Spatial Patterns of High-Frequency Oscillations in the Rat Cerebellar Cortex

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Abstract— **Rhythmic signals in the brain have always intrigued neuroscientists and the cerebellum is not an exception. Cerebellar high-frequency oscillations have been explored over many decades, but underlying mechanisms have remained unclear. In this study, we have recorded spontaneous and evoked potentials from the cerebellar surface with chronically implanted, multi-electrode arrays. Evoked and spontaneous signals during behavior showed highly synchronized oscillations at ~150 Hz. Furthermore, this rhythmic activity displayed directional preference on the cerebellar surface. This preliminary study demonstrates the presence of highly synchronized cerebellar oscillations in high-frequency band that emerge episodically in anesthetized animals by sensory stimulation as well as during face cleaning in awake animals.**

Keywords: Cerebellum, evoked potentials, multi-electrode arrays, micro EcoG.

I. Introduction

Cerebellar morphology constitutes an ideal platform to study spatial and temporal characteristics of local field potentials (LFPs) with its crystallite structure. It is divided into three layers; densely packed granule cells at the deepest level, molecular layer that contains parallel fibers (Pfs) running transversely near the surface, and highly organized layer of Purkinje cells (PCs) in between.

Cerebellar signals can express peculiar frequency bands (1-30 Hz) associated with sensorimotor control in rodents and humans [1-3]. Synchronization in cerebellar signals in the 7-25 Hz band was reported in freely behaving animals [2]. It was also shown that the cerebellum can sustain coherent theta (7-8 Hz) activity during sensorimotor processing [3]. While other sub-cortical networks can drive these oscillations, potential sources include the internal circuitry within the cerebellar cortex. Olivo-cerebellar system, which elicits complex spikes on PCs, can show firing patterns at 2-4 Hz and 6-9 Hz [4, 5], whereas granule layer neurons are able to fire in the 7-25 Hz range [6].

A large number of cerebellar studies have investigated low-frequency oscillations, but high frequency activity of the cerebellum is poorly understood. There is only few reports that show high-frequency oscillations in the cerebellum [7-10], revealing rich information content particularly in the 30-200 Hz region [9-11]. To date, it is still speculative whether these oscillations are related to the task or the stimulus.

In this study, we have chronically implanted rats with flexible substrate, multi-electrode arrays (MEAs) on the paramedian lobule (PML) of the posterior cerebellum. Spontaneous and evoked signals were recorded under anesthesia and during wakefulness. We observed that very fast oscillations (100-250 Hz) recorded from the cerebellar surface can be evoked by sensory inputs as well as being generated spontaneously in behaving animals. Furthermore, these rhythms reveal a preferred direction on the cerebellar cortex when they synchronize episodically.

II. Methods

A. *Surgical Procedure*

Flexible multi-electrode arrays were chronically implanted in ten 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, 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 (top panel; Fig.2.B). 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 end of the lead wires was placed on the skull and fixed in place using dental acrylic and stainless steel screws.

B. *Recording Procedures*

Evoked and spontaneous potentials were recorded under anesthesia and in wakefulness, respectively. 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, TBSI, NC) inserted into the micro connector on

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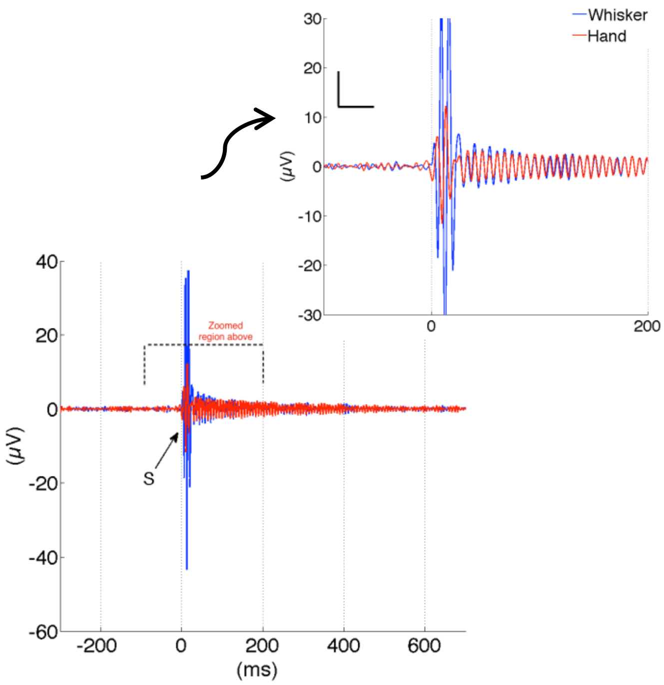

Fig. 1. Evoked potentials are filtered between 100-250Hz and averaged across 20 s epochs and for ipsilateral (to the side of electrode implantation) whisker and hand stimulations. Pre-post stimulus windows show rhythmic signals. In both recordings, oscillations become highly synchronized immediately after the initial EPs and start ringing around 150Hz. Stimulation arrives at t=0ms. Scale bar 40ms, 10μV in the inlet.

the head. The signals were sampled at 16kHz and collected in 20 s episodes. Video images were captured simultaneously with neural recordings to confirm the behavioral state of the animal in awake recordings retrospectively.

Evoked potentials (EPs) were induced by a mechanical tap applied by a tapering 1 mm, cotton-tipped, wood stick attached to the membrane of an audio speaker driven through the computer. Stimulation was applied at 1 Hz in 20 s recording episodes bilaterally on the dorsal hand and whisker areas. Only ipsilateral stimulation results were included in this paper. Recorded neural activities were filtered between 100-250Hz for all plots but power estimation analysis (10Hz-1kHz). All data analysis was performed in Matlab.

III. RESULTS

Sensory evoked potentials stimulated from two different peripheral areas were analyzed for high frequency content (100-250 Hz) in anesthesia (Fig.1). Cerebellar potentials (EPs) evoked by both stimulation of the hand and the face contained high-frequency components in the ~30ms window post-stimulus. While temporal features were similar, whisker EPs were several times larger in amplitude than that of the hand stimulation. Immediately after EPs cessation, a long lasting ringing was observed. Local field potentials featured a nearly perfect sinusoidal shape at ~150Hz and lasted >500ms in both recordings (Bottom panel, fig.1). Two characteristics of the rhythms were noted. First, the pre-stimulus period (-300ms) in the averaged 20 epochs failed to show any evidences of this rhythmicity in any of the animals studied (Top panel, fig.1). Second, oscillation amplitudes were independent of the initial segment of the EP amplitudes. Despite the fact that the EP responses for whisker and arm stimulations were contained within ± 40μV and ± 20μV, respectively, rhythmic activity was under ± 5μV.

Next, we investigated whether there was a spatial relationship to this rhythmic activity. As an advantage, multi-electrode array allowed spatio-temporal correlation analysis on the PML surface (Fig.2B). We searched for correlation changes between all recorded channel pairs in a moving time window after the stimulus arrival. Correlation coefficient was $r \leq 0.3$ in either medio-lateral or rostra-caudal directions in the pre-stimulus period (Fig.2.A and fig.2.C).

Oscillations became synchronized ($r \geq 0.9$) with induced stimulation and lasted for 300-500 ms post-stimulus. While rapid tuning was observed for the initial ~30 ms of the EPs period, it showed no directional preferences. Rhythmic activity specifically made the channels correlate in the medio-lateral direction (Second row, Fig.2.A and C). Interestingly, both hand (blue dot, Fig.2.A) and whisker area (green and black dots, Fig.2.C) stimulations displayed directionality in the same direction. Temporal patterns of high-frequency content continued for 200 to 500ms depending on the stimulation site. Highly correlated oscillations regressed to baseline values ($r < 0.3$) in all recorded channels once the rhythmicity ceased.

Similar but weaker rhythmicity was noted in the rostro-caudal direction for whisker stimulation (black dot; bottom panel, Fig.2.C). Maximum correlation reached to ~0.6 within a relatively shorter time frame (≤ 200ms). In contrast, hand stimulation was absent of the oscillations in this orientation

Spatio-temporal analysis was also applied to the wakefulness state (Fig. 3). Face cleaning behavior involves cyclic arm-hand movements on the perioral whisker and face areas. Electrodes aligned transversely indicated higher synchronization for periods of activity during a 10 s epoch (right; Fig.3). Correlation coefficient varied between 0.2-0.8 in eight channels recorded medio-laterally, which were also associated with evoked potentials from the hand (blue dot; Fig.3). At rest, none of the electrode clusters showed any synchronization (left).

Frequency content of spontaneous signals in awake animals was investigated in a broader bandwidth (10Hz-1kHz). High-frequency oscillation at ~150Hz was confirmed as a bump in the power spectra of face cleaning epochs (red trace; Fig.4). Signal power of the characterized frequency content was 10 fold larger than the baseline power (30Hz-1kHz). An elevated low-frequency band is also noted around the beta band (~20Hz) in the face-cleaning recordings. Resting data did not present any spectral peaks (black trace).

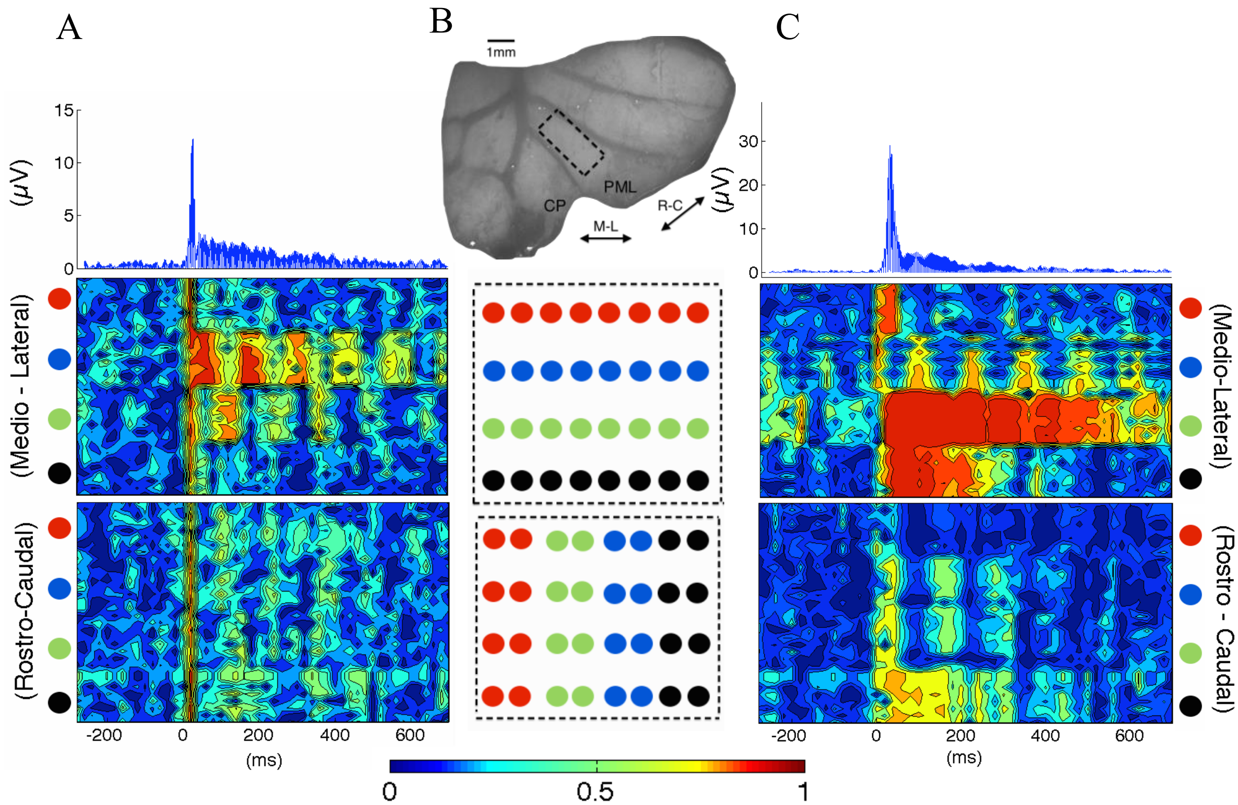

Fig.2. A, C, Synchronous activity (100-250Hz) is shown for pre-/post stimulus periods in response to hand and whisker area stimulations, respectively under anesthesia. Mean cross-correlation coefficients between contact pairs in selected orientations are calculated within a one-second window and incremented in 20 ms steps. Transversely (Second row) and sagittaly (Third row) averaged over 20 repetitions. **B**, Electrode contacts on the MEA are marked with same color dots (Second and third column; Center panel) and matched with correlation plots (Left and right panels). Rectified-averaged signals (100-250Hz) are shown on top to indicate the time and magnitude of the recorded field potentials (Blue traces). Stimulation arrives at t = 0 ms and initiation of rhythmic activity is at t=30 ms (All).

IV. DISCUSSION

High frequency oscillations in cerebellar signals recorded following a sensory stimulus and in a behavioral state were investigated in this study. High-frequency ringing in spontanous cerebellar signals were reported by earlier as well as recent investigations [8-10, 12, 13], though, induced and task related spectral modulations are poorly investigated in the cerebellum literature.

We found that LFPs recorded from cerebellar surface can contain rhythmicity at ~150Hz frequency band following hand and face stimulations (Figs.1 and 2). Temporal pattern of this spatially correlated signals was long-lasting (300ms-500ms) and may be dependent on stimulation characteristics; e.g. stimulus amplitude and peripheral area (Fig.1). Increased spatial synchrony in high frequency oscillations (200-300 Hz) during sensory stimulations was reported by earlier groups [14]. Our results expanded these findings to stimulation of different peripheral areas. Interestingly, both arm and face EPs elicited almost an identical amplitude of rhythmic activity, despite the difference in EP magnitudes (see zoomed region in Fig.1). However, the temporal pattern showed a difference in terms of the duration of synchrony. This may be explained by the strength of the EPs which can elicit longer sustained rhythmicity.

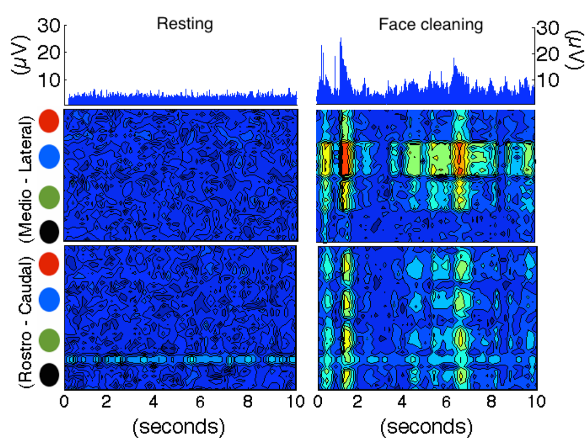

Fig.3. Analysis shown in Fig.2 is applied to quiescent and behavioral conditions in awake animals. Similar to sensory evoked responses; face-cleaning activity demonstrates intermittent synchrony in the same frequency band (100-250Hz). Electrode channels that recorded high rhythmicity following hand stimulation are also the most active during face cleaning.

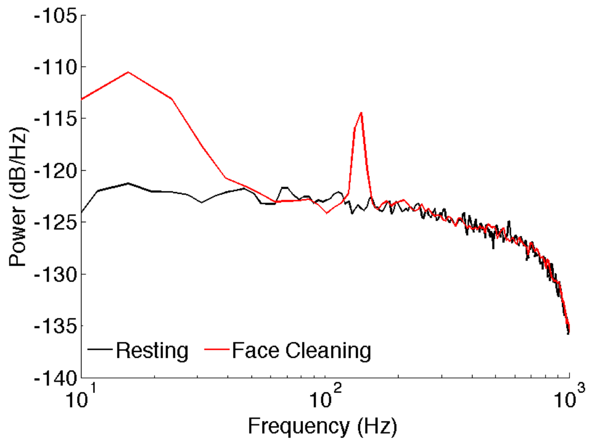

Fig.4. Power spectrum estimation on the same data as in Fig.3. Spectral analysis in face-cleaning indicated a sharp peak at ~146Hz, which is probably associated with the oscillations shown in EPs (Red trace). An elevation at low-frequency band (1-30Hz) is also seen. Resting animals failed to demonstrate any active frequency band during 10s recording episode (Black trace).

D'Angelo and Isope et al. suggested that these rhythms may originat in the molecular layer by parallel fibers [6, 8]. Parallel fibers run along the transverse plane in the most superficial layer of the cerebellar cortex and synapse on PCs. Thus, parallel fiber related potentials are likely to be one of the major sources of recorded LFPs on the cerebellar surface.

In both face and arm stimulation recordings, electrode contacts aligned in medio-lateral direction showed a higher coherent activiy succeding the EPs (Fig.2). The source giving rise to these signals may be the simple spikes, which have typically a maximum of ~250Hz firing frequency [15] and are predominantly synchronized in the paramedian lobule during behavior and largely restricted to PCs oriented along the Pf beam [16, 17].

The same electrode group that showed high rhythmicity with hand stimulation was also highly active at ~150Hz in behaving animals (Right, Fig.3). It may be argued that face cleaning behavior involves largely arm movements. This may be explained by the fact that the Purkinje cells are tonically active and they exhibit ongoing spontaneous discharges that are modulated by incoming sensory information during the face cleaning behavior [18].

V. Conclusion

Our data presented a high frequency rhythmicity in the cerebellar activity recorded from the PML surface primarily in the medio-lateral direction. It was observed that this spectral modulation could be activated by sensory stimulations as well as during face-cleaning behavior. Though these rhythms were observed in multiple animals and sessions, our data does not conclusively explain the source this activity. These results suggest that the surface MEA recordings can be a useful tool to investigate the very fast oscillations of the cerebellum in the spatio-temporal domain.

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