

# Characterizing Neuronal Activation of Melanotan II, a Novel Pharmaceutical with Potential to Enhance the Efficacy of Autism Therapy



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## BACKGROUND

- Autism affects 1 in 68 children in the United States yet there is no effective pharmaceutical drug to palliate the social deficits caused by this disorder<sup>1</sup>
- Brain regions of the social salience network (SSN) coordinate to process social information<sup>2</sup>

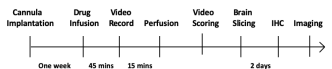


The Social Salience Network<sup>3</sup>

- Oxytocin (OT) signaling modulates brain activity in the SSN, but cannot cross the blood brain barrier
- Melanotan II (MTII) is an alternate pharmacological compound that evokes endogenous OT release and affects social behavior<sup>4,5</sup>
- Dose response study showed increase in side effects with an increased dose
- Analyzing MTT II induced neuronal activation in the paraventricular nucleus (PVN)

## METHODS

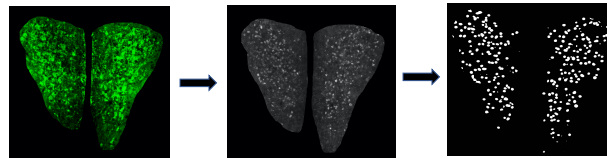
24 adult male prairie voles received doses of MTII ranging from 0.1 nmol to 1pmol in the lateral ventricles



An online machine learning program called DeepFLaSH<sup>6</sup> that is trained to analyze c-FOS expression was used followed by imajej software to count the cells

## RESULTS

### Neuronal Activation in the PVN at Different Doses of Melanotan II



OT receptor activation to identify the PVN

C-FOS activation in the PVN

Map of c-FOS activation created by DeepFLaSH

**Dose-Response Analysis of MTII Induced Neuronal Activation in the PVN:** OT receptor is labelled using OT antibody in the PVN, the PVN is identified and selected using the ImageJ software. This selection is used to identify the PVN in the c-FOS activated image. The image is then uploaded on DeepFLaSH which creates an automated segmentation mask that outlines all the c-FOS activated cells. This segmentation mask is uploaded on ImageJ software and created into a binary image followed by running watershed. Then the activated cells are analyzed by the ImageJ software and counted.

### Automating Cell Counting to Analyze c-FOS Expression

Slice	Hand Count	DeepFLaSH Count	Percentage Difference
Slice 1	405	360	11.76
Slice 2	311	274	12.65
Slice 3	243	215	12.23
Slice 4	373	327	13.14
Slice 5	330	286	14.29

**Manual and Automated Cell Count:** 5 slices were analyzed for c-FOS activation using two ways: manual way by hand counting it and automated way by running it on DeepFLaSH. The manual and the automated count show a consistent percentage difference with a standard deviation of 0.97.

## CONCLUSION

The manual and automated cell count analysis suggest that DeepFLaSH is viable option for analyzing c-FOS expression.

## FUTURE DIRECTIONS

- Examine MTII-induced activation of c-FOS expression in other regions of the social salience network at the highest dose
- Examine MTII induced neuronal activation of c-FOS expression with and without a social stimulus

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