

4-9-2016

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"The Effect of Early Life Social Stress on Anxiety-like Behaviors and Ethanol Drinking in Female Long-Evans Rats" (2016). *Stander Symposium Posters*. 771.

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The Effect of Early Life Social Stress on Anxiety-like Behaviors and Ethanol Drinking in Female Long-Evans Rats

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Introduction

- Among women with Alcohol Use Disorders (AUDs), women have higher rates of anxiety-disorders and are more negatively influenced by early life stress compared to men.
 - Preclinical models studying the relationships between early life stress, anxiety-like behavior, and ethanol (EtOH) intake and preference have mainly use male subject.
 - Many attribute stress's influence on brain development to the role of the hypothalamic-pituitary- adrenal (HPA) axis, a part of the neuroendocrine system controlling stress reactions, which is still developing well into adolescence. Early life social stressors led to long-lasting effects in corticosterone alterations in male rats (McCormick et al., 2005).
 - This study investigated the long term effects of chronic social instability on anxiety-like behavior in the elevated plus maze (EPM) and in self-administered EtOH consumption in females. We modified a model by McCormick et al. (2004), as it has previously shown usefulness in studying drug abuse vulnerability for nicotine.
- Hypothesis: female Long Evans rats, when exposed to adolescent social instability, would exhibit more anxiety-like behavior and EtOH intake later in life than those in stable social situations.

Methods

Subjects:

32 Female Long Evans Rats arrived PND 21.

Social Instability Procedure (4 experimental groups):

- Social Isolation (Iso):** rats raised 1/cage only handled for routine maintenance.
- Social Stability (SS):** rats raised 2/cage only handled for routine maintenance.
- Chronic Social Instability (CSI):** rats raised 2/cage, received one hour of isolation a day followed by a re-pairing with novel partner for the next 24 hours for 16 days.
- Acute Social Instability (aSI):** rats raised 2/cage, received one hour of isolation on the last day and were then re-paired with novel partner for 24 hours (Day 16).

Anxiety- Like Behavior Measure: Elevated Plus Maze:

- Time spent in open vs. closed arms, arm entries. Measured 5 min.

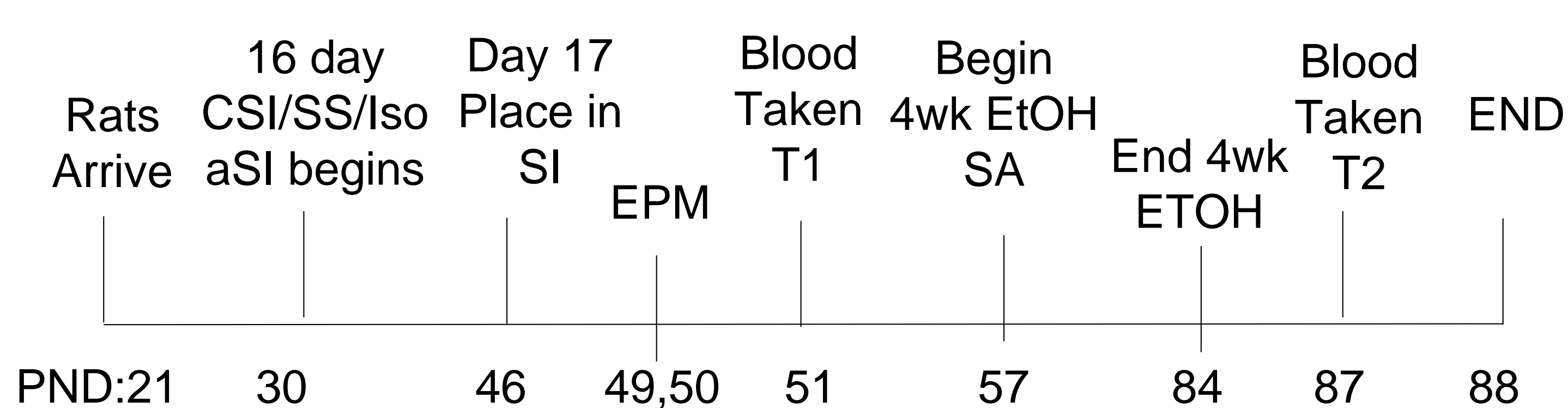
CORT (corticosterone) Measurements:

- Blood via tail nick to obtain plasma for CORT measurements.
- Taken after behavior testing (Timepoint 1/T1) and after EtOH self-administration (Timepoint 2/T2)
- CORT measured using a 96 well plate competitive enzyme immunoassay containing a polyclonal CORT antibody (Immunodiagnostic Systems).

At the completion of behavioral testing, all of the cohort participated in EtOH self-administration:

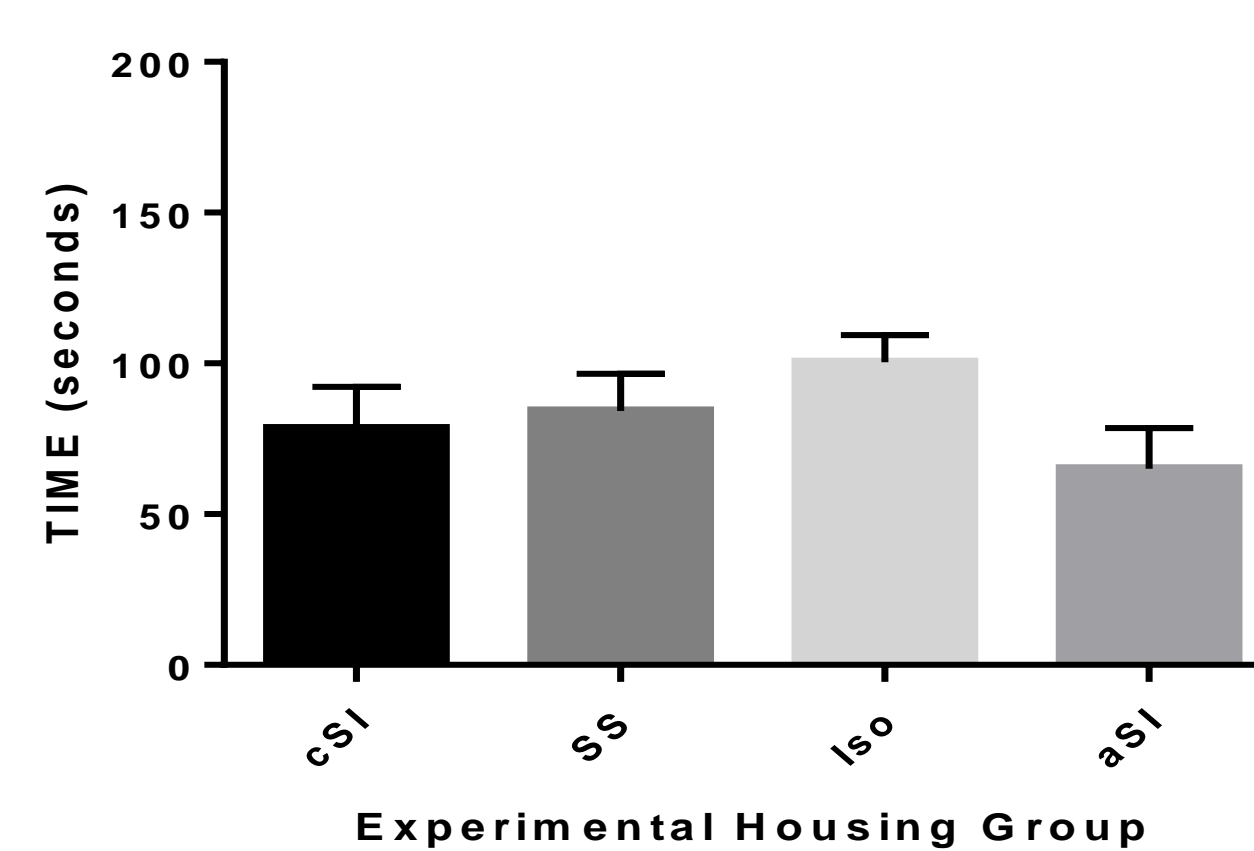
- 20% EtOH/water given M, W, F for 4 weeks.
- 2 bottle choice- controlled for location preference.
- EtOH intake and preference were measured at 30min and 24h.

Figure 1. Experimental Timeline



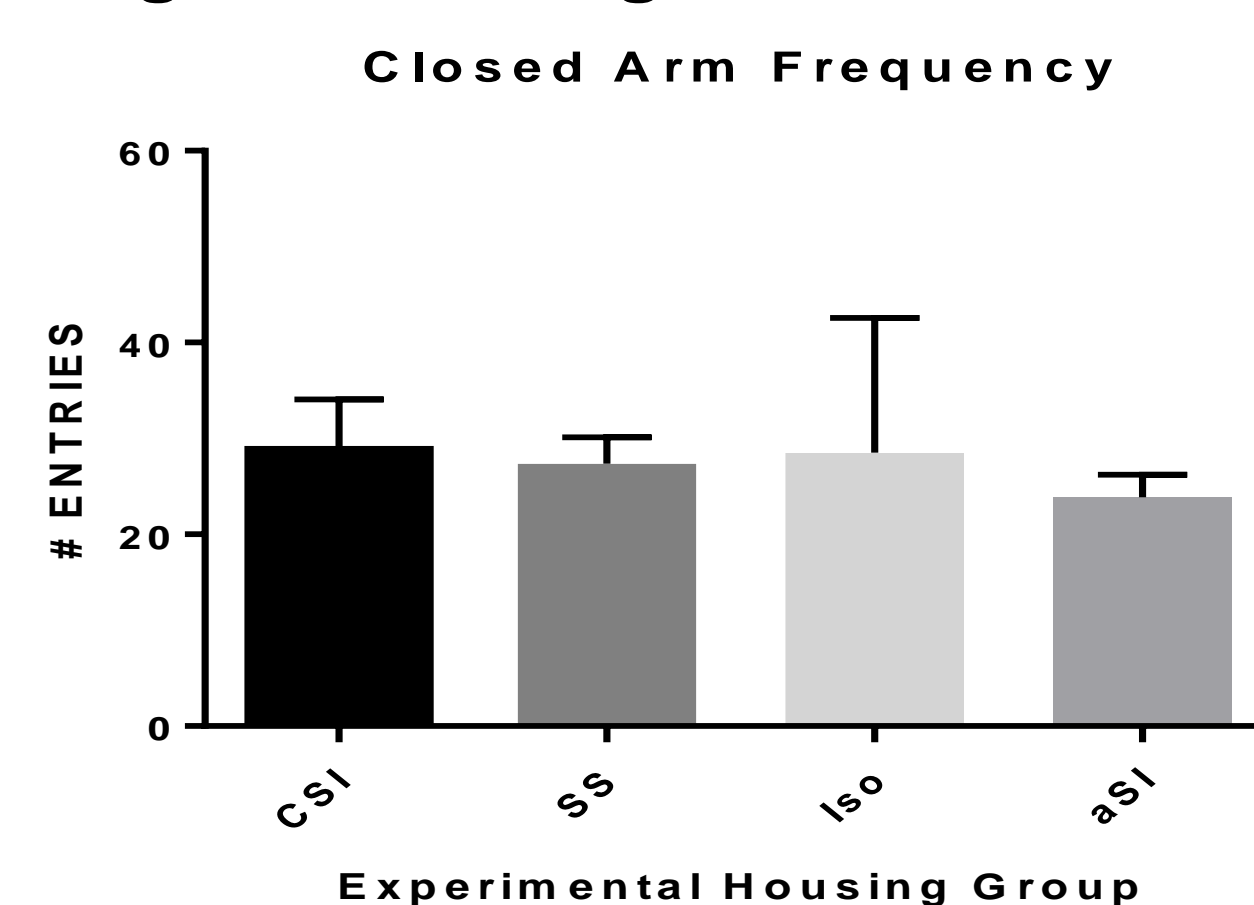
Experimental Timeline. aSI, acute social instability; CSI, chronic social isolation; EPM, elevated plus maze; EtOH, alcohol; Iso, isolation; PND, post natal day; SS, social stability; SA, self-administration; T1, timepoint 1; T2, timepoint 2.

Figure 2. No significant differences in open arm time



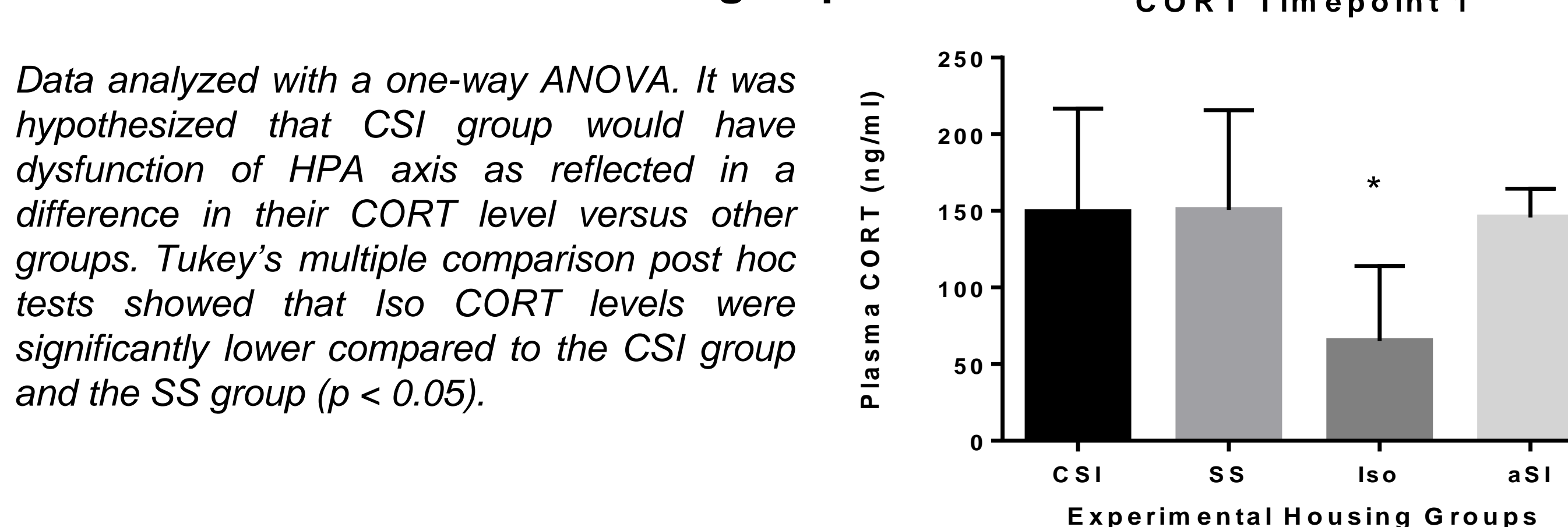
Data were analyzed with a one-way ANOVA. It was hypothesized that CSI would show greater anxiety-like behavior, in that they would spend less time in open arms than other groups. However, this hypothesis was not supported.

Figure 3. No significant difference in time spent in closed arm.



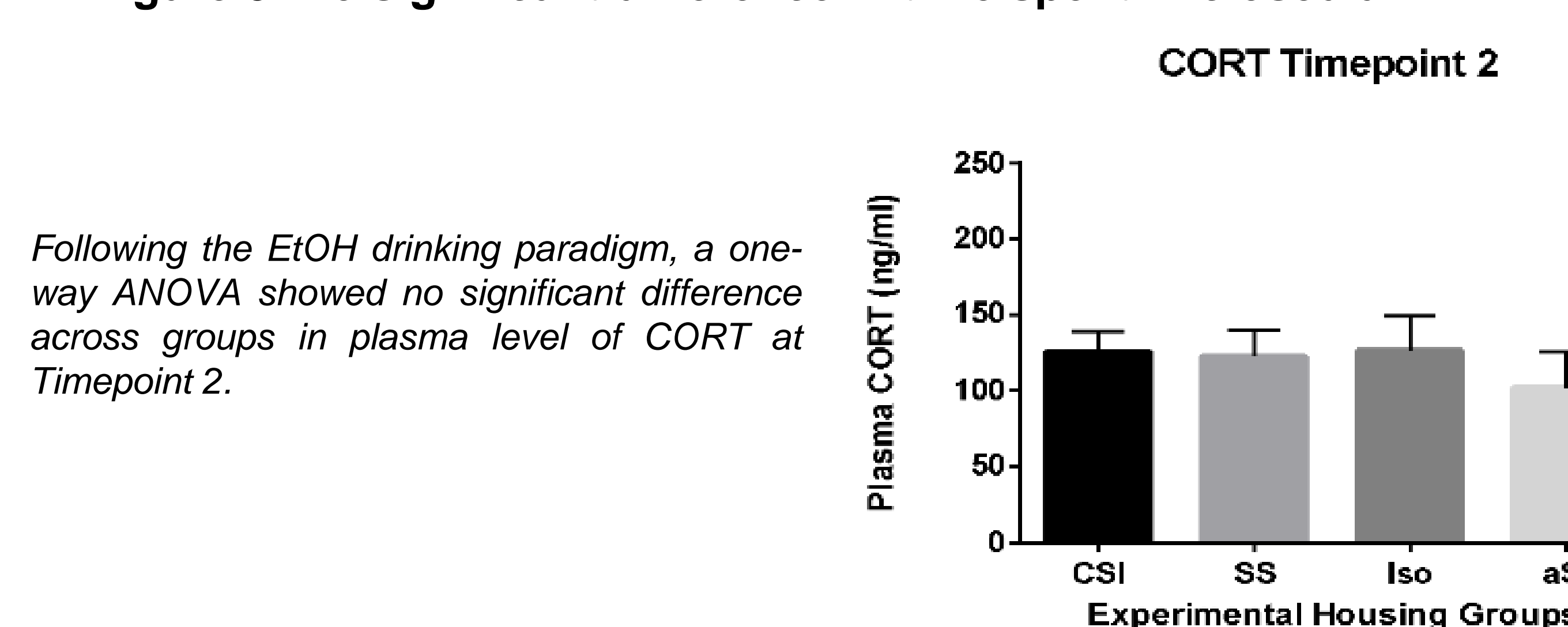
Data were analyzed with a one-way ANOVA. Closed arm frequency is a measure of locomotion. There was no difference of locomotion across groups.

Figure 4. Iso CORT levels significantly lower compared to CSI and SS groups



Data analyzed with a one-way ANOVA. It was hypothesized that CSI group would have dysfunction of HPA axis as reflected in a difference in their CORT level versus other groups. Tukey's multiple comparison post hoc tests showed that Iso CORT levels were significantly lower compared to the CSI group and the SS group ($p < 0.05$).

Figure 5. No significant difference in time spent in closed arm



Following the EtOH drinking paradigm, a one-way ANOVA showed no significant difference across groups in plasma level of CORT at Timepoint 2.

Figures 6-9. No significant interaction or main groups effect for intake or preference at 30 minute and 24 hour time points

Figure 6.

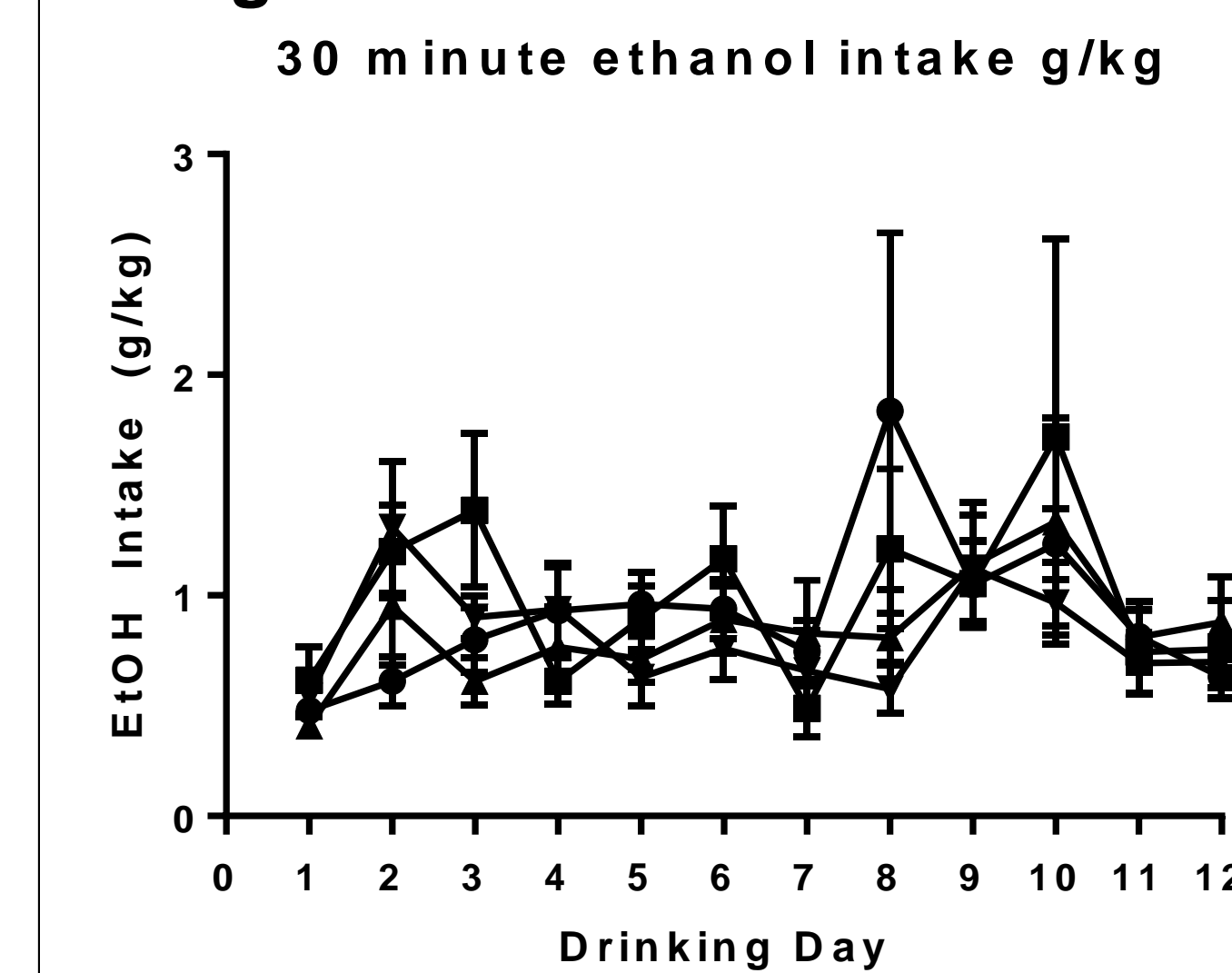


Figure 7.

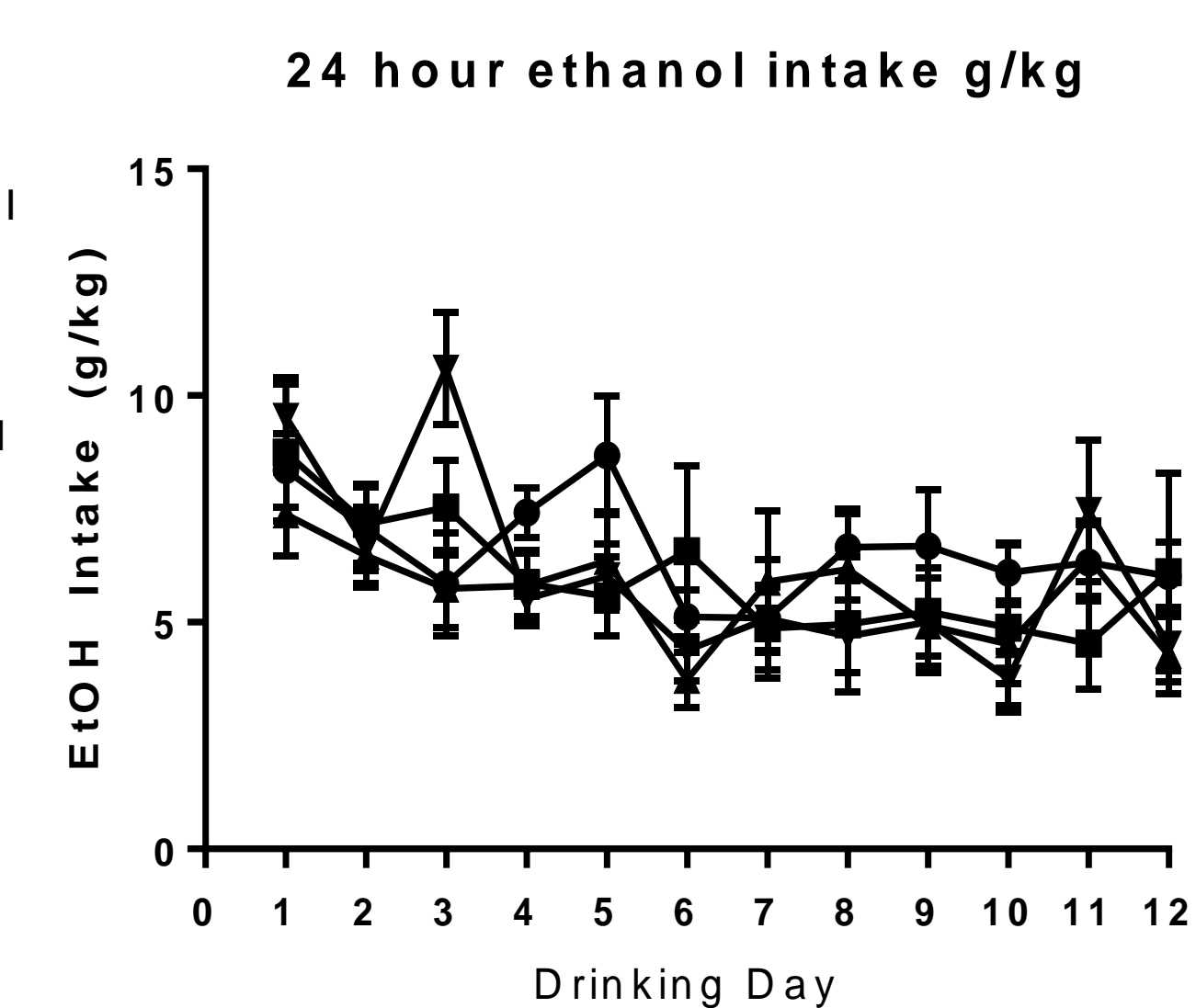


Figure 8.

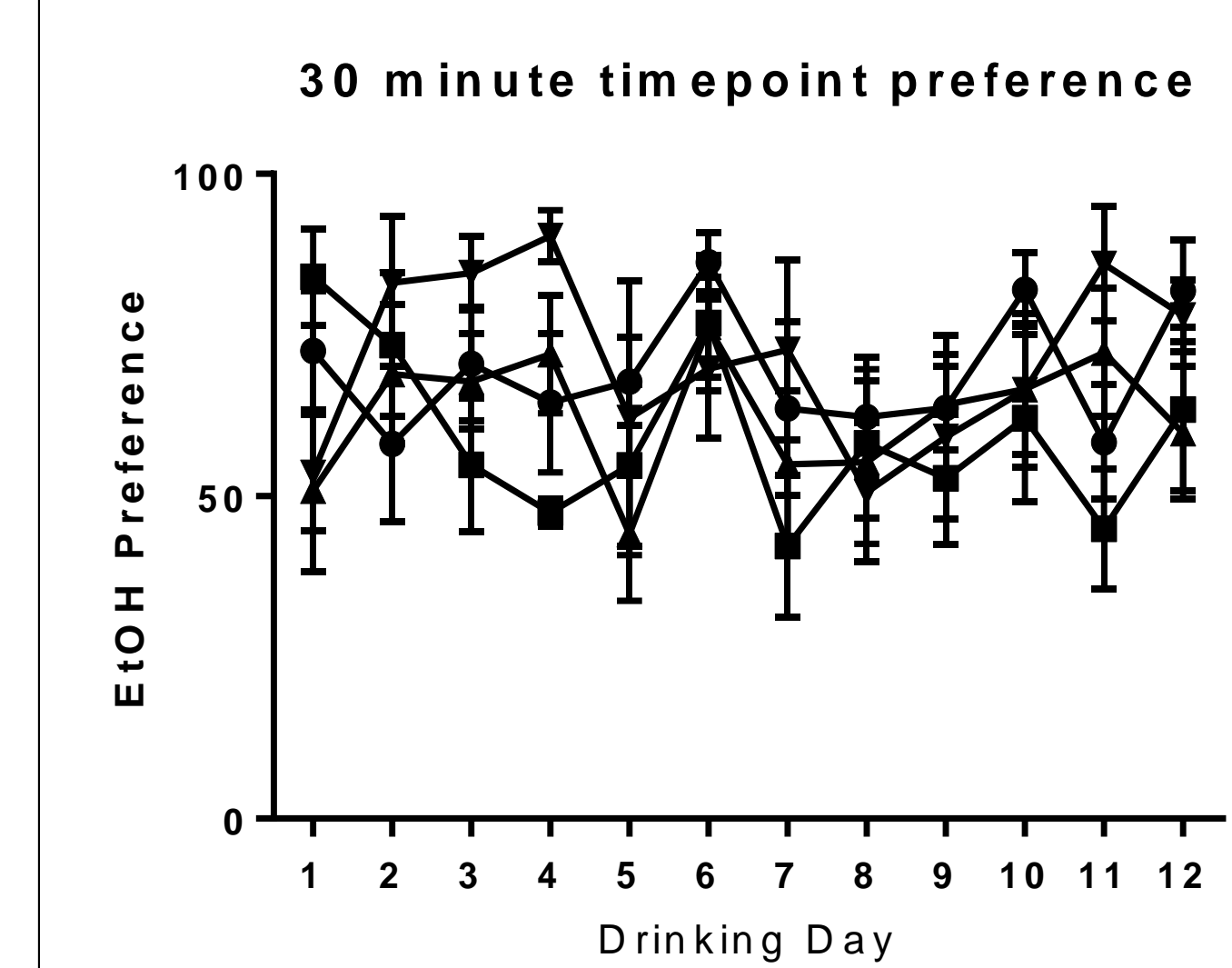
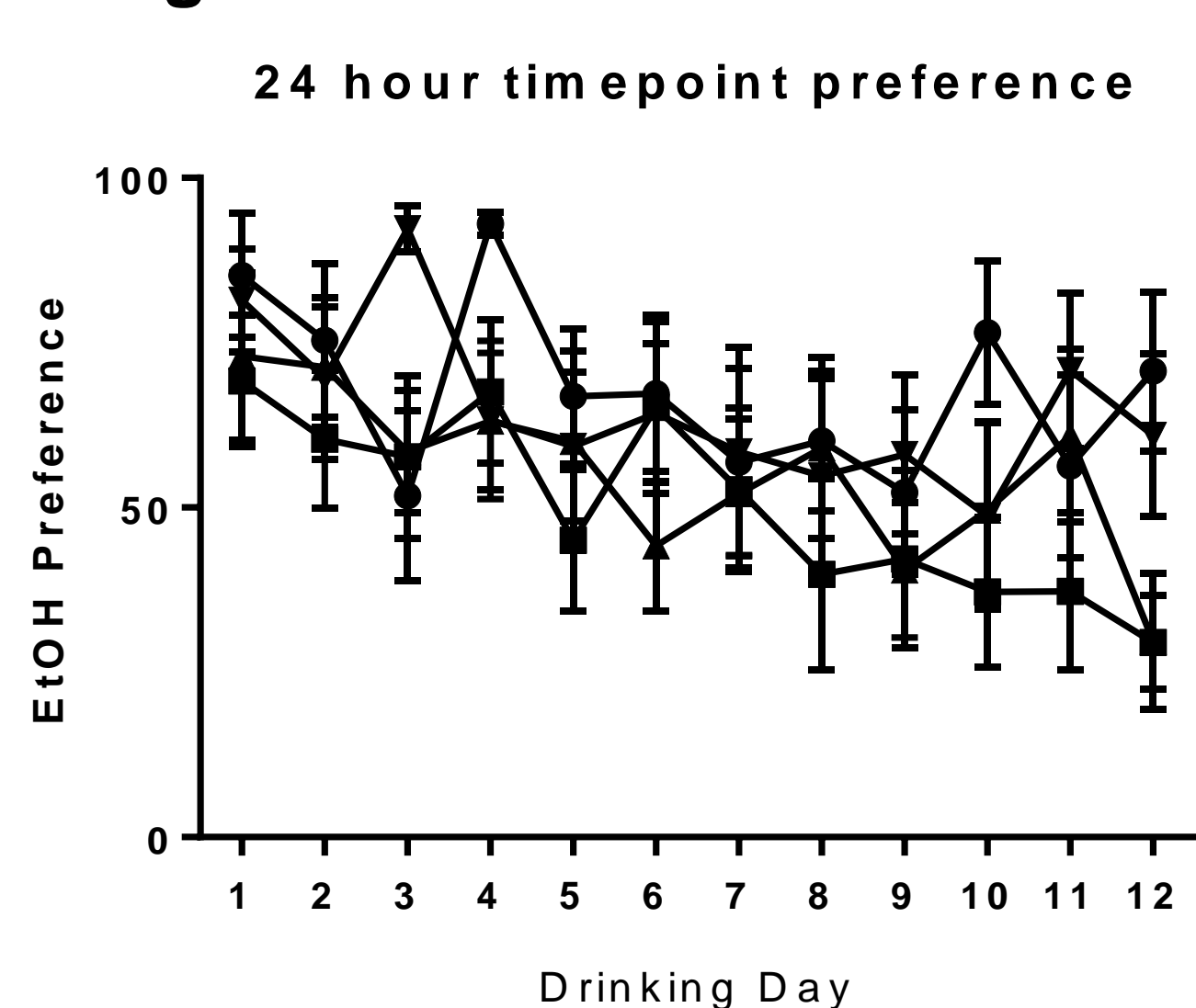


Figure 9.



Figures 6-9. Ethanol intake and preference. For each dataset 6-9, a two-way RM ANOVA revealed no significant interaction, main effect of group, or main effect of drinking day for intake and preference measures at the 30 minute and 24 hour time points. Data presented as mean +/- SEM.

Conclusion

- Elevated Plus Maze:** No significant differences in time spent on open arms or closed arm frequency.
- CORT:** ISO females had significantly lower levels of CORT at Timepoint 1. The importance of this is unclear, but was taken in a single moment that may have influenced this result. There were no significant differences at Timepoint 2, suggesting no long-lasting HPA axis dysregulation.
- EtOH:** No significant group differences for intake or preference.
- This data suggests that housing type does not produce a significant increase in anxiety-like behaviors and EtOH drinking in female rats.
- Future work will expand upon these findings, utilizing different models of stress to induce an EtOH addiction-like phenotype in female Long -Evans rats.

Acknowledgements

Many thanks to Dr. Tracy Butler, who served as the advisor for this honors thesis. Also thanks to the psychology department and the biology department for their support of this research, the engineering department for the manufacturing of the elevated plus maze, and the rest of the Butler lab, especially Anissa Maffett. This work was supported by the University of Dayton College of Arts and Sciences Summer Dean's Fellowship funding the University of Dayton's Honors Program funding