Exercise as a Stress-Intervention Method in a Mouse Model of Autism

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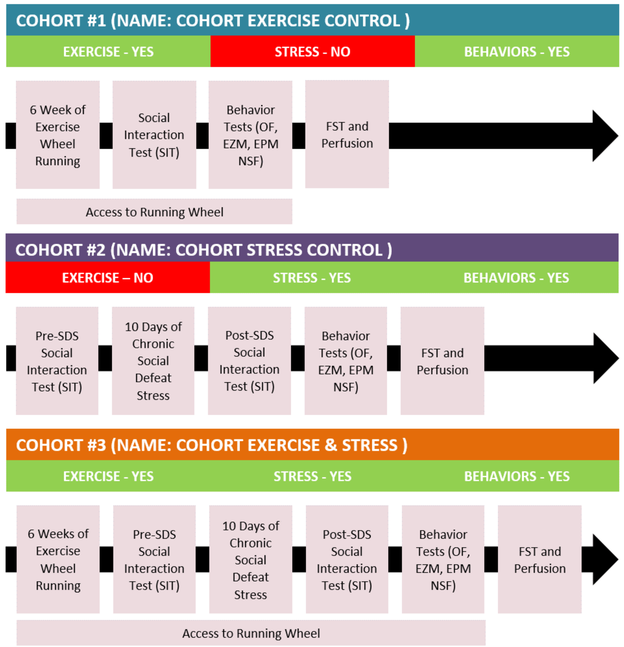
Background

Autism Spectrum Disorder (ASD) is an extremely prevalent neurodevelopmental disorder that is characterized by challenges with social skills, repetitive behavior, and communication deficits[1]. Individuals with ASD are also more susceptible to stress, and thus high rates of comorbidity exist between ASD and psychological disorders such as depression and anxiety[2]. Past research has shown that mutations in the Engrailed-2 (EN2) gene lead to behavioral and anatomical phenotypes similar to those of people with ASD, allowing us to use mice with the EN2 knock-out (KO) gene as a neurodevelopmental animal model of autism[3]. With this model, we are able to better understand the impact of stress.

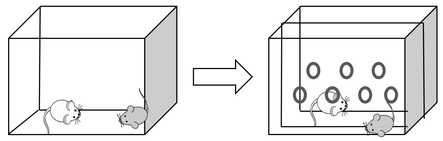
Previous work in our lab has suggested that EN2 KO mice are more susceptible to stress than their wild-type (WT) counterparts, and subsequently display more anxiety-like and depressive-like behaviors after 10 days of Chronic Social Defeat Stress (CSDS). Our question then becomes: Is there any way to reduce susceptibility to stress in EN2 KO mice? Past research in the exercise science field demonstrates that exercise is capable of reducing anxiety-like behaviors and depression-like behaviors, while improving memory and learning in rodents. Thus, the aim of this study is to determine whether exercise has preventative and/or ameliorative effects in populations that are highly susceptible to stress. To better understand the effects of exercise, a traditional CSDS cohort, an exercise and CSDS cohort, and an exercise with no CSDS cohort were run. The behaviors from each cohort were then compared to determine the effect of exercise. We hypothesized that exercise can improve anxiety-like and depressive-like symptoms in stressed EN2 KO mice.

Methods: Experimental Setup



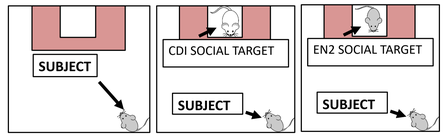
Methods: Behavioral Assays

Chronic Social Defeat Stress

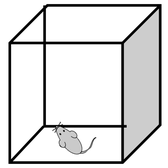


The mouse is exposed to a larger and more aggressive strain (CD-1) for 5 minutes per day for 10 days. These exposures result in multiple incidents of attack and defeat by the more aggressive mouse.

Social Interaction Testing

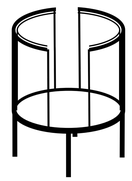


The mouse explores the chamber three times for 2.5 minutes per trial. The mouse is alone (no target), paired with a CD1 (target), and paired with a novel EN2 mouse, respectively.



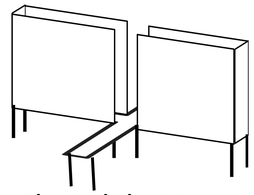
Open Field

The mouse explores a brightly-lit, empty chamber for 30 minutes to test for general exploratory abilities and anxiety-like behaviors.



Elevated Zero Maze

The mouse explores a circular enclosure that has two open sections and two closed off sections for 5 minutes.

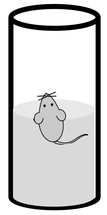


Elevated Plus Maze

The mouse explores a plus-shaped enclosure that has two open arms and two closed arms for a single 10 minute trial.

Novelty Suppressed Feeding

After a period of fasting, the mouse is placed in a brightly-lit chamber with a food pellet in the middle for 6 minutes. The NSF tests for conflict between the anxiogenic environment and hunger-induced behavior.

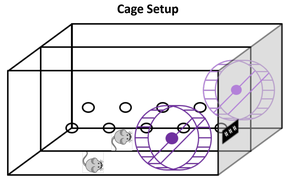


Forced Swim Test

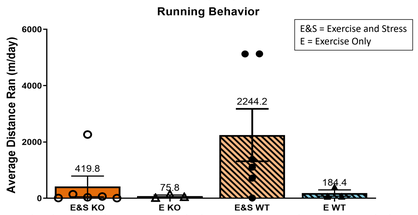
The mouse is placed in chamber half-filled with water for a period of 6 minutes and is exposed to forced swimming.

Results

1. RUNNING BEHAVIOR

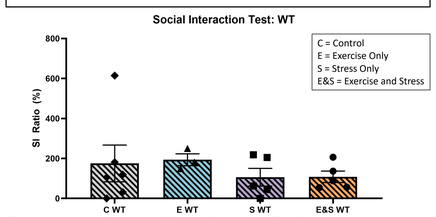


Mice were pair-housed, separated by a plexiglass divider. Both mice had access to its own wheel. A modified pedometer was used to track the number of rotations every day.

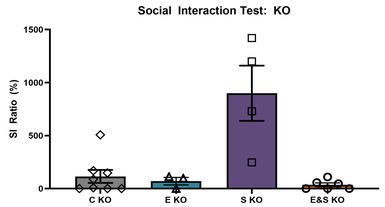


Trends showed that WTs tended to run more than KOs and that both KOs and WTs in Cohort 3 (Exercise and Stress) tended to run more than those in Cohort 1 (Exercise Only). Mann-Whitney test showed no statistically significant data. E&S KO n=6, E KO n=3, E&S WT n=6, E WT n=3.

2. SOCIAL INTERACTION TEST

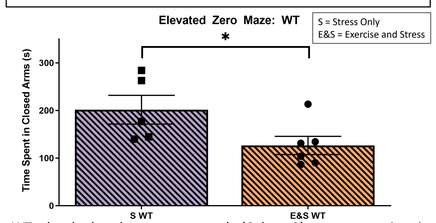


Trends showed Control WTs and Exercise WTs (Cohort 1) spent more time in the social interaction zone than WTs that had undergone stress (Cohorts 2&3). Mann-Whitney test showed no statistically significant data. C WT n=6, E WT n=3, S WT n=5, E&S WT n=6.

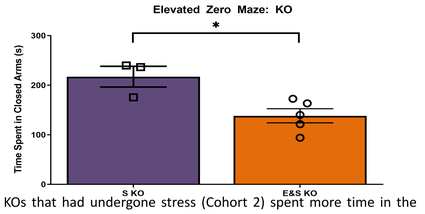


The same trends as exhibited by the WTs (above) were not seen in the KOs. Mann-Whitney test showed no statistically significant data. C KO n=8, E KO n=3, S KO n=4, E&S n=6.

3. ELEVATED ZERO MAZE

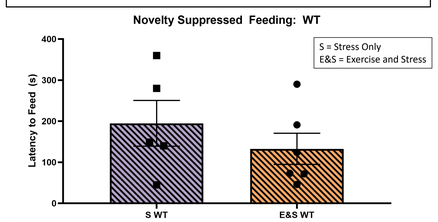


WTs that had undergone stress only (Cohort 2) spent more time in the closed arm of the arena than WTs that were exposed to exercise and stress (Cohort 3). (Mann-Whitney U = 3, S WT n= 5, E&S WT n = 6,P = 0.0303 two-tailed)

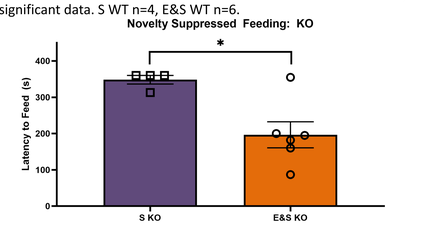


KOs that had undergone stress (Cohort 2) spent more time in the closed arm of the arena than KOs that were exposed to exercise and stress (Cohort 3). (Mann-Whitney U = 0, S KO n= 4, E&S KO n = 6,P = 0.0357 two-tailed)

4. NOVELTY SUPPRESSED FEEDING



Trends showed WTs that had undergone stress (Cohort 2) displayed a greater latency to feed than WTs that were exposed to exercise and stress (Cohort 3). Mann-Whitney test showed no statistically significant data. S WT n=4, E&S WT n=6.



KOs that had undergone stress (Cohort 2) displayed a greater latency to feed than KOs that were exposed to exercise and stress (Cohort 3). (Mann-Whitney U = 1, S KO n= 4, E&S KO n = 6,P = 0.0190 two-tailed)

Discussion and Conclusions

- The aim of this study was to look at the effects of exercise on EN2 KO mice.
- Comparison of running behaviors between KOs and WTs showed that WTs tend to run more. This may be due to the fact that EN2 KOs have cerebellar deficits, which impacts the development of their motor skills, affecting muscle strength, motor coordination, and motor learning[5].
- In the SIT, EN2 KOs did not display an increase in social interaction after having access to an exercise wheel in the same manner as the WTs. Unlike other behavioral paradigms, the SIT measures exploratory behavior in the presence of an aggressive CD1, which could have created a difference in behavioral response in the stressed EN2 KOs.
- Both WT and KO mice that had access to an exercise wheel before going through CSDS displayed a decrease in time spent in the closed arm of the EZM (indicative of less anxiety-like behaviors), and had a shorter latency to feed time in the NSF (indicative of less hesitancy to explore a novel environment).
- Positive results from this study support the role of exercise as a possible intervention approach for subjects that are highly susceptible to stress, such as the EN2 KO mice.
- One major limitation of this study was the small sample size.

Future Directions

1. Look for neuroanatomical differences between KO mice with access to exercise wheels and those that did not. Regions of interest: Locus Coeruleus, Amygdala, Anterior Cingulate Cortex, & Hippocampus
2. Evaluate the effects of exercise in KO mice that have undergone early life stress.
3. Use BDNF to measure neurogenesis in the hippocampus of exercised KO mice

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Acknowledgements

- Thank you to the Aresty Research Center.
- Thank you to the Rutgers University, Honors College.
- Thank you to the Samuels Lab and the Psychology Department.

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