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Effect of Anesthesia on Spontaneous Activity and Evoked Potentials of the Cerebellar Cortex

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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 and xylazine combination. It is not clear how the cerebellar cortical circuitry is affected by anesthesia. In this study, we have recorded spontaneous and evoked potentials from the cerebellar surface with chronically implanted, flexible-substrate, multi-electrode arrays. The frequency contents of the spontaneous activity suggest that ketamine/xylazine anesthesia suppresses most of the components except those below 30 Hz. This preliminary study also showed that multi channels of cerebellar cortical activity can be recorded using flexible multi-electrode arrays in behaving animals, which is very challenging task with single microelectrodes.**

Keywords: cerebellum and anesthesia, multi-electrode arrays, micro EcoG.

I. Introduction

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. Ketamine/xylazine combination has commonly been utilized as an anesthesia regime in electrophysiological experiments conducted in rats, although anesthesia undoubtedly affects the neuronal circuitry in the cerebellar cortex. Bengtsson and Jorntell showed that ketamine/xylazine anesthesia can substantially reduce parallel fiber and climbing fiber activity [1].

Recordings of neural activity in unanesthetized animals is challenging because of the limited lifetime of electrodes in the harsh conditions of the body environment in addition to the mechanical stresses induced by the animal's movements. Multi-electrode recordings of the cerebellar activity in behaving animals have a potential to provide tremendous insights about the cerebellar function, as acknowledged by many review articles in the field. Despite the abundance of the reports with data collected using single micro electrodes, there are only a few studies where multi-channel signals are recorded in behaving animals [2-4].

Computational power of the cerebellar cortex is found in the structures around the Purkinje cell and its organelles. The Purkinje cells and their dendrites are located in the layers within 300µm from the pial surface. Thus, cerebellar cortex presents a perfect paradigm to use non-penetrating, surface electrodes for recordings of multi-channel neural activity from the most important cells of cerebellar function with minimal disturbance to the local neurons. 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 all the characteristic volleys that were observed with penetrating microelectrodes [5, 6].

In this study, we have chronically implanted rats with flexible substrate multi-electrode arrays (MEAs) on the paramedian lobule of the posterior cerebellum. Spontaneous and evoked signals were recorded under anesthesia and during wakefulness for comparison. An air puff applied to the face was used as a stimulus to evoke cerebellar signals. The results suggest that anesthesia has a very strong effect both on the spontaneous signals and the evoked potentials. The spiking ability of the Purkinje cells must be compromised by ketamine/xylazine anesthesia as suggested by the power density plots.

II. Methods

A. *Surgical Procedure*

Flexible multi-electrode arrays were chronically implanted in two Sprague-Dawley rats (300-400g) 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 paramedian cortex. Electrode contacts were 50µm in diameter and located 300µm apart from each other in a 4x8 configuration. Gold contacts were electrochemically coated with PEDOT to reduce their impedances. The electrode array was fixed in place to the pia mater using very small amounts of octyl cyanoacrylate tissue adhesive (Nexaband, WPI, Inc., FL) at four corners. The Omnetics micro connector at the other end of the lead wires was placed on top of the skull and fixed in place using dental acrylic and stainless screws.

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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 100mg/kg ketamine and 10mg/kg xylazine mixed and diluted in normal saline. The recordings were performed through a 34-channel head-stage amplifier (Gain 100, Triangular Biosystems International, NC) inserted into the micro connector on the head. The signals were sampled at 16kHz and collected in 5s episodes. Video images were captured simultaneously with neural recordings to confirm that the animals were not moving during data collection retrospectively.

Evoked potentials were induced by an air puff (30psi) stimulation of the periphery, e.g. the left or right dorsal forearm, whiskers, face, and perioral areas. Multiple trials were averaged to reduce background activity against the evoked signals. All data analysis was performed using Matlab.

III. RESULTS

Spontaneous activity of the cerebellar cortex was analyzed for frequency content in anesthetized and awake states (Fig. 1). Ketamine/xylazine anesthesia suppressed the cerebellar cortical activity at all frequencies above 30Hz. Interestingly, the large components at low frequencies seemed to be unaffected by the anesthesia regime.

Average coherence between all adjacent electrodes in the array was also computed to further investigate the effect of anesthesia (Fig. 2). The coherence values were very large especially above 60Hz up to about 1kHz in the awake-quiet animals. Interestingly, a lower coherence frequency band was consistently observed in the plots around 20-40Hz.

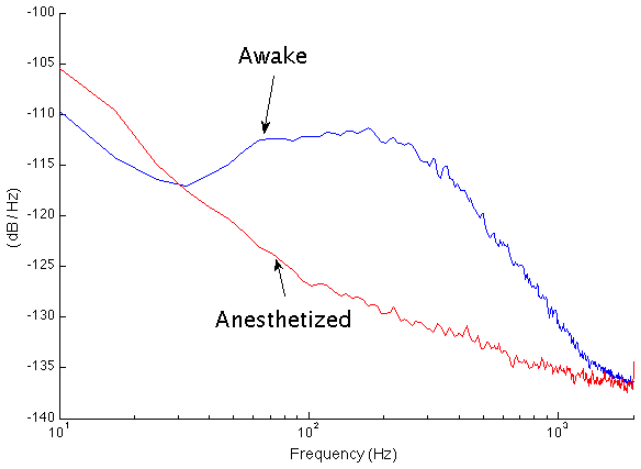

Fig. 1. Power spectra of the spontaneous activity recorded from the paramedian cortex of the cerebellum in anesthetized and awake-quiet animals. Multiple epochs of 5 sec long spontaneous activity were collected on different days in 2 rats. First, all 32 channels of recording were combined into one power spectrum as a representative spectrum of all the channels and then the spectra from multiple epochs (N=5 for each state in each animal) were averaged (Welch's method) for a robust analysis of frequency content.

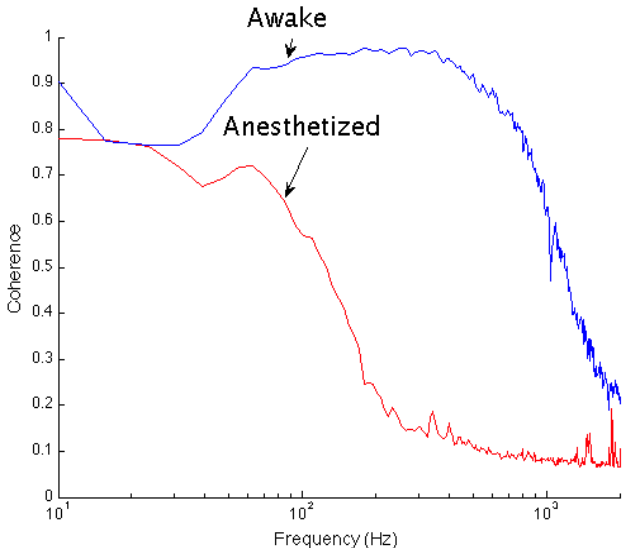

Fig. 2. Average coherence between the adjacent electrode contacts of the array (300µm in medio-lateral or rostro-caudal direction) in anesthetized and awake-quiet animals. No stimulus was applied. Eight epochs in anesthetized and 7 epochs in awake-quiet states from two rats were averaged. Note that the frequency resolution of the plot is about 8Hz.

Anesthesia substantially reduced the coherence at all frequencies except where the lower coherence band was seen in awake animals (20-40Hz).

Next we studied the evoked potentials under anesthesia and wakefulness by applying an air puff to the facial areas without whiskers and to the arms (Figs. 3). In the

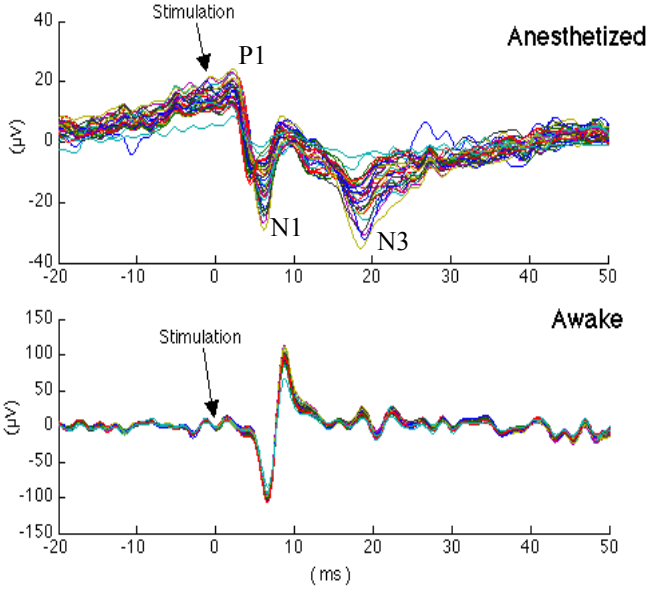

Fig. 3. Cerebellar signals evoked with an air puff to the face (at t=0) in anesthetized and awake states. Traces from all 32 electrodes are shown. Twenty trials were averaged for each trace. The bottom signals were collected in the same rat 90 min after the anesthesia was induced.

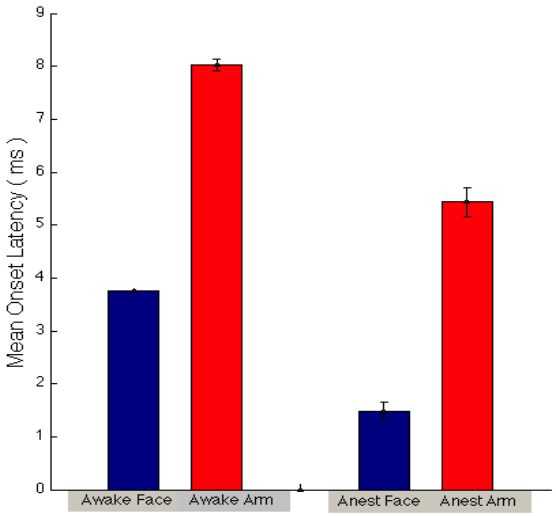

Fig. 4. Arrival times for the first volley in the evoked potentials from the stimulus onset. The air puff, i.e. the stimulus, was applied either to the face or the ipsilateral arm in anesthetized and awake states in two rats. Bars show the mean±std (N=20 stimulus for each bar).

anesthetized state, the evoked potential amplitudes varied across the 32 electrodes in the array as indicated by different colors in the top plot in Fig. 3. The amplitude variation across the array disappeared and all the evoked potential amplitudes became almost the same as the effect of anesthesia wore off in about 90 minutes (bottom plot). The amplitude of the first volley was increased by about 5 fold while the components with longer delays were no longer detectable.

The arrival times of the first volley was further studied in Fig. 4. In general, the signals evoked by arm stimulation always arrived with larger delays than the face-evoked signals. This is expected because the neural pathway from the face, through the trigeminal nerve, to the cerebellum is shorter than that of the arm. However, the fact that the arrival times were longer in the awake state than the anesthetized state was not expected. The differences were large and statistically significant.

IV. DISCUSSION

The electrode array was attached on the cerebellar pial surface in this study. Thus, the recordings must predominantly contain field potentials from the molecular layer, which include signals from Purkinje cells, the inhibitory cells to Purkinje cells, and the synaptic potentials of parallel fibers onto Purkinje cells. Armstrong and Drew electrically stimulated the cutaneous afferents to the snout in decerebrated rats and showed that all the components of the extracellular field potentials generated within the cortex by the mossy fiber input were detectable with surface ball electrodes [5]. The field potential volleys shown in Fig. 3 are in good agreement with their surface recordings, and especially the two negative peaks (N1 and N3) are strikingly similar to those in their Fig. 2; except that our signals are more spread out in time. This may be attributed to anesthesia since Armstrong and Drew used decerebrated preperations. The usage of air puff as a peripheral stimulus, as opposed to electrical stimuli, may increase the variation in evoked potential arrival times and thus spread the signals out in time also.

Bengtsson and Jorntell 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 [1]. 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 of anesthesia used in their (33/1.7 mg/kg of ketamine/xylazine) and our case (100/10 mg/kg). Alternatively, the evoked potentials observed in the awake animals may contain other components, e.g. the spiking activity of Purkinje cells.

The lengthening of the arrival times in the awake animal (although a decrease would be more intuitive) also suggests that the large evoked response observed in the awake animal is not the mossy fiber activity as in the P1-N1 volley of the anesthetized case, but perhaps it is the response of the Purkinje cells in the form of simple and/or complex spikes.

The N3 potential in Fig. 3 is smaller than N1 in amplitude but wider in duration. Bengtsson and Jorntell also reported that the N3 field potential, which was interpreted as the excitatory post synaptic potentials of parallel fibers on the Purkinje cell dendrites, were greatly reduced as well as the climbing fiber activity at the given dose above.

The power spectrum of the signals recorded with tetrodes in the Purkinje cell layer in unanesthetized rats had a sharp peak around 254Hz [2]. This study demonstrated that network oscilations of simple spike activity is due to recurrent inhibitory connections between Purkinje cells. The broad spectral elevation in higher frequencies in our case can be the average of signals from multiple networks of Purkinje cells oscillating at slightly different frequencies.

Fig. 1 suggest that most of the activity in the superficial layers of the cortex is abolished by our anesthetic regime. An alternative 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. Because we are recording local field potentials from the surface, randomized spiking of all Purkinje cells can appear as no activity in the the signals averaged by the medium. However, supression of simple and complex spike activity is a more likely explanation, rather than a mere desynchronization of the simple spike activity, in light of the discussion on the evoked potential response above.

V. CONCLUSIONS

Our results in general agreed with previous reports that ketamine/xylazine anesthesia substantially reduces the spontaneous and evoked signals in the cerebellar cortex. This clearly demonstrates the need for electrode technology that can record the cerebellar activity in behaving animals. Another conclusion is that multi-electrode arrays chronically implanted on the cerebellar surface can record field

potentials in awake animals. Through the use of multi-electrode arrays we were able to analyze signals from a large area of the cerebellar cortex, which was not possible before with single microelectrodes. This can provide a powerful tool to study cerebellar function in behaving animals after training them for various tasks. 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 surface, non-penetrating electrodes can record field potentials with large amplitudes and high frequency components from the pial surface.

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