STATE-DEPENDENT NEURAL INHIBITION BY EXTRACELLULAR STIMULATION

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ABSTRACT

Electric stimulation, such as tCDS and DBS, is used widely in clinical and academic settings in the treatment of neurological diseases and disorders. However, little is known about the mechanisms that underlie electrical stimulation on a cellular level. Our research utilizing the *Aplysia californa* buccal ganglia neurons built upon previous findings concerning the presence of neuronal activity states, but demonstrate that these states play a role in the cell's responsiveness to electrical stimulation. It was demonstrated that fast-firing neurons are more resistant to inhibitory stimulation as compared to slow-firing neurons. NEURON computational modeling revealed differences in ion channel dynamics that may underlie the differences in stimulation responsiveness that are associated with neuronal states. Our findings call upon further investigation into neuronal state-dependent stimulation as clinical application of electrical stimulation progresses.

INTRODUCTION

- Recent evidence indicates that state of the nervous system may play a significant role in the outcome of electric and magnetic stimulation (termed "state-dependent"). Examples: electrical stimulation of the ventral tegmental area in rhesus monkeys produced different responses, depending on if the animal is awake or under anesthesia (Murris, Arsenault et al. 2020). Magnetic stimulation produces different perceptual or behavioral outcomes that may depend on the excitability levels of specific neuronal population (Silvanto and Muggleton 2008). Instantaneous brain state can be used to promote efficacious plasticity induction by the TMS (Stefanou, Baur et al. 2019).
- Direct monitoring of neural activity provides initial evidence that the level of neural activity determined the outcome of electric stimulation. Example: Recording of extracellular spikes and local field potential from cat visual cortex following TMS has demonstrated that the response to TMS depends on the state of network activity (Pasley, Allen et al. 2009). These observations suggest that stimulation effects of the electric and magnetic fields could be dependent on the active state of individual neurons. Ultimately, any intervention (i.e. TMS or tDCS) modifying brain function must affect single neuron activity.
- However, state-dependent neural control with electric stimulation has rarely been studied at the single cell level, and its underlying molecular mechanisms are unclear.

REFERENCES

- Lu, H., Chestek, C. A., Shaw, K. M. & Chiel, H. J. Selective extracellular stimulation of individual neurons in ganglia. *Journal of neural engineering* **5**, 287-309, doi:10.1088/1741-2560/5/3/003 (2008).
- Murris, S. R., Arsenault, J. T. & Vanduffel, W. Frequency- and State-Dependent Network Effects of Electrical Stimulation Targeting the Ventral Tegmental Area in Macaques. *Cereb Cortex* **30**, 4281-4296, doi:10.1093/cercor/bhaa007 (2020).
- Pasley, B. N., Allen, E. A. & Freeman, R. D. State-dependent variability of neuronal responses to transcranial magnetic stimulation of the visual cortex. *Neuron* **62**, 291-303, doi:10.1016/j.neuron.2009.03.012 (2009).
- Silvanto, J. & Muggleton, N. G. New light through old windows: moving beyond the "virtual lesion" approach to transcranial magnetic stimulation. *NeuroImage* **39**, 549-552, doi:10.1016/j.neuroimage.2007.09.008 (2008).

METHODS

- *Aplysia californa* and buccal neurons
- Intracellular recording
- Electric stimulation with extracellular electrode
  - -Cathodic stimulation protocol (i.e., used in tDCS)
  - -High frequency stimulation protocol (i.e., used in DBS)
- Computer simulation with multi-compartment NEURON model

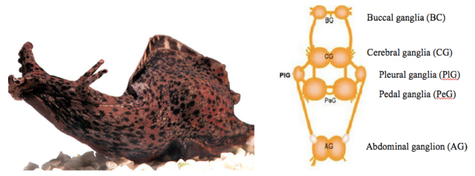

Figure 1. *Aplysia californa* and its central nervous system

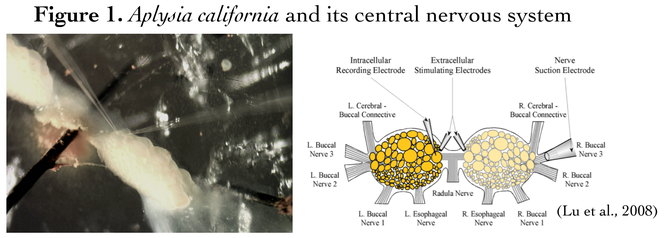

Figure 2. Extracellular stimulation of the soma. One glass electrode positioned close to B4 soma for stimulation, one intracellular electrode for controlling and recording B4 activity

RESULTS

1. Neuron characterization via morphological & electrophysiological profiles

A. Neuron depolarization

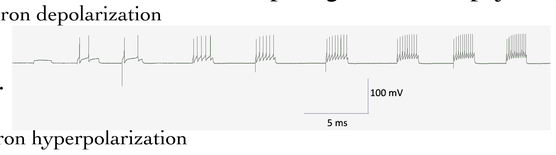

Figure 3. Electrophysiological profile of the B4 neuron. A. Action potentials in neuron elicited by depolarization current injected into the soma. B. Post-inhibitory rebound in neuron elicited by hyperpolarization current injected into the soma.

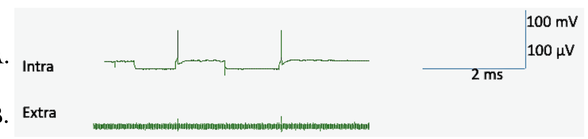

Figure 4. Close position of the extracellular electrode to the soma allow direct detecting of neural activity with the extracellular electrode. A. Depolarization induced activity; B. hyperpolarization induced activity.

2. State-dependent neural inhibition with cathodic inhibition (i.e., tDCS)

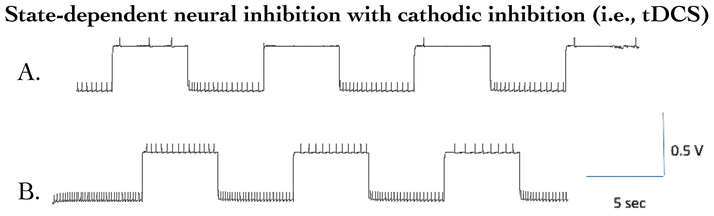

Figure 5. Cathodic inhibition is state-dependent. A 4.5 nA current is applied to the extracellular electrode for cathodic inhibition. A. The neuron was inhibited when its baseline activity is relatively low; B. The neuron is only partially inhibited when its baseline activity is high.

3. State-dependent neural inhibition with high-frequency stimulation (i.e., DBS)

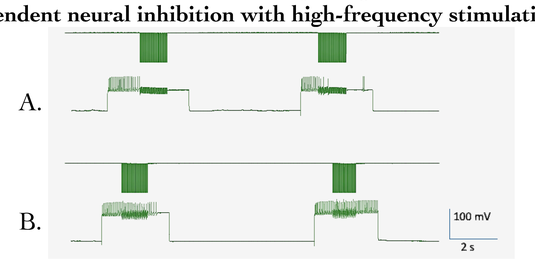

Figure 6. State-depend inhibition with high frequency stimulation (100 Hz). The B4 neuron was elicited to fire action potential with various depolarizing currents.
A. Activity in the low active neuron was completely inhibited by the stimulation.
B. Activity in the high active neuron was minimally affected by the stimulation.

NEURON: COMPUTATIONAL MODELING

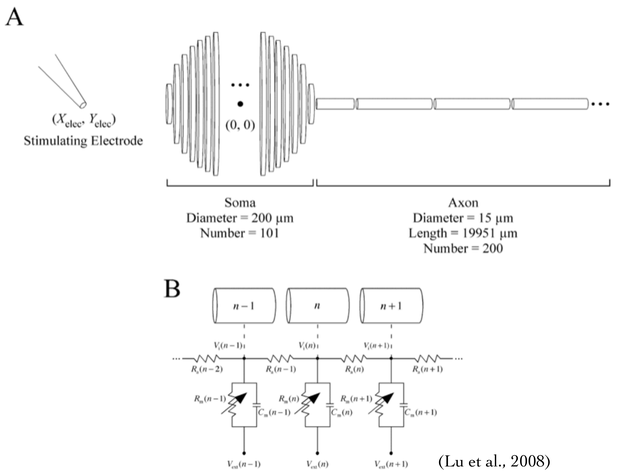

Figure 7. Multi-compartment NEURON model of a single neuron under a point electrode stimulation.
A. Model configuration; B. Hodgkin and Huxley type channels

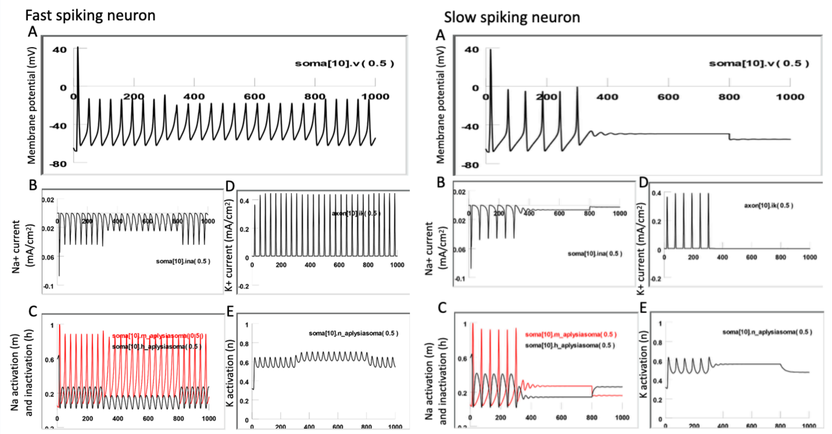

Figure 8. Ion channel dynamics in fast firing neurons under cathodic stimulation.
A. Cathodic stimulation causes slight depolarization of the membrane potential, but the neuron maintains its firing capability.
B. Stimulation causes a reduction of the fast inward Na+ current due to the reduction of the driving force for the Na current.
C. During stimulation, the Na+ channel could be fully activated (m). The inactivation (h) can not reach its original high value due to the slight, constant membrane depolarization.
D. During stimulation, K+ current was not affected.
E. Cathodic stimulation cause a slight increase in the voltage-dependent potassium activation (n).

Figure 9. Ion channel dynamics in slow firing neurons under cathodic stimulation.
A.Cathodic stimulation causes depolarization of the membrane potential and a complete blockage of the action potentials.
B.Fast inward Na+ current disappeared. Instead, stimulation caused a small, constant inward Na+ current.
C. During stimulation, the Na+ channel is not fully activated (m). The inactivation (h) can not reach high value, indicating the failure of sufficient inactivation of the channel.
D. During stimulation, K+ current disappeared.
E. Cathodic stimulation causes a slight increase in the voltage-dependent K+ activation (n). However, K+channels are not fully activated due to the lack of action potentials.

CONCLUSION AND DISCUSSION

1. Neural inhibition by electric stimulation depends on the activation state of the neuron. It is more difficult to inhibit neurons with high activity.
2. Ion channel dynamics that sustain the action potentials are easier to be manipulated by the electric stimulation in the low active neurons than in the high active neurons.
3. Clinic implications: direct continuous monitoring of neural activity (EEG or fMRI) is essential for optimal stimulation outcomes.