1Hz 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=0ms), and averaged across both multiple stimuli and 32 channels to show
characteristic waveforms generated by arm and face stimulation. Raw signals were filtered between
10Hz-300Hz with a 5th order Butterworth filter.

Fig. 8. Amplitude distribution of air-puff evoked potentials from all 32 electrode contacts as the animal is
recovering from ketamine/xylazine (55mg/kg and 12mg/kg respectively, IP) anesthesia in A through C.
The first recording was at 20 minutes after anesthesia injection (A), and then at 45 minutes later (B), and
finally during wakefulness (C). Note the increase in the evoked signal amplitudes from A to C.

Fig. 9. Somatotopic mapping of the PML area studied with peripherally evoked potentials under
anesthesia. Placement of the electrode array (4x8) is as shown in Fig. 1 on the cerebellum. Towers
indicate the amplitude mean±std (dark and light blue parts) of signal volleys that arrive around 12-13ms
recorded with each electrode in the array, averaged from 10 different acquisitions made on the same or
different days. Multiple air-puffs were applied (N=20) in each acquisition, either to the dorsal arm (A) or
to the wisher free face area (B).
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Figure 2.
Figure 2.

B

Cerebellum

Awake — w Mean
Anest — w/o Mean
Figure 2.

C

M.Cortex

Awake  w Mean
Anest   w/o Mean
Figure 3.

![Graph showing coherence over frequency for different brain regions.]

- **Cerebellum** with Mean
- **M. Cortex** without Mean

**Graph Details:**
- Coherence on the y-axis
- Frequency (Hz) on the x-axis
- Logarithmic scale from $10^1$ to $10^3$ Hz
Figure 3.

Cerebellum

Awake  Med – Lat
Anest   Rost – Cau
Figure 3.

C

M.Cortex

Coherence

Frequency (Hz)

Awake

Anest
Figure 4.

A

Cerebellum

(Rost − Cau) Distance (µm)

(Med − Lat) Distance (µm)

Coherence

0.05

0.1

0.15

0.2

0.25

0.3

0.35

0.4

0.45

0.5

0.55

0.6
Figure 6.

A

Cerebellum

Awake

Anest

B

M. Cortex

Awake

Anest
Figure 7.
Figure 8.
Figure 9.