No evidence for synaptogenesis due to tDCS during reach training in rats

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INTRODUCTION:

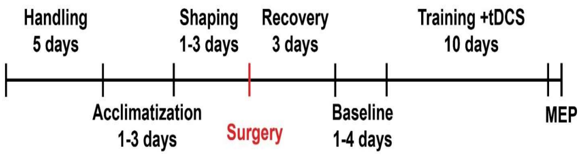
Electric fields generated by transcranial direct current stimulation (tDCS) are thought to cause lasting behavioral effects by modulating synaptic efficacy. One possible mechanism by which synaptic efficacy is increased is by inducing an increase in the number of synapses. In the present study, we determined the effects of anodal tDCS delivered over the motor cortex on synapse number. We (Farahani et al, in review) and others have shown that tDCS improves motor learning in animals and humans. Here, we ask if tDCS augments motor cortex synapse number in association with motor learning.

We studied this question using rats trained to perform a pellet reaching task while receiving either anodal tDCS over the motor cortex or no stimulation during a period of 10 days. Motor cortex synapse counts were made using high-resolution confocal microscopy, on tissue prepared for immunohistochemistry for synapse markers.

METHODS:

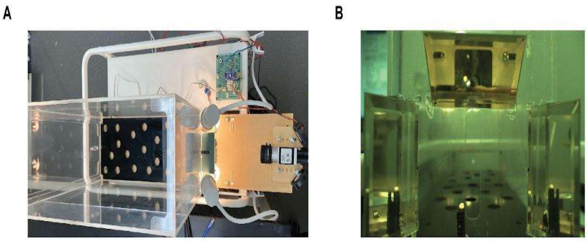
Animals (N=29) were trained to perform a single pellet reaching task. Once rats were acclimated to the task environment and the sugar pellet reward, they were trained 20 minutes a day, for 10 consecutive days, with or without concurrent tDCS at 150 μA centered over the forelimb area of motor cortex. Performance measure was the number of successful reaches.

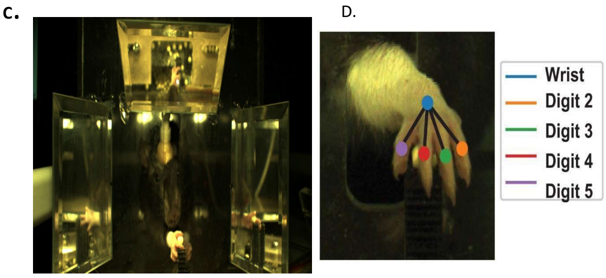
Experiment Timeline



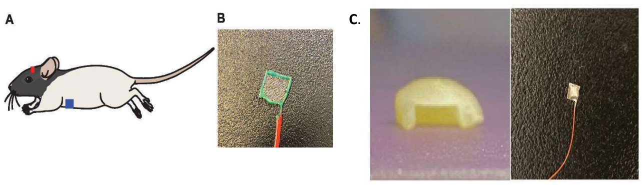
At the completion of training, animals were euthanized and perfused with saline and 4% PFA. The brain was extracted and the frontal lobe was dissected bilaterally and sliced (40μm thick frozen sections). Brain sections were processed for immunohistochemistry for the following protein markers:

- Synaptophysin (SYN)-presynaptic marker
- Postsynaptic density-95 (PSD95)-postsynaptic marker
- NeuN-neuron marker

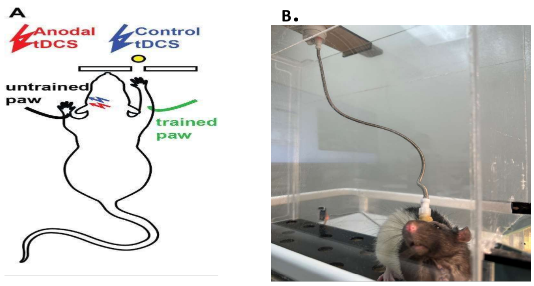




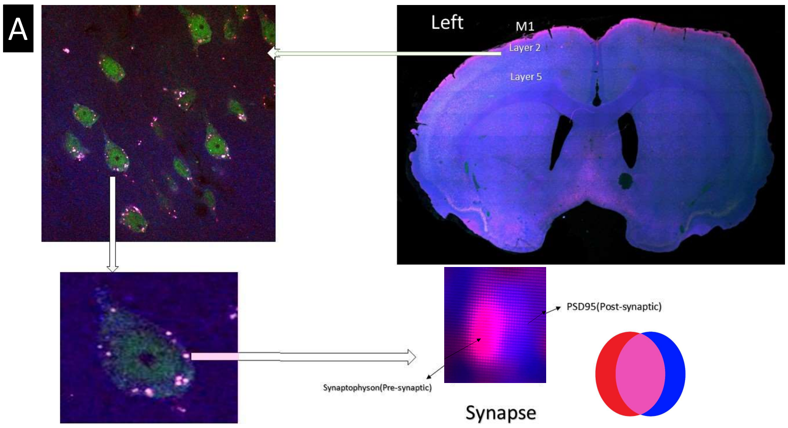
A,B: Images of upper (A) and frontal (B) views of training chamber. **C:** TDCs session: Rat reaching for pellet. **D.** A snapshot of a paw with the colored dots indicating the position labels



A: Schematic of the montage (red, anode over M1; blue, cathode) **B:** Cathode grid electrode implanted in the chest. **C:** Left: custom-made epicranial electrode holder made from dental cement, right: M1 electrode 3x3 mm² platinum plate.



A: The "trained" paw is the one with which the animal learned to grab the food pellet (yellow) through a narrow slit. tDCS electrode is placed over the contralateral motor cortex of the trained paw and delivered during 10 days of training. B: Image of a rat tethered to the wire linked to tDCS stimulation while on the training chamber



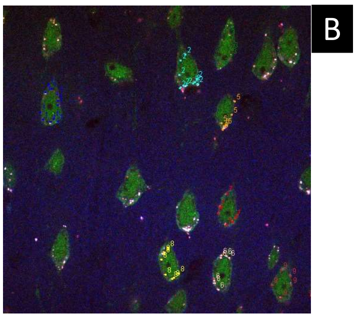
A.Confocal microscopy:

- -Zeiss880
- -Settings : 63X , thickness 0.7um, overlap 10%, zstack 55 slices
  - SYN-AF647 (Red)
  - PSd95-AF405 (Blue)
  - NeuN-AF555 (Green)
- -Imaging of 29 animal brain tissues , 2 slices per animal , 2 hemispheres , primary motor cortex M1 , 2 layers (Layer 2 and 5) .

B.Synapse counting:

Process: neurons in layer 2 and 5 imaged separately; identify pyramidal neurons by morphology (shape, nucleus) and NeuN; identify triple labeled synapses (SYN+PSD+NeuN) with all three imaging channels on; turn off NeuN and count SYN-PSD.

- -ImagJ/Fiji cell counting and marking with number
- -10 neurons analyzed for Layer 2 and 10 neurons for layer 5 for each section
- -Difference computed: synapse number for MCX contralateral to reaching minus ipsilateral side



RESULTS:

Overview: Subsequent to handling and acclimatization, rats were food restricted during shapping for 6 days. Pre-surgery training was concluded after 10 successful reaches (rat reaches for pellets and eats each). This was done to determine the preferred paw for contralateral implantation of the motor cortex (M1) anodal electrode .

Contralateral = trained side with and without tDCS

Ipsilateral=untrained side with and without tDCS; this is used as control

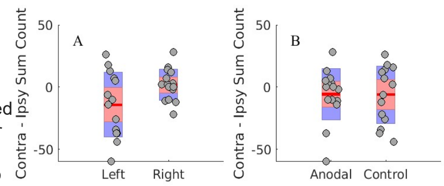
Left-pawed rats used their right arm to assist during reaching. Right-pawed rats were unidextrous.

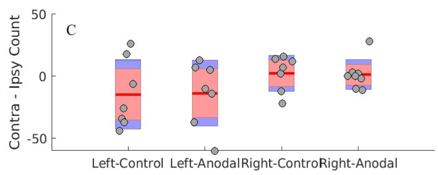
->We found that right-pawed animals had a larger contralateral - Ipsilateral difference in synapse number for Layer 2, compared to left-pawed animals (one-sample t-test: $t(26)=2.2$, $p=0.037$). There was no effect of tDCS on synapse count, nor any effects in Layer 5, or for the left-pawed animals. We also found no significant correlation of synapse count with the number of successful reaches.

In summary

Reaching: Training increased successful reaches and rat performance .

Effect of training + tDCS: No effect on synapse count for Layer 5 or the left pawed animals; right-pawed animals had a larger contralateral - Ipsilateral difference in synapse number for Layer 2, compared to left-pawed animals.





Synapse count: contralateral synapse number minus ipsi-lateral number for 20 neurons per hemisphere. Each point is one animal. (A) separated by paw preference (B) separate by stimulation condition (anodal tDCS vs control) (C) separated by paw preference and stimulation condition.

"Reaching M1": Contralateral to reaching arm without and with stimulation.

"Non_reaching M1": Ipsilateral to reaching arm without and with stimulation

CONCLUSION:

- Training combined with tDCs improved rat performance, in terms of rate of successful reaches.
- tDCS did not have a significant effect in training-induced synaptogenesis.

In a related study (Farahani et al., in review), we did find tDCS to have a significant effect on reaching skill acquisition. The significant effect observed for right-pawed rats may be due to their unidextrous performance strategy. Experiments are in progress to determine if there are any quantitative differences in synapse morphology.

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