Study of Purkinje Cell Response to alternating current stimulation (ACS) as a treatment modality for Cerebellar ataxia: An *in vitro* approach

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Cerebellum plays an important role in movement coordination and balance. One possible treatment for cerebellar ataxia is transcranial electrical stimulation (tES) a non-invasive procedure to alleviate cerebellar ataxia. However, the underlying mechanism of tES is not fully understood. In the last two years we have studied the response of Purkinje cell to the direct current stimulation (DCS) at the cellular level.

In this project, *in vitro* experiments were conducted to test the hypothesis that ***ACS used as a treatment for cerebellar ataxia changes the excitability of the Purkinje cell by decreasing its firing rate during and after the treatment.*** With this effort, we can have a better understanding about the mechanism of tES.

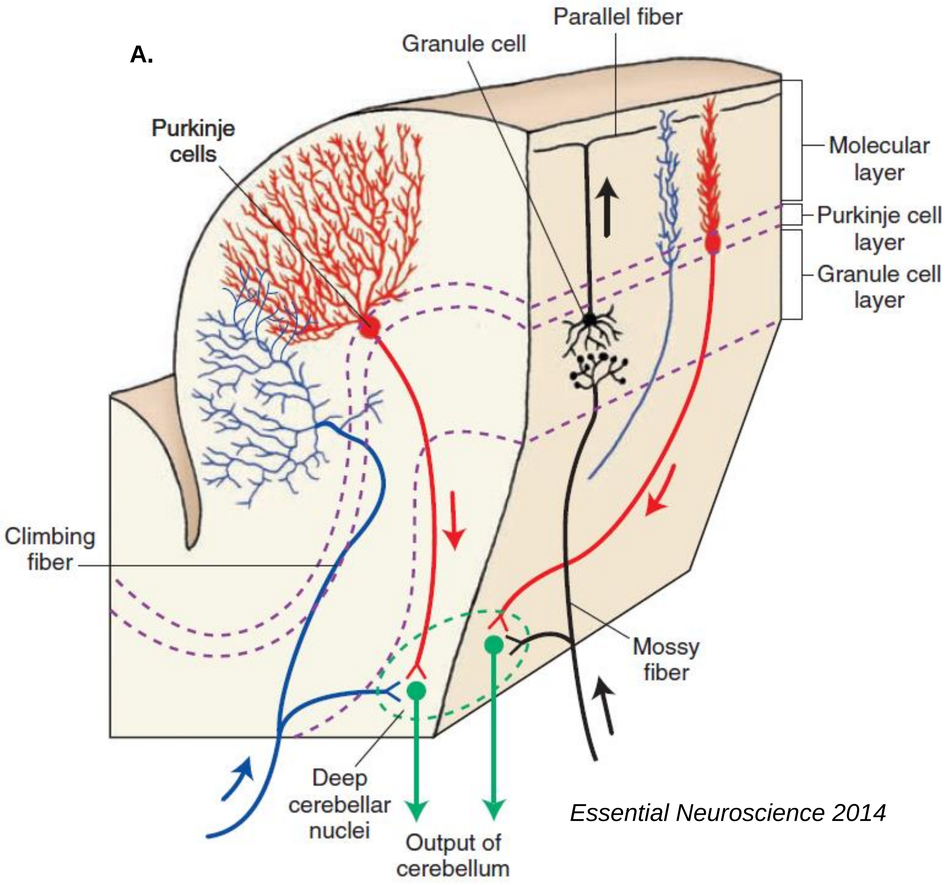

Figure 1. Cerebellum
A. Visual representation of the cerebellar network that Purkinje cells are found in.

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Cerebellar Tissue Preparation

This study follows the protocol (#A17-009) approved by the PCOM IACUC. Sprague-Dawley (SD) rats were used for all experiments. Young pups were used to maximize the quality of recording. Each rat was placed in an induction chamber with isoflurane for 2-4 minutes.

Rats were then weighed and decapitated using a guillotine. An incision was made along the midline of the dermis using surgical scissors. Excess cervical muscles and fascia at the base of the skull was excised. The parietal bone was then removed and the cerebellum was cut with a scalpel. Vessels and meninges were removed from the specimen and placed in ice-cold artificial cerebrospinal fluid (ACSF) before being staged onto the vibratome loading block.

Parasagittal sections of 200-300 μm in thickness were obtained. Slices were then incubated in a 37◦C chamber.

A.

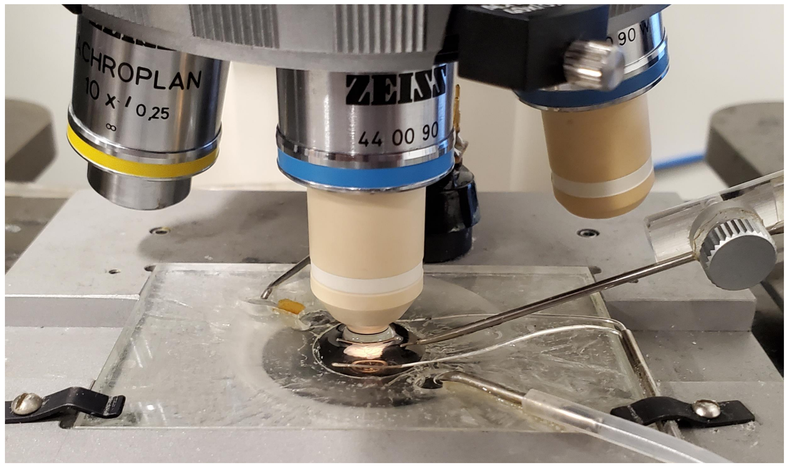

Figure 2. Whole-Cell Patch Clamp Recording Chamber
A. These two wires in parallel induce a uniform electric field across the cerebellar slice. Voltage gradient is indicated by false color in the background. Tips of recording and ground electrodes will be aligned (dotted line) to have the same gradient. Notice that, the Purkinje cells can be oriented differently by following the folds in each folium.

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Cell Recording

In vitro recordings of Purkinje cells were obtained using whole-cell patch-clamp and Axoclamp-200B amplifier. A ground electrode and a glass pipette filled with internal solution was used to patch the cell prior to applying alternating current stimulation (ACS, 20 Hz). Pre-, during- and post- stimulation recordings were obtained.

While establishing a membrane potential to between -60 mV and -75 mV with bias current, a series of current commands (-0.5 nA to +0.5 nA at 0.1 nA per step) were given to test the basic properties of the cell under the control condition. In addition, spontaneous activity was recorded without bias current.

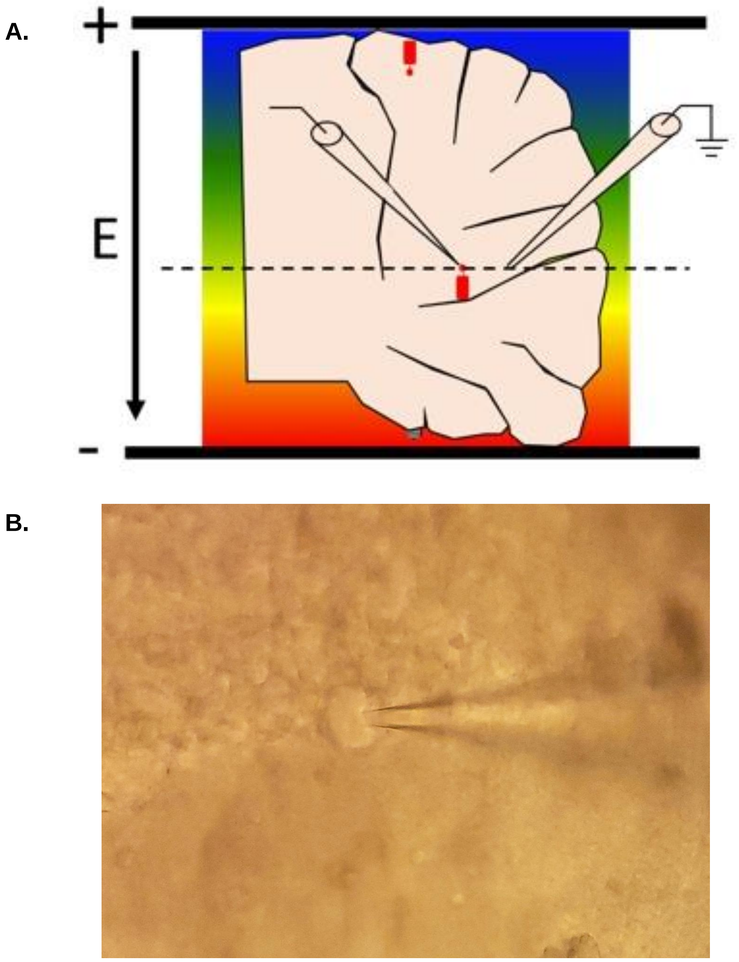

Figure 3. Whole-Cell Patch Clamp Recording Cell
A. Relationship between stimulation electrodes and cerebellar slice. **B.** Micropipette coming in contact with the surface of a Purkinje cell.

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Data Analysis

Statistical significance was determined via Student *t*-Test.

Frequency plots to present a visual representation of the changes in firing pattern was processed using a mathematical computer software program, MATLAB.

Traces from pre-, during- and post- stimulation were processed with number of spikes with the best trace.

Corresponding frequency plots were generated to analyze changes in firing rates.

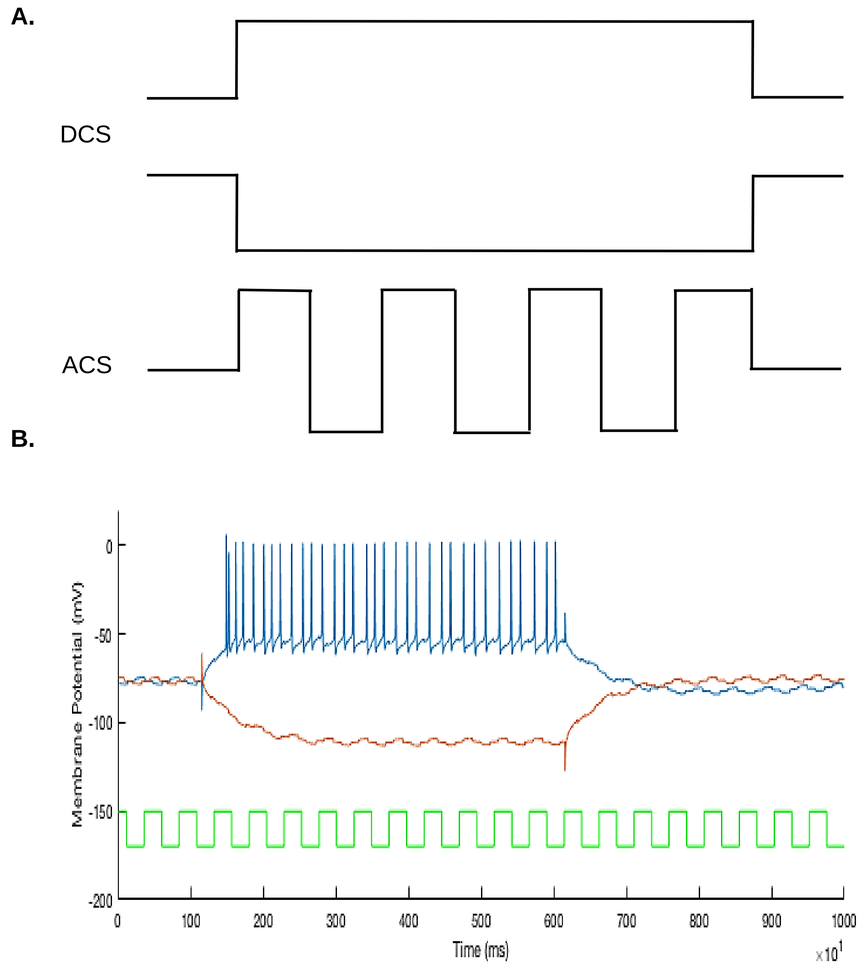

Figure 4. Stimulation Pattern
A. Current stimulation paradigm given to cell in ACS compared to DCS (-200 µA to 200 µA). **B.** Basic property of Purkinje cell during ACS stimulation and its corresponding graph representation of current changes of ACS.

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Purkinje Cell Firing Rate Change

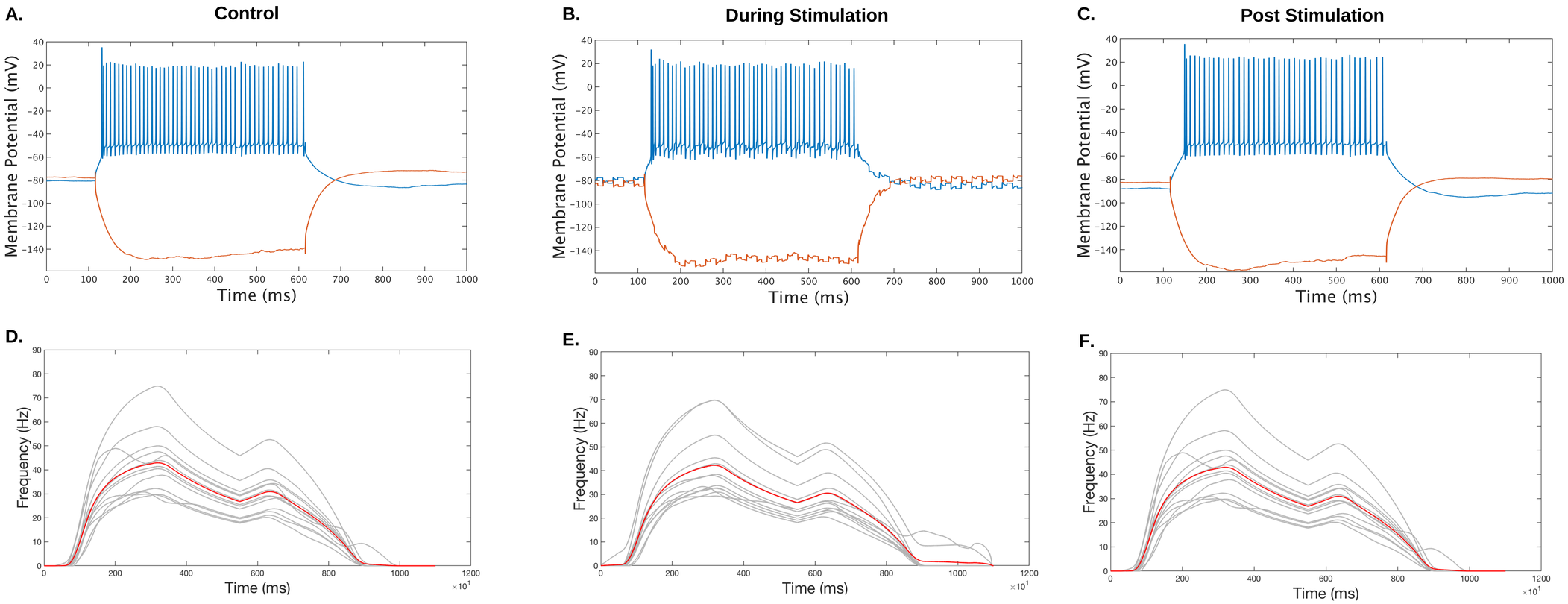

Figure 5. Basic properties and firing rate changes of Purkinje cell before, during, and after stimulation
Control recordings with current injections at -0.7 and +0.2 nA. Recordings with the same current injections; **A.** Pre-ACS. **B.** During-ACS. **C.** Post-ACS. **D.** Firing rate changes of 10 Purkinje cells under control settings. **E.** Firing rate changes of 8 Purkinje cells during ACS. **F.** Firing rate changes of 10 Purkinje cells post ACS. Average firing rate is shown in red.

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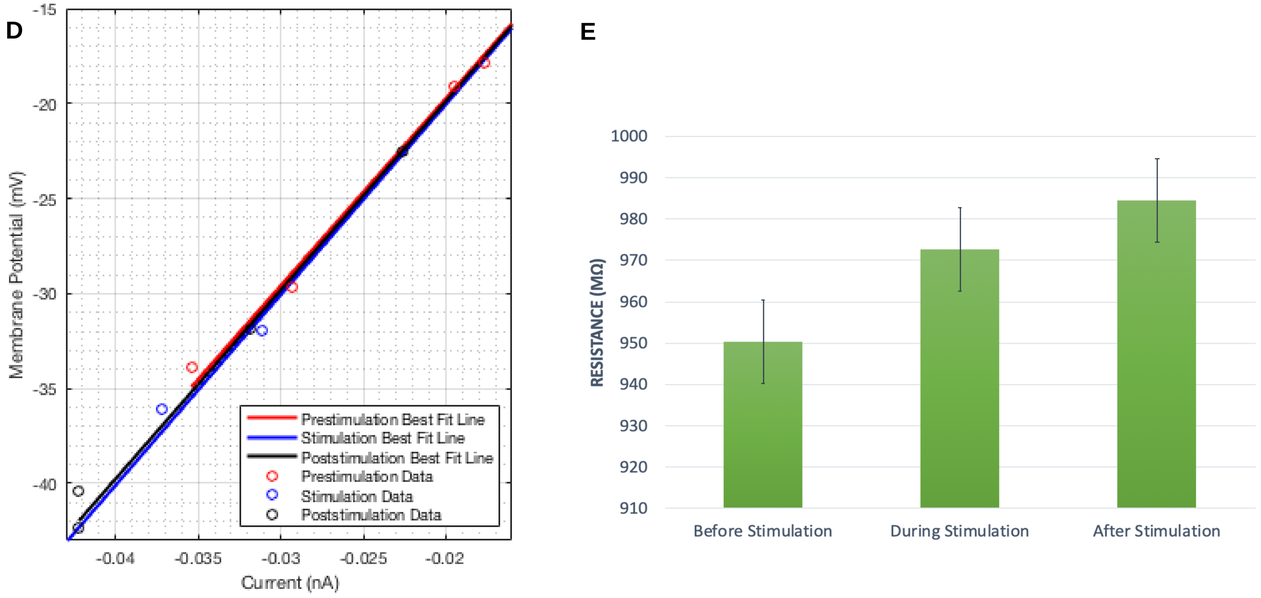
Membrane Resistance Change Responses to ACS



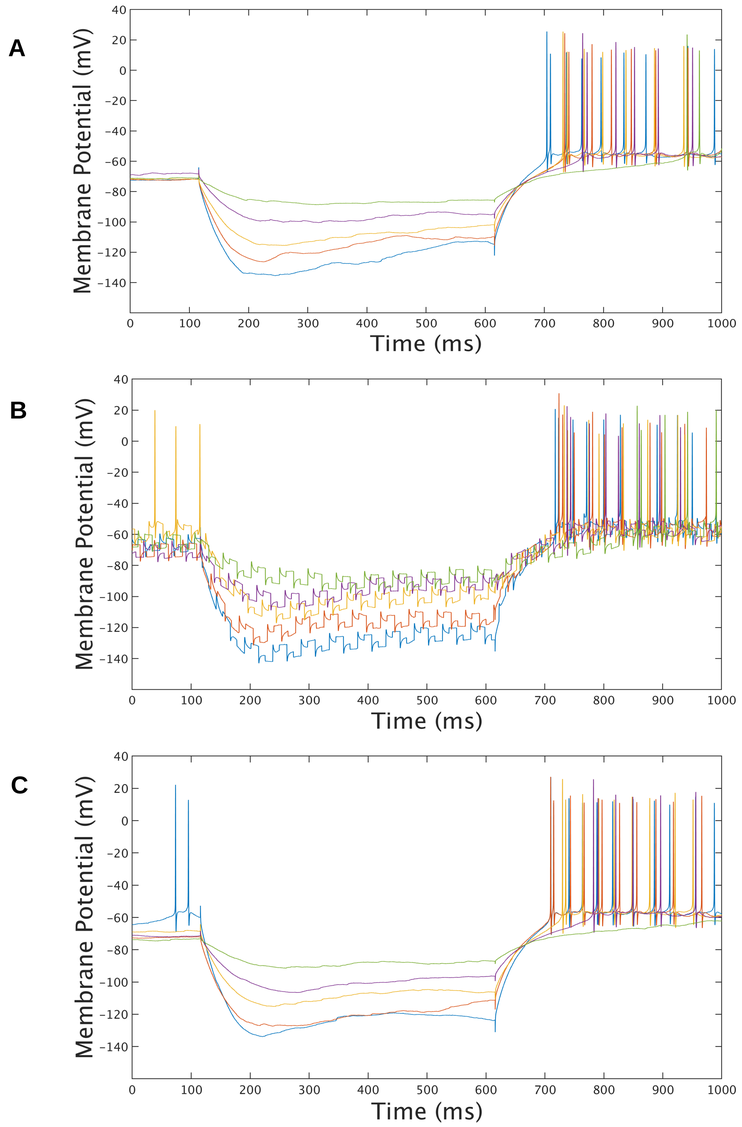


Figure 6. Membrane resistance change in Response to ACS
Five traces of basic properties in response to negative current injection during **A.** Pre-ACS. **B.** During-ACS. **C.** Post-ACS were used to obtain voltage change at the steady state. **D.** Membrane resistance (MΩ) of Purkinje cell is generated by best fit line using the V/I plot. **E.** Comparison of membrane resistance (MΩ) of Purkinje cells (n=6) before, during, and after stimulation.

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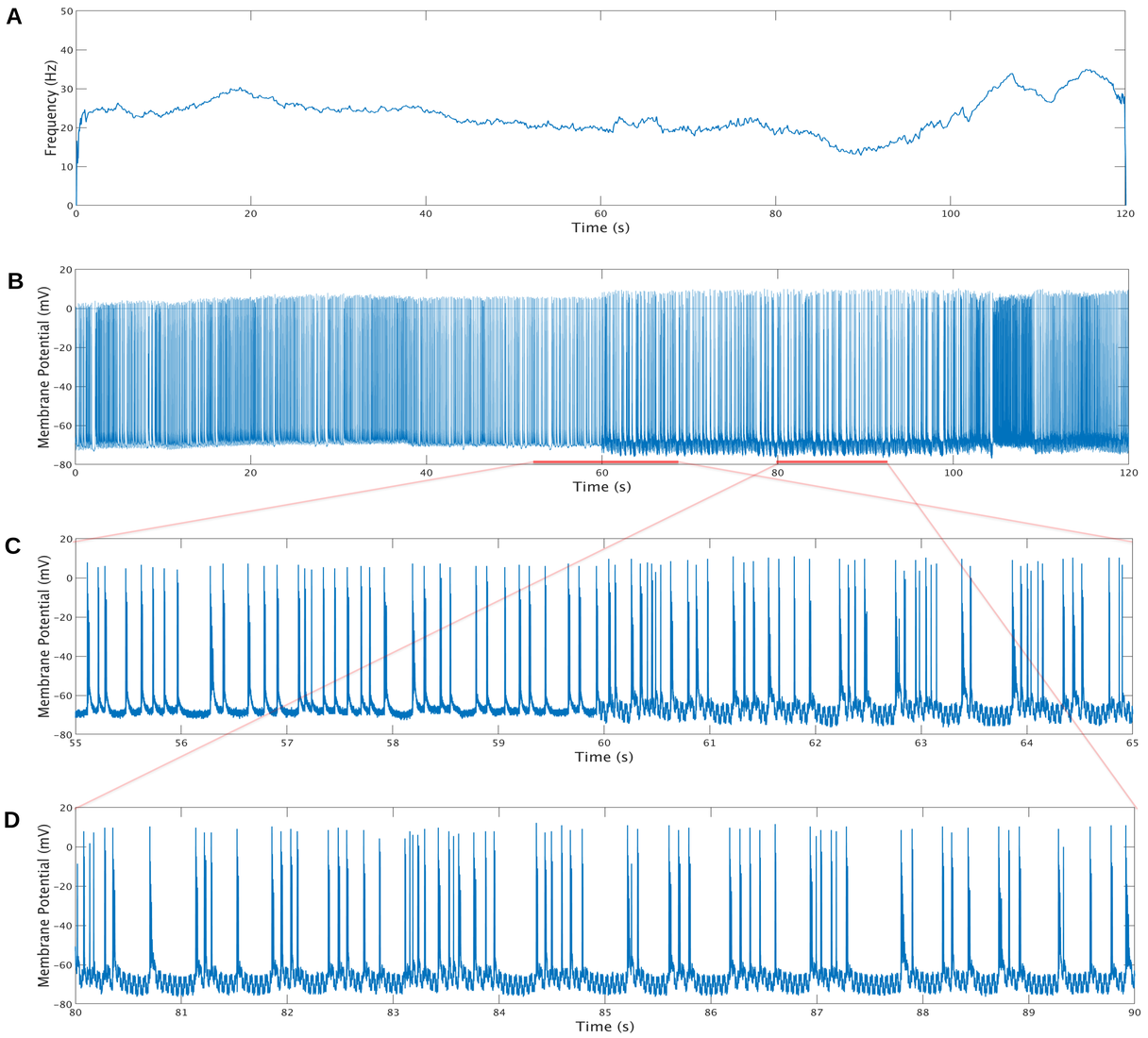

Spontaneous Activity Changes of Purkinje Cells by ACS

Figure 7. Changes of spontaneous activity of Purkinje cell by ACS

A. Purkinje cell firing rate changes with stimulation at the 60 second mark.

B. Original trace of action potentials to generate the plot in A.

C and **D.** A closer look of recordings under beginning and during ACS.

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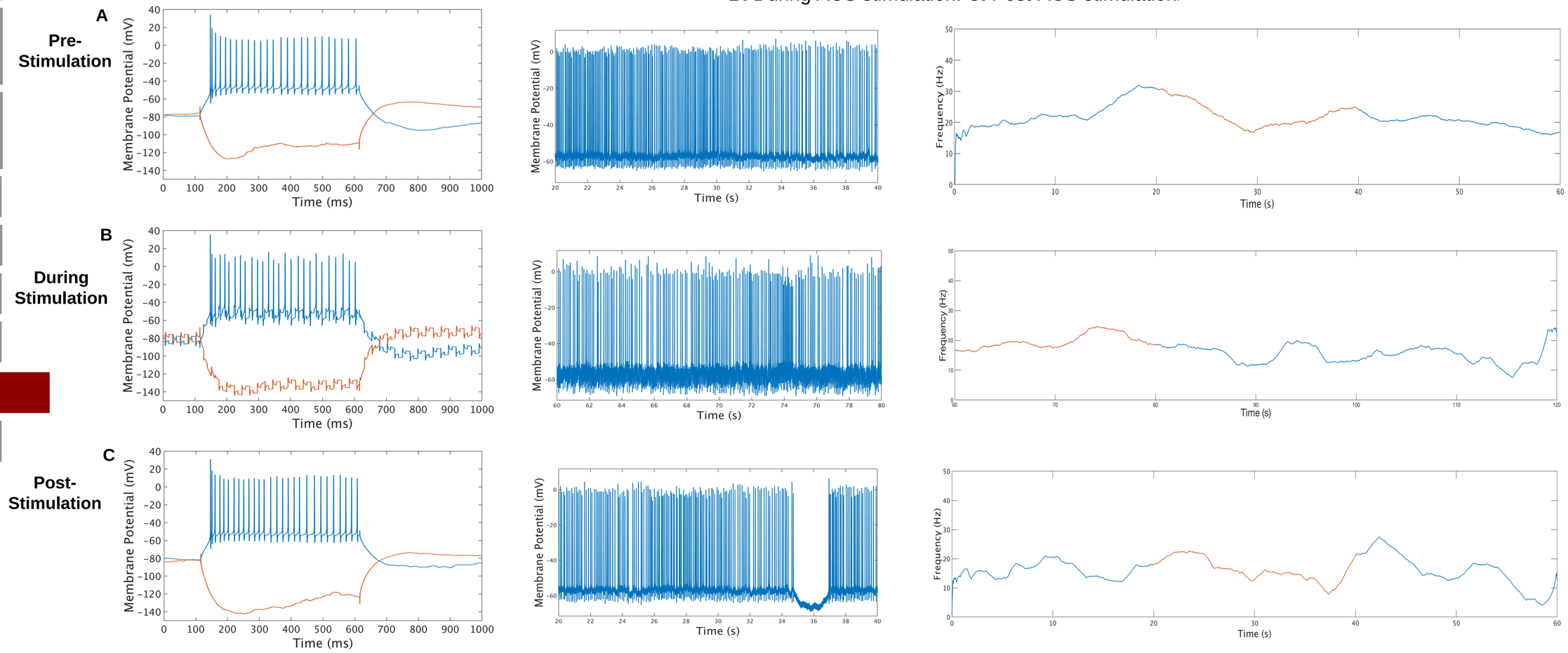
Example of Purkinje Cell Response to ACS

Figure 8. Purkinje cell responses to ACS
Basic property, spontaneous activity, and change in firing rates in **A.** Pre-ACS stimulation **B.** During ACS stimulation. **C.** Post-ACS stimulation.



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Summary and Conclusion

1. Thirteen sets of data were recorded before, during, and after ACS to monitor the average firing rate of Purkinje cells.
2. Average firing rate of Basic Property decreased after ACS (p=0.000799, n=8).
3. No significant changes were observed in average firing rate before and during ACS (p=0.399, n=5).
4. No significant difference was observed for spontaneous firing rate change before and after ACS (p=0.662, n=5).
5. No significant difference was observed in membrane resistance before, during, and after ACS.
6. The future direction of this study is to include frequency change and input resistance of Purkinje cells. This tests our hypothesis, that the dendrite tree of individual PC orientation of each folium determines the final output change caused by ACS. This experiment can be used as a basis for future behavioral studies.