

Hypothesis

Experimental Design

Surgical Procedure

Neural Recording

Data Analysis

Results 1

Results 2

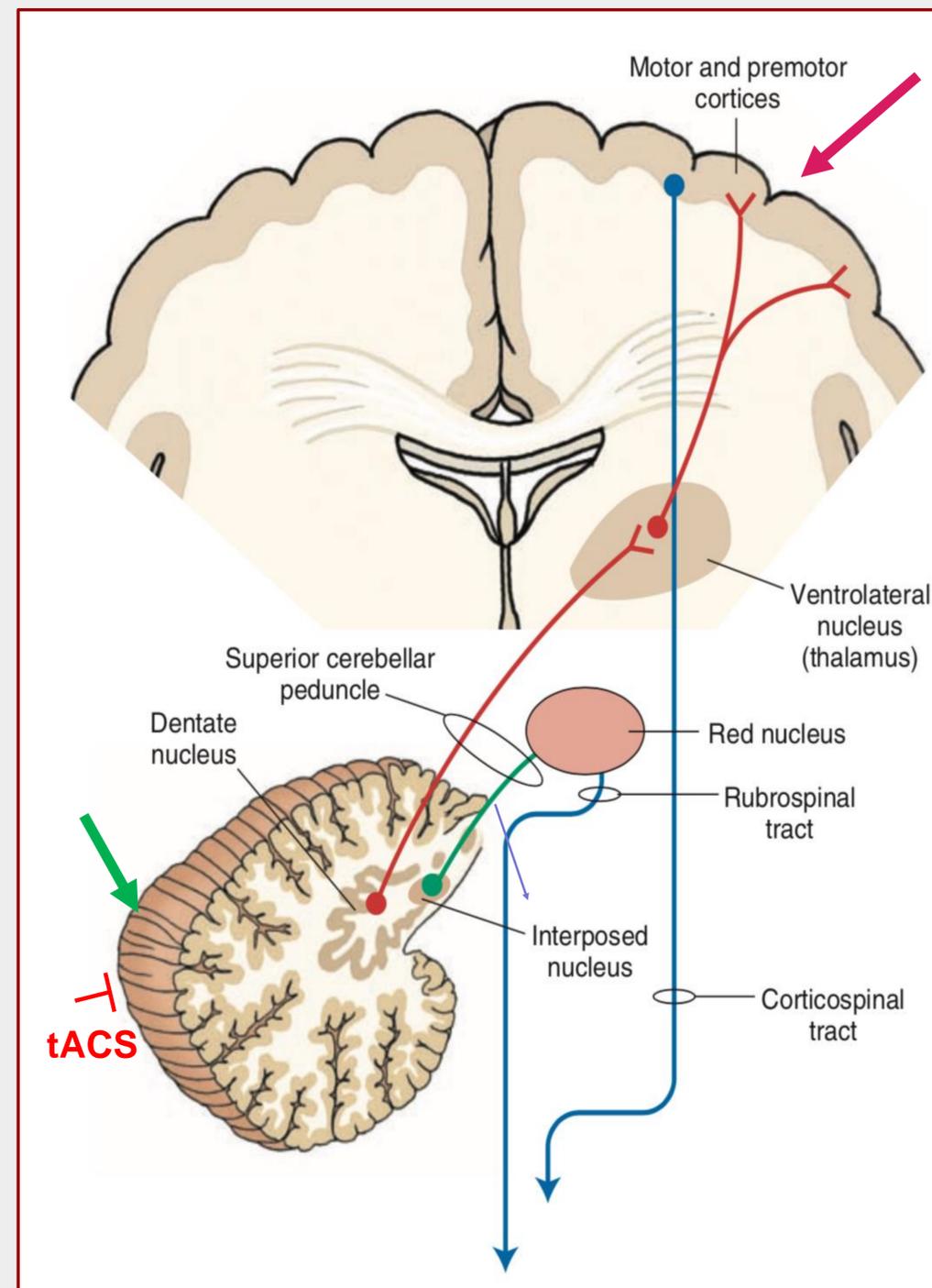
Results 3

Conclusion

Cerebellar ataxia is characterized by uncoordinated muscle movements and often manifests as abnormal gait and lack of fine motor control in the patient population. Although there is currently no approved cure, recent studies suggest transcranial direct electric stimulation (tES) to be a potential therapy for cerebellar ataxia and similar movement disorders in humans. Our previous studies have explored effects of anodal and cathodal transcranial direct current stimulation (tDCS) on Purkinje cell firing rate and local field potential (LFP) in the cerebellar and cerebral cortices. The effect of transcranial alternating current stimulation (tACS), in addition to direct current stimulation, is examined in this study in order to better understand the complete effects of tES.

We hypothesize that tACS modifies the activity of the Purkinje cell and results in an altered effect on motor cortex activity

To test this hypothesis, isolated recordings from cerebellar and motor cortices were collected to observe the electrical firing rate of Purkinje cells and LFPs. Power spectrum analyses were used to investigate cerebellar cortical activity in response to tACS. The average spiking rates were analyzed, along with the power spectrum analyses on LFP recordings. Cross correlation and coherence between the motor and cerebellar LFPs were examined.





Investigation of a Potential Therapy for Cerebellar Ataxia: Transcranial Alternating Current Stimulation (tACS) Using an *in vivo* Approach

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Experimental Overview

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Surgery

Sedation administered to induce loss of consciousness, minimizing distress

Anesthesia administered

Animal placed in stereotactic surgical apparatus

Skull over cerebellar and cerebral cortices exposed; sagittal midline incision made in the skin and soft tissue

Holes drilled in the skull

Neural Recordings

Electrodes Placed

Control recordings conducted for 10 minutes

tACS administered and stimulatory activity recorded for 20 minutes

Post-stimulatory activity recorded for 10 minutes

Ground electrode placed on contralateral cheek

Metal wire placed on skin immediately posterior to cerebellum hole

Recording electrodes placed in cerebellar cortex and motor cortex holes



Investigation of a Potential Therapy for Cerebellar Ataxia: Transcranial Alternating Current Stimulation (tACS) Using an *in vivo* Approach

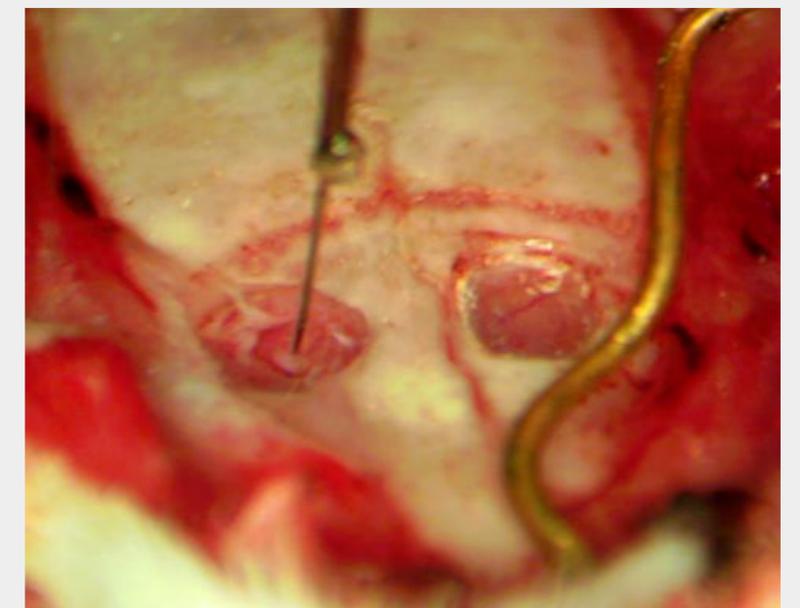
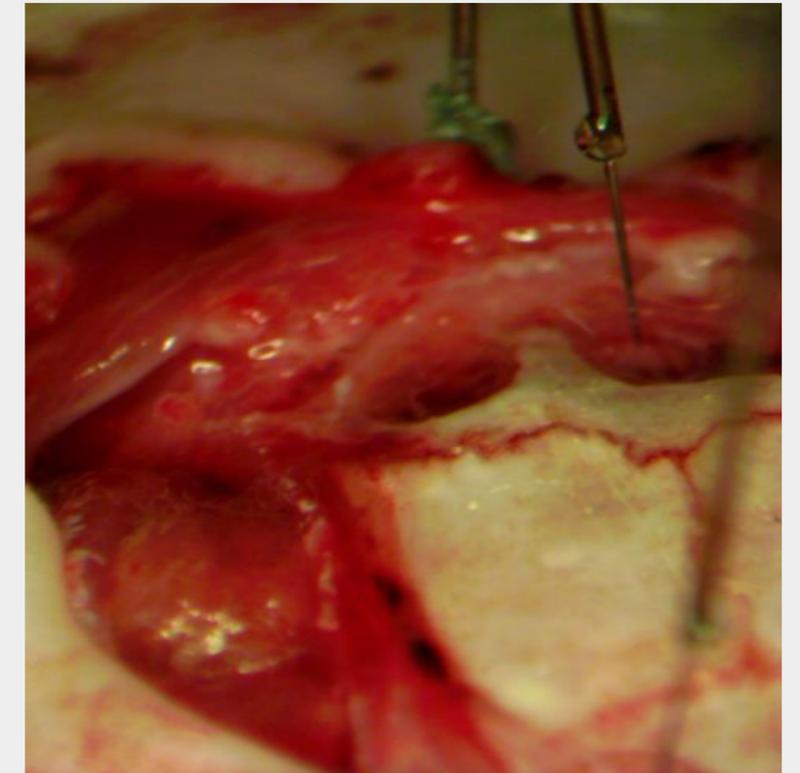
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- Data Analysis
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- Results 2
- Results 3
- Conclusion

Surgery

All cranial surgical procedures were performed according to the Bower lab methods (Bower et al. 1981). Protocols used in this project have been approved by the IACUC of PCOM. Each rat, prior to anesthesia, was placed in a induction chamber with isoflurane (4% with Oxygen at 1 L/min by calibrated vaporizer). This allowed the rat to lose consciousness with minimal distress. Afterwards, the animal was anesthetized via ketamine/xylazine/acepromazine cocktail injected into the intraperitoneal cavity. Supplemental doses of anesthetic were given throughout the duration of the experiment as determined by the animal's vitals and strength of the pedal reflex (response to pain). The animal was then placed in a stereotactic apparatus on a custom built surgical table with heating pad in place. Core body temperature and heart rate were monitored to determine stability of the animal. A sagittal midline incision was made in the skin and the soft tissue was removed to expose the skull over the cerebellar and cerebral cortices. Four holes were drilled into the skull, two over the crus of the cerebellum, and two over the contralateral motor cortex. Using a needle, the dura was excised to expose the brain parenchyma; the skin posterior to the cerebellum was kept intact for the tACS probe. The two cleanest drill holes of the cerebellar and contralateral cerebral cortices were chosen for electrode placement. The ground electrode was placed on the contralateral cheek area. To generate the tACS, a metal wire (1-2 mm² diameter) was placed on the skin immediately posterior to the hole prepared over the cerebellum, the same isolator was used to be triggered by a pulse generator at 20 Hz under bipolar mode. The current was delivered using a stimulus isolator (WPI A365, 100-200 μ A) for 20 minutes.



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Neural Recording

Platinum/iridium monopolar electrodes were used (1 MΩ, UEPSEGSXN4G, FHC, Bowdoin, ME). Neural signals were amplified (A-M Systems model 1700), digitized (Digidata 1300A, Axon Instruments), and recorded with Clampex 9 (Axon Instruments). An electrode was positioned into the motor cortex (1.6 mm anterior to the bregma, 3 mm lateral to the midline, depth = 1 mm). A second electrode was positioned into the surface of the cerebellar cortex and a single unit of Purkinje cell activity was isolated. In order to confirm the cell was stable for recording, the single unit activity was monitored for 2 minutes. After confirming stability, the filter was opened to be 1 Hz - 10 kHz. A pre-tACS control recording was conducted for 10 minutes. tACS was applied and activity recorded for 20 minutes. Lastly, a post-stimulation activity was recorded for 10 minutes.

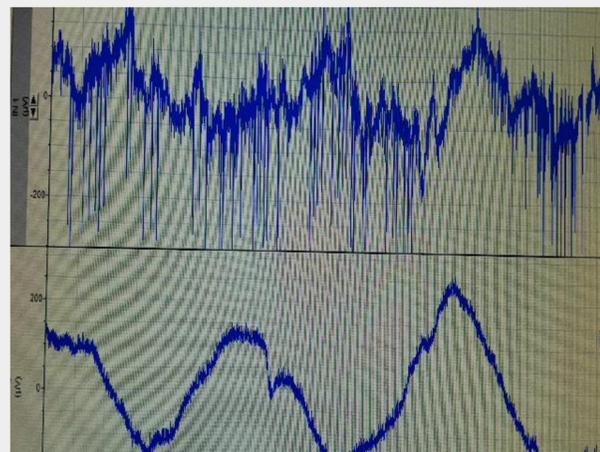
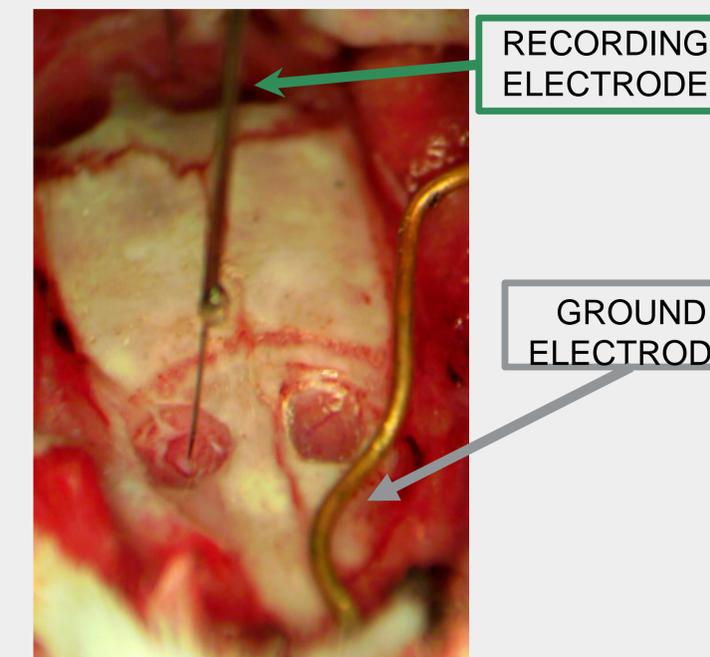
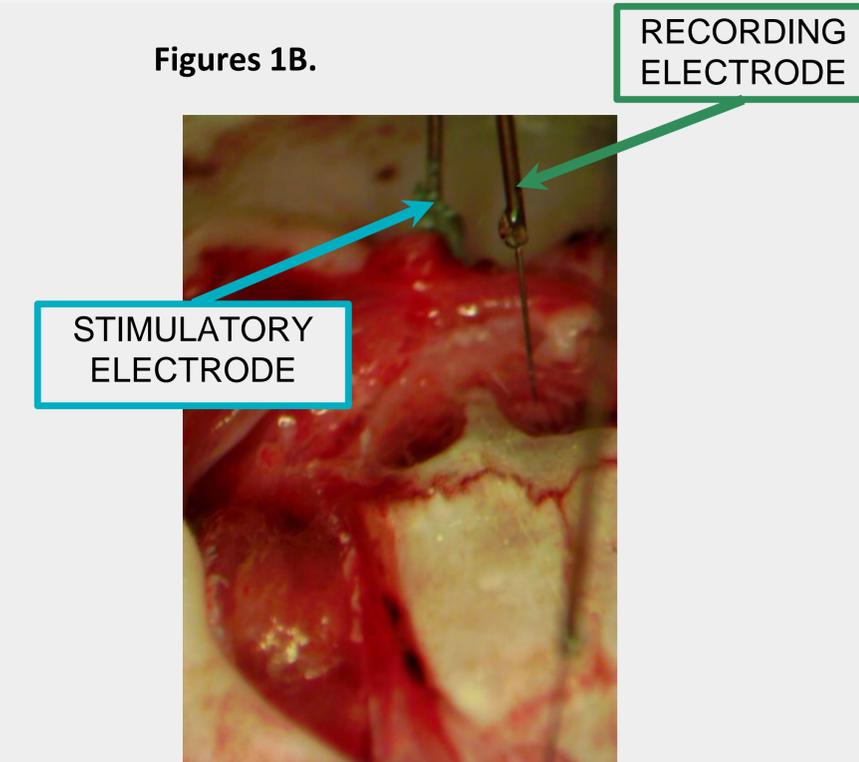


Figure 1A.



Figures 1B.



Figures 1. Cerebellar pathway, circuit, and experimental set-up.

A. (above) Variable orientation of Purkinje cell dendrite trees can be found under tACS stimulation (red, figure is modified from Francis S, et al., *Pharmacological Review*. 62: 525-563. September 2010).

B. (right) tACS location (blue arrow), cerebellar and motor recording (green arrows); ground electrode (gray arrow)

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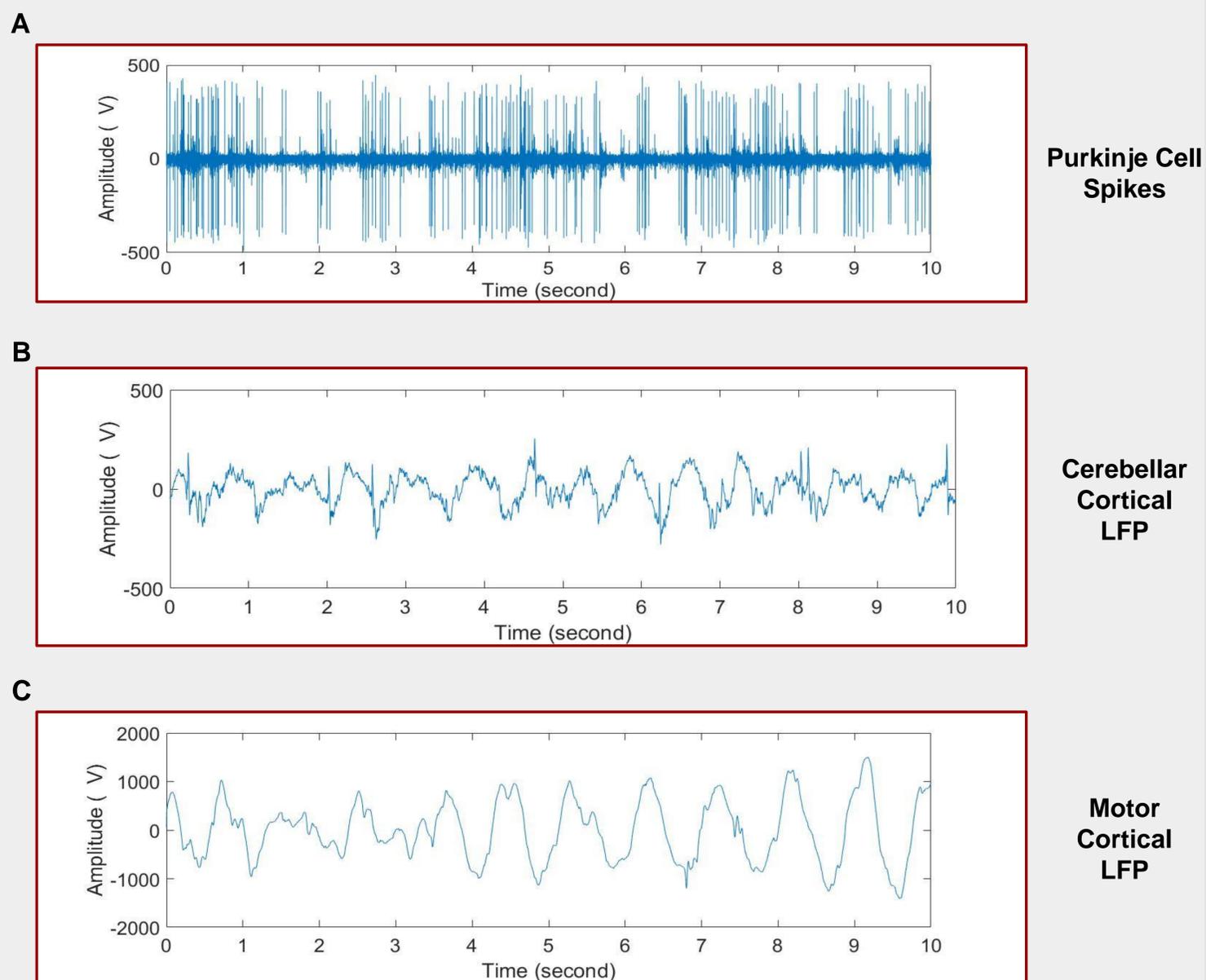
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Data Analysis

Data analyses were performed using MATLAB, a mathematical software program. Artifacts were removed. For each data set, both Purkinje and motor, a plot displaying local field potential (LFP) of cell simple spike activities before, during, and after stimulation were generated. LFPs, ranging from 1-100 Hz, were examined for the general cerebellar and motor cortical cellular activities. Power spectrum analyses were performed for each data set, and surface plots were generated. Cross-correlation and coherence analyses were performed using a custom MATLAB function, which utilized built-in MATLAB commands for cross-correlation and magnitude squared coherence, *xcorr* and *mscohere*, respectively. Filtered data were imported into MATLAB to be processed via this function, and normalized plots were generated to demonstrate correlation and coherence between cerebellar and motor cortices. These quantitative methods have been widely used in the data analysis of brain activities (Adhikari *et al.*, 2010; Brazhnik *et al.*, 2012).

Figure 2. Examples Recordings from Cerebellar and Cerebral Cortices

A. Band filtered from the cerebellar recording, 300 Hz to 10 kHz to show the single unit activity from the isolated Purkinje cell; **B.** LFP (1-100 Hz) events from the cerebellar cortex; **C.** LFP events from the motor cortex using the same filter.



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Results

Purkinje Cell Spiking Rate Change by the tACS

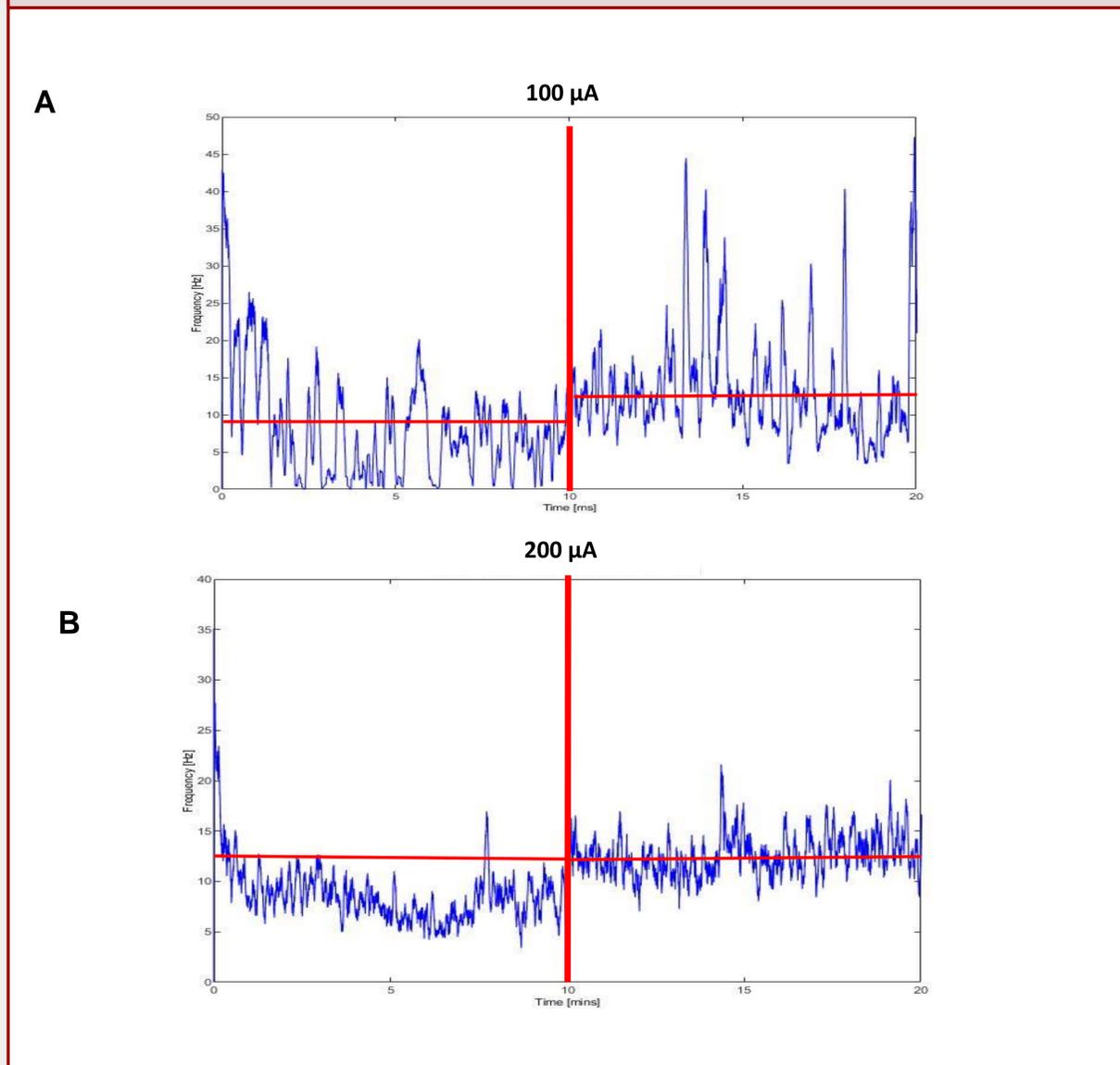


Figure 3. Comparison of Purkinje cell spiking rate in response to 100 μ A and 200 μ A stimulation.

Examples are used to show spiking rate change in Purkinje cell before (0-10 min), and after (10-20 min) tACS. The average Purkinje cell spike rate before and after tDCS is illustrated in red lines. **A.** 100 μ A **B.** 200 μ A.

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Experimental Design

Surgical Procedure

Neural Recording

Data Analysis

Results 1

Results 2

Results 3

Conclusion

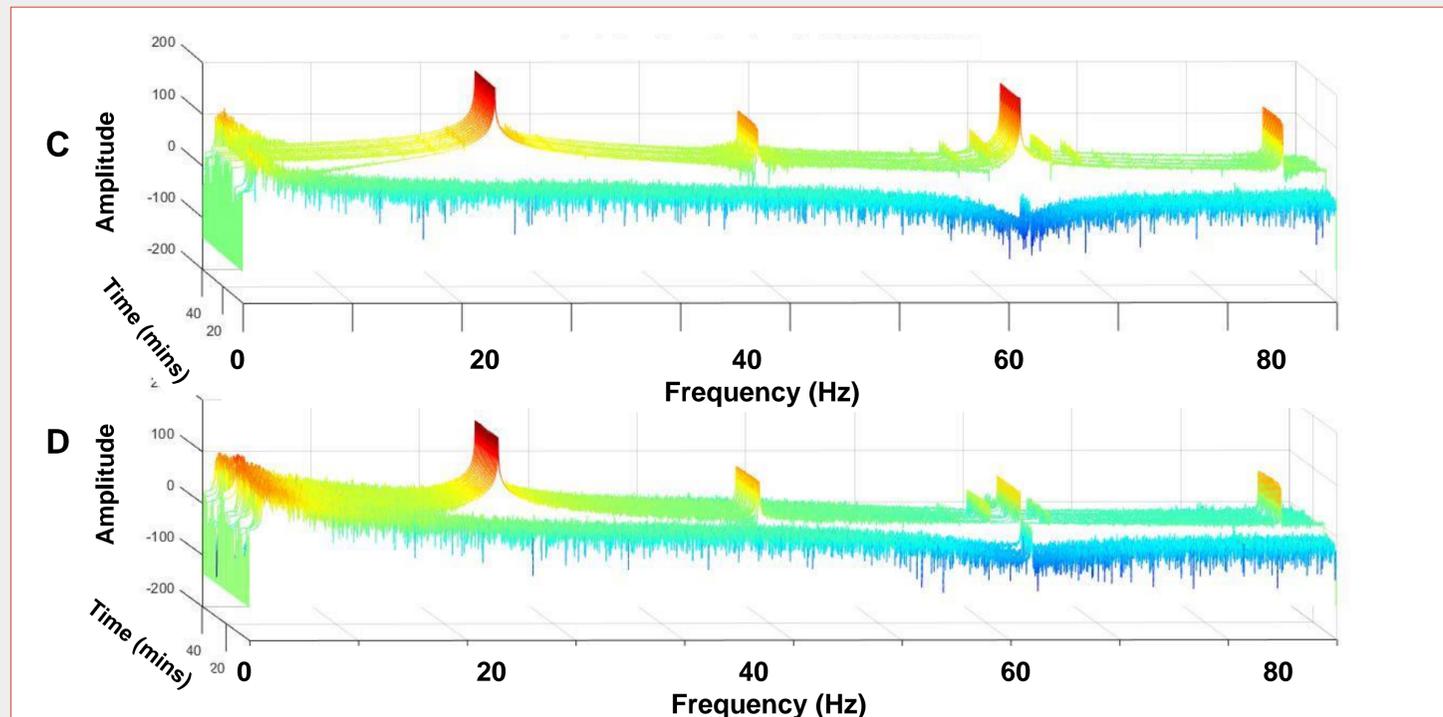
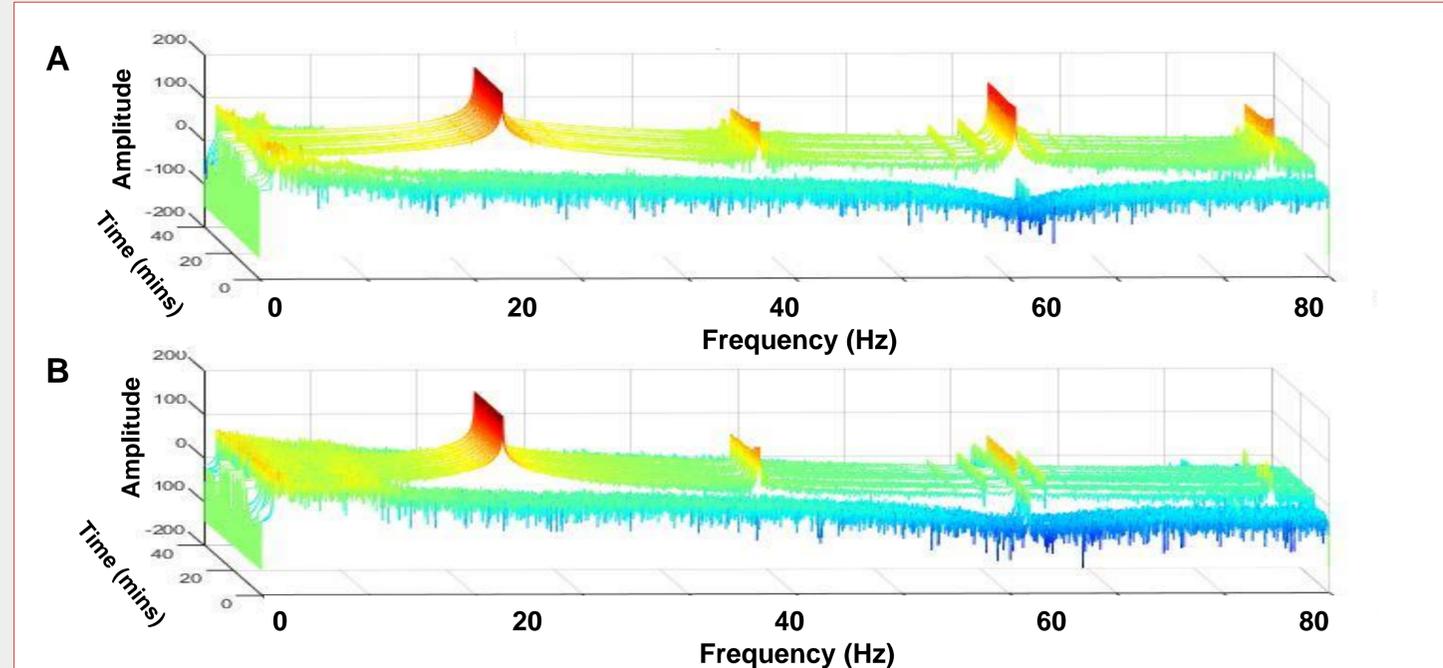


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Power Spectrum Analysis



Results

Figure 4. Changes of cerebellar and motor cortical LFPs in response to 100 μ A (A-B) and 200 μ A (C-D) stimulation

A. Power spectrum of the cerebellar cortex LFP before, during, and after tACS (100 μ A) stimulation; **B.** Power spectrum of the motor cortex LFP before, during, and after tACS using the same parameters.

Recordings done under 100 μ A stimulation revealed that tACS decreases the peak amplitude at low-frequency (2 Hz) in 4/7 cells, while 2 cells showed an increase at 2 Hz, and 1 cell showed no change. Motor cortex remains unchanged **C.** Power spectrum of the cerebellar cortex LFP before, during, and after tACS (200 μ A) stimulation; **D.** Power spectrum of the motor cortex LFP before, during, and after tACS using the same parameters. For recordings done under 200 μ A stimulation revealed that tACS decreases the peak amplitude at low-frequency (2 Hz) while the motor cortex remains unchanged. This decrease was observed in 8/10 recordings.

Hypothesis

Experimental Design

Surgical Procedure

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Results 2

Results 3

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Cross-Correlation and Coherence Comparison

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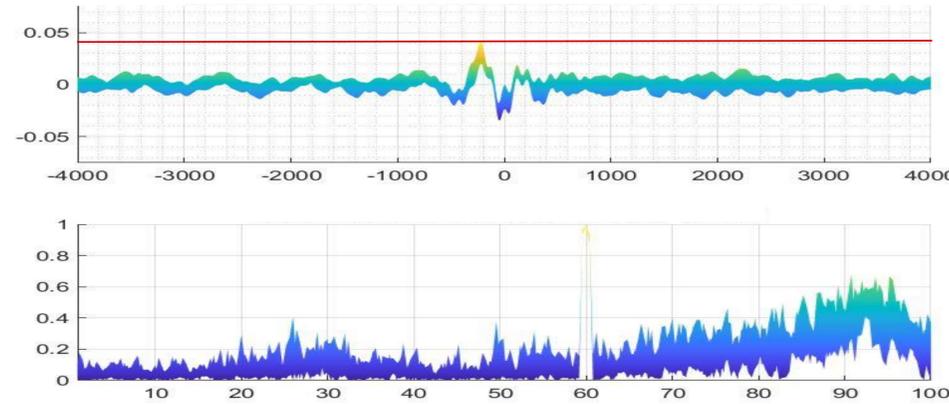
Data Analysis

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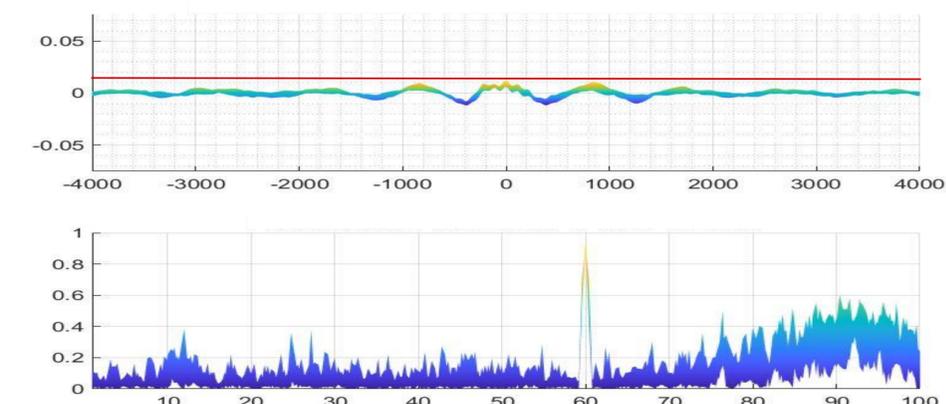
Results 2

Results 3

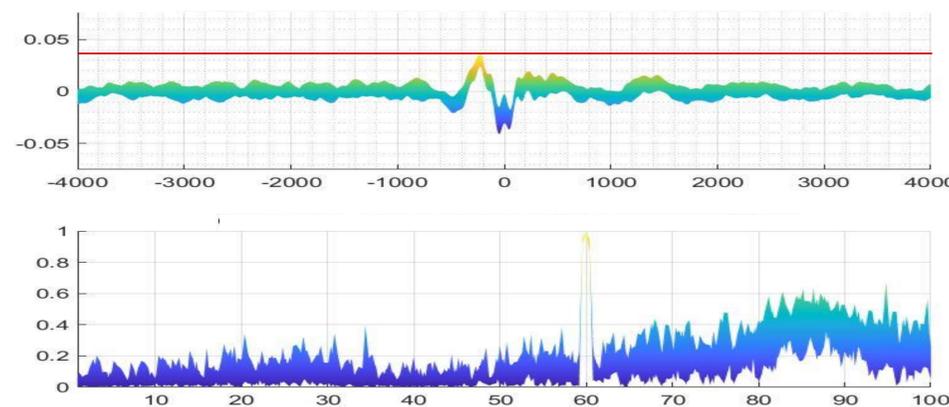
Conclusion



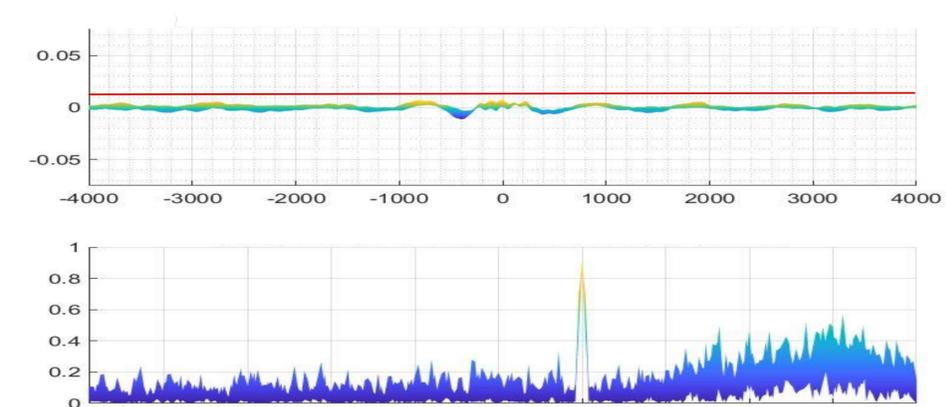
lag: -2.21 ms
corr: 0.042



lag: -2.0 ms
corr: 0.013



lag: -2.23 ms
corr: 0.036



lag: 2.0 ms
corr: 0.008

Results

Figure 5. Cross correlation and coherence changes of LFPs before and after tACS at 100 and 200 μA.

A. Cross correlation and coherence between cerebellar and motor cortex LFPs before tACS (100 μA); **B.** Cross correlation and coherence after 20 minutes of tACS. The cross correlation level between cerebellar and cerebral cortical LFPs under 100 μA demonstrated an increase in 4/8 recordings. Two of the recordings showed a decrease, and 2 recordings were unchanged. Nine recordings showed coherence between the two brain regions at 10 Hz, 5/9 displayed either an increase or decrease. Eight out of 9 recordings showed additional coherence from 85 to 95 Hz. **C.** Cross correlation and coherence between cerebellar and motor cortex LFPs before tACS (200 μA); **D.** Cross correlation and coherence after 20 minutes of tACS. The cross correlation level between cerebellum and cerebral LFPs under 200 μA demonstrated an increase in 4/7 recordings, while 1 decreased, and 2 were unchanged. Five recordings showed coherence between the two brain regions at 10 Hz, with all 5 displaying either an increase or decrease.



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Summary & Conclusion

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1. When subjected to a 100 μ A AC stimulation (n=6), Purkinje cell firing rate increased post-stimulation, while (n=2) Purkinje cells exhibited a decrease in firing rate. When Purkinje cells were subjected to 200 μ A stimulation (n=5), cells showed an increase in overall firing rate post-stimulation, while (n=4) exhibited an decrease in firing rate post-stimulation.
2. The findings from this study suggest tACS can induce a change in cerebellar cortical activity at low-frequency. Subsequent changes in the motor cortex at the same low-frequency were observed, but not as strongly. This was supported by power spectrum analyses used to study changes in cerebellar cortical activity; results from this analysis showed a decrease in peak amplitude at a low frequency of 2 Hz in 12 of the 17 cells tested.
3. Cross correlations between the cerebellar and cerebral LFPs were found to increase post-stimulation, indicating that tACS altered communication between the cerebellar and motor cortices. Changes in coherence values were observed at low-frequency, 10 Hz, suggesting that tACS synchronizes cerebellar and cerebral cortical activities at a preferred frequency.
4. Overall, tACS was shown to cause an increase in the firing rate of Purkinje cells. Power spectrum analyses revealed a decrease in amplitude of low frequency activity of LFP. Additionally, cross correlation and coherence analyses suggest the increase in primary motor cortical activity is related to the increase in Purkinje cell firing rate. These analyses suggest the cerebellar tACS altered primary motor cortical activity due to a change in cerebellar output.
5. Future analyses should focus on comparisons between tDCS and tACS as treatment modalities in diseased and symptomatic animal models. This could provide a better understanding of the clinical applications of tES in the treatment of cerebellar ataxia.