Role of 5-HT2C receptors in hyperreflexia post-spinal cord injury in mice

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Background

Spasms post-chronic SCI can inhibit patients' functional recovery and decrease independence.[1,2] After SCI, serotonin 2C receptors (5-HT2CR) undergo a conformational change and become constitutively active.[3] The unregulated 5-HT2C signaling facilitates persistent inward currents (PICs) and increases motoneuron excitability.[4,5] The subsequent physiological presentation is hyperreflexia and spasms in individuals post-SCI.

Research Objectives & Hypothesis

This pilot study further investigates mechanisms underlying involuntary motor behaviors post-injury by studying hyperreflexia in vivo in a mouse transgenic line lacking 5-HT2CRs.

- *Hypothesis:* Following SCI, we hypothesize hyperreflexia responses will be diminished in 5-HT2CR knockout mice (KO) when compared to wild type mice (WT).

Methods

SCI: At 10-weeks-old, WT and KO mice underwent complete transection injuries to the spinal cord at vertebral level T10, corresponding to spinal segments T12 and T13.[6]

Hyperreflexia: Electrical stimulation (e-stim) in the form of small electrical currents was delivered to the plantar side of the hind paw of mice.[7] Semmes-Weinstein monofilaments were applied to the plantar side of the hind paw.[8] For both sensory inputs, EMG responses from the withdrawal reflex were recorded from the lateral gastrocnemius (LG) and tibialis anterior (TA) muscles.

Thresholds were determined as the lowest current or force inducing motor activity for e-stim and monofilament, respectively. Mice were tested at 5x threshold for e-stim and the next higher force for monofilament.

Analysis: Threshold and EMG (integral) data were collected and processed in Spike2 software (CED), and data were analyzed in SPSS (IBM). Mann-Whitney U tests were run to determine significant differences between groups.

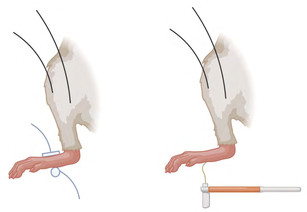
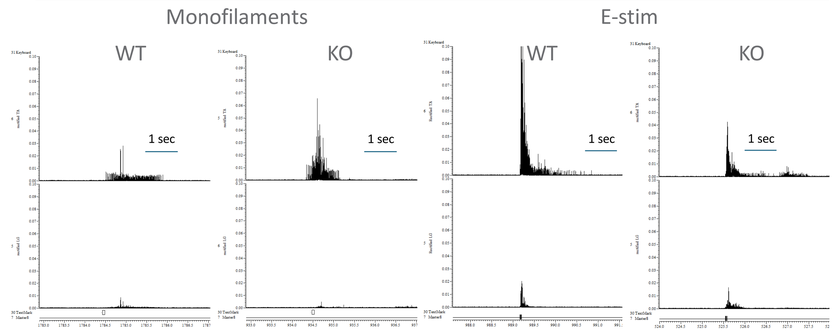


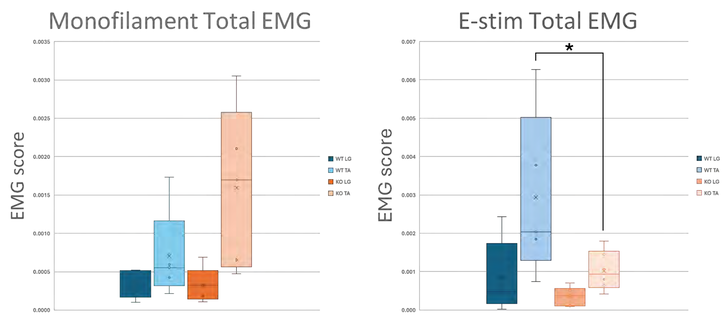
Figure 1. Image of recording electrodes in tibialis anterior and lateral gastrocnemius and placement of stimulating electrodes (left) or monofilaments (right).

Figure 2. Signal graphs for monofilaments and e-stim



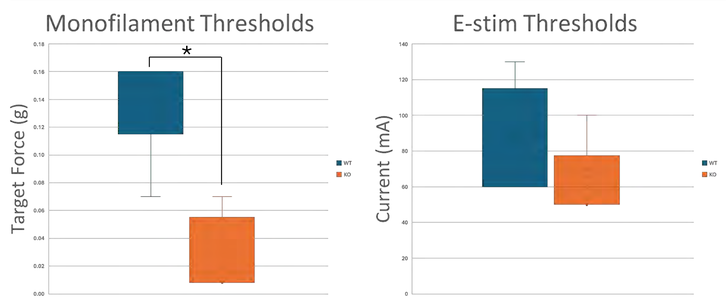
Significant differences were seen in TA (e-stim only), and no differences found in LG.

Figure 3. Monofilament and E-stim Total EMG



Box-and-whisker plots with data for monofilament and e-stim total EMG. A statistically significant difference was seen in the TA between WT and KO groups.

Figure 4. Thresholds for monofilament and E-stim



Box-and-whisker plots for monofilament and e-stim thresholds to determine values used during testing. A significant difference in threshold was seen for monofilament force between WT and KO groups.

Results

- Total EMG signal for TA after electrical stimulation was significantly higher in WT as compared to KO (U=4, p=0.045).
- No significant differences were found in the LG EMG signals (*i.e.,* the hyperreflexia response).
- The monofilament threshold was smaller for the KO group (0.03 g) than the WT group (0.14 g, U = 0.5, p = 0.009).

Conclusions

- Supporting our hypothesis, the significant difference in TA EMG signals between the WT and KO mice highlights the role 5-HT2CRs play in modulating the hyper-reflexive response post-SCI.
- The significantly lower EMG response in the TA of the KO mice led us to expect that monofilament threshold would be higher in KO vs. WT. However, the opposite was observed, indicating that the KO group had higher excitability.
- The discrepancy between EMG signal and monofilament threshold in KO mice can be explained by differences in EMG vs. monofilament spinal circuits.[9]
- Understanding the mechanism of involuntary motor behaviors can lead to pharmacological advances in treatment and improve functional recovery.

Limitations

- Pilot project
  - ➢ Underpowered
- Test subjects: Mice
  - ➢ Difficult to assess difference in sensory stimulations

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