AUTOMATED TRACKING OF LOCOMOTOR ACTIVITY IN MICE SHOWS INDIVIDUAL DIFFERENCES IN ALCOHOL SENSITIZATION

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INTRODUCTION

- Sensitization to chronic alcohol reflects changes in mesocorticolimbic dopamine signaling that is integral to both reward and locomotion, which has repeatedly been demonstrated following chronic use of psychostimulants that directly engage this dopamine pathway (Abrahao et al., 2009).
- Mice that develop locomotor sensitization have been shown to have greater preference for alcohol.
- Additionally, our lab has previously shown a relationship between voluntary alcohol intake and $GABA_A$ receptor expression, and we wished to determine whether locomotor sensitization to ethanol was associated with similar changes in GABAergic signaling.
- We hypothesized that individual differences in locomotor sensitization would reflect neuronal changes in $GABA_A$ expression and would be associated with alcohol-induced stress response changes.
- To investigate these correlations, we returned to archived videos of mice that were chronically treated with ethanol. To quantify mouse locomotion in these archived videos, various object-tracking programs were compared and a video processing workflow was generated.

METHODS

Subjects

- 20 C57BL/6J mice (5 mice/sex/treatment)

Procedure

- Mice received 1x daily injection of ethanol for 14 days. On the first and last day mice received 2.0g/kg ethanol (20%v/v; I.P) and on days 2 thru 13 they received 2.5g/kg doses, I.P.
- Control mice received saline on all days.
- On days 1 and 14 of injections, the mice performed the locomotion task immediately after their injection.

Comparison of particle tracking programs

- This virtual project used archived videos of animals chronically treated with ethanol for a different purpose. In order to determine whether these mice displayed locomotor sensitization, a software capable of reliably tracking these animals' locomotor response to ethanol had to be found.
- Tracking program user interface was also assessed for efficiency and functionality.

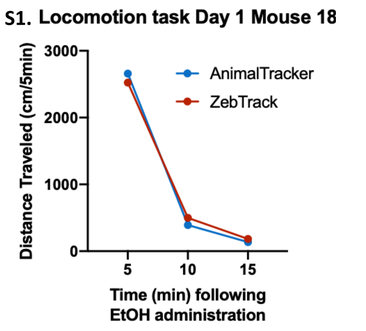
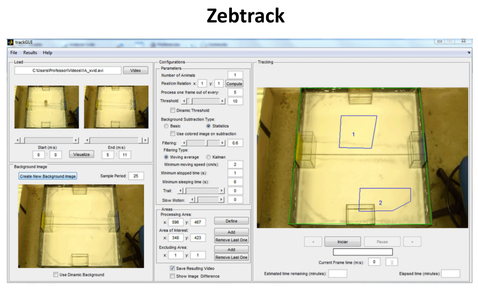
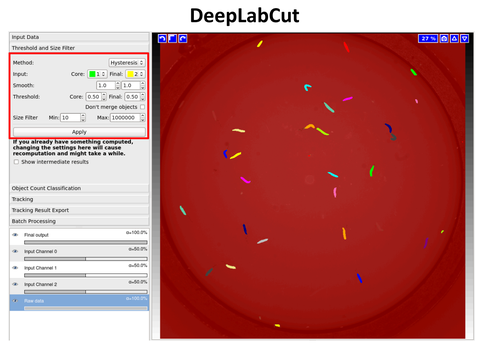


Fig. S1. Multiple object tracking programs were compared to determine the most effective one to use for mouse locomotion quantification for archived videos. These are the similar locomotion tracks of the Zebtrack and AnimalTracker programs, where the distance of mouse 18 was recorded and broken into 5-minute segments.





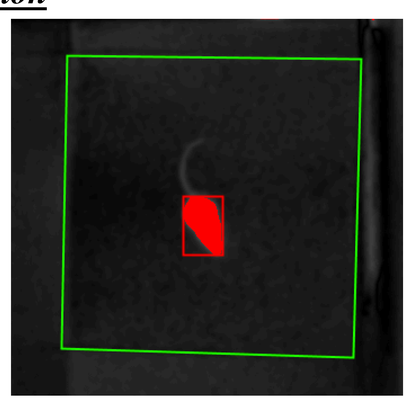
Automated video processing workflow

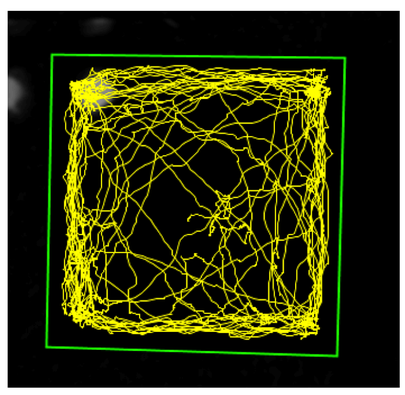
zoom and crop videos → batch update video format → upload video to ImageJ → process video

AnimalTracker

- AnimalTracker is an ImageJ-based image processing program used to track and analyze animal behavioral videos (Gulvás et al., 2016).
- The coordinate tracking program is based on color thresholding to allow for blob detection
- The program generates a coordinate for each frame of the archived video and then calculates the speed, direction and total distance traveled.
- After researching many object tracking programs, we chose to use AnimalTracker because it was efficient, easy to use and offered versatile functions.

AnimalTracker processing of locomotion task video: color threshold and blob detection





RESULTS

I. Ethanol-induced locomotor sensitization in female mice

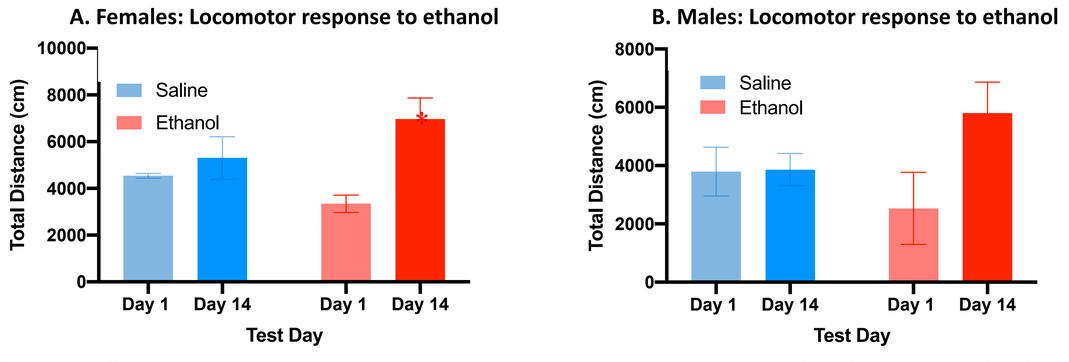


Fig. 1. (A) Female mice show a significant interaction of day and treatment, as ethanol treated females displayed enhanced locomotor response to ethanol on day 14 ($p<0.05$, compared to Day 1, *Sidak corrected posthoc*). (B) Males show marginal increase in activity, and variable acute response to ethanol on day 1.

II. Shift from hypolocomotion to hyperlocomotion following chronic ethanol

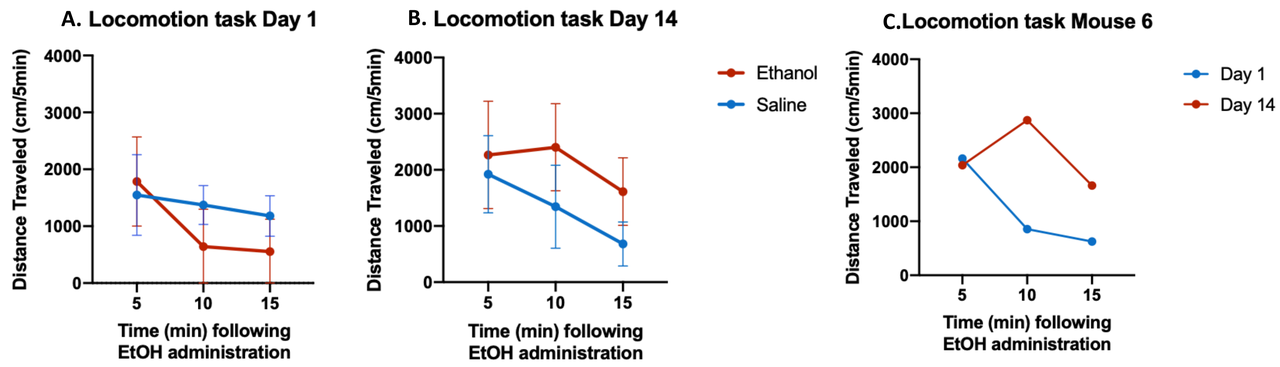


Fig. 2. Temporal pattern of locomotor response to ethanol changes following repeated exposures. (A) On day 1 of ethanol injections, ethanol-treated mice show hypolocomotion 10 minutes following drug administration (when compared to saline treated controls). (B) After 14 days of ethanol injections, mice show hyperlocomotion 10 minutes after their ethanol injection. (C) This divergence in locomotor response between test days is shown in the locomotion pattern for female ethanol-treated mouse 6, as well as the movement tracks visualized for both days (D).

III. Locomotor sensitization associated with anxiety-like behavior during protracted withdrawal

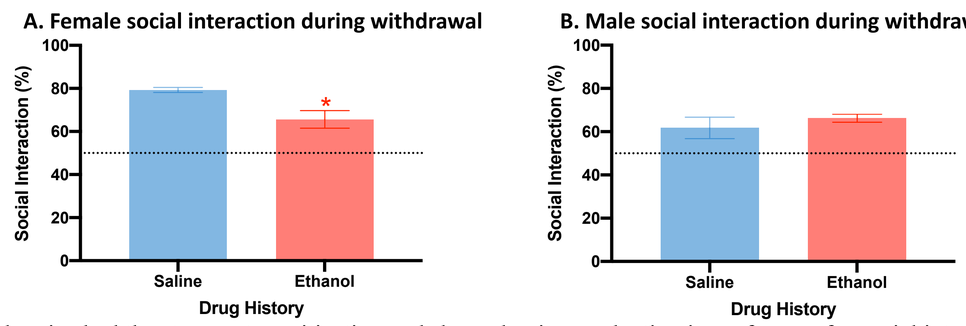


Fig. 3. Female mice had the strongest sensitization and showed unique reduction in preference for social interaction during protracted withdrawal. Following 14 day ethanol treatment, mice remained in home cages for a 14 day abstinence period and were tested in a variety of behavioral tasks, including social interaction assays. (B) Males do not show change in preference for social side of the box during withdrawal. (A) Females show significant ($p<0.05$) reduction in preference for the social side of the apparatus.

IV. GABAergic plasticity in the reward and stress pathways in sensitized mice

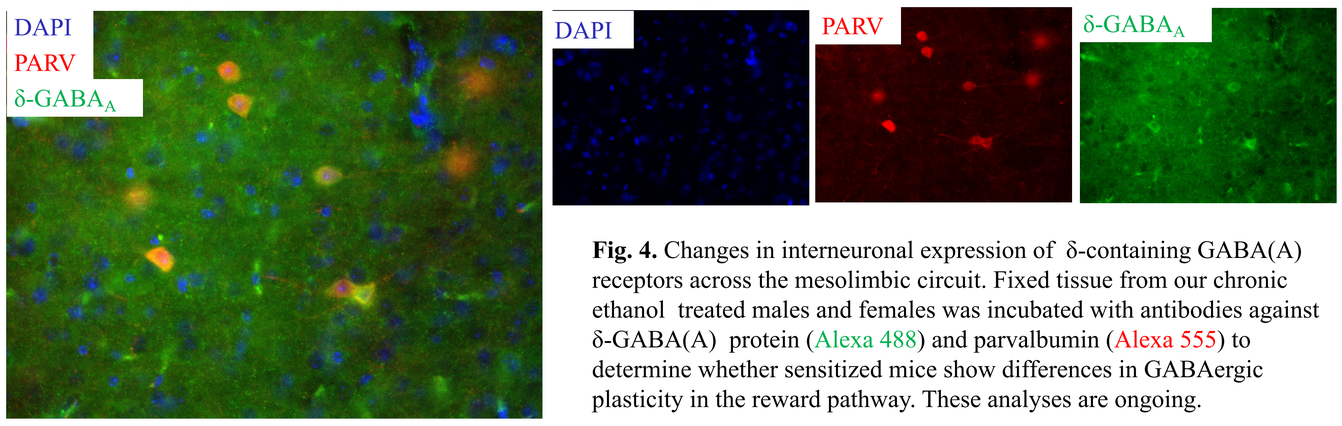


Fig. 4. Changes in interneuronal expression of δ-containing GABA(A) receptors across the mesolimbic circuit. Fixed tissue from our chronic ethanol treated males and females was incubated with antibodies against δ-GABA(A) protein (Alexa 488) and parvalbumin (Alexa 555) to determine whether sensitized mice show differences in GABAergic plasticity in the reward pathway. These analyses are ongoing.

SUMMARY AND CONCLUSIONS

- We found AnimalTracker to be the most effective free object-tracking program and developed a workflow that can be used for data collection on a variety of behavioral tasks in the future
- Female mice chronically administered alcohol showed a significant increase in total distance traveled following the drug.
- Further analyses will compare this locomotion data to the neurobiological data collected with these mice, including mesocorticolimbic immunohistochemical assays for δ-$GABA_A$ receptors and parvalbumin, along with blood corticosterone levels to help determine other factors such as stress markers or changes in GABAergic signaling that may correlate with locomotor sensitization to alcohol
- By connecting both the dopaminergic signaling and changes in stress reactivity to a mouse model of alcohol sensitization, we can increase understanding of the common mechanisms that may underlie alcohol's effects on reward and stress.

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