Project 3: ANOVA

11/18/2020
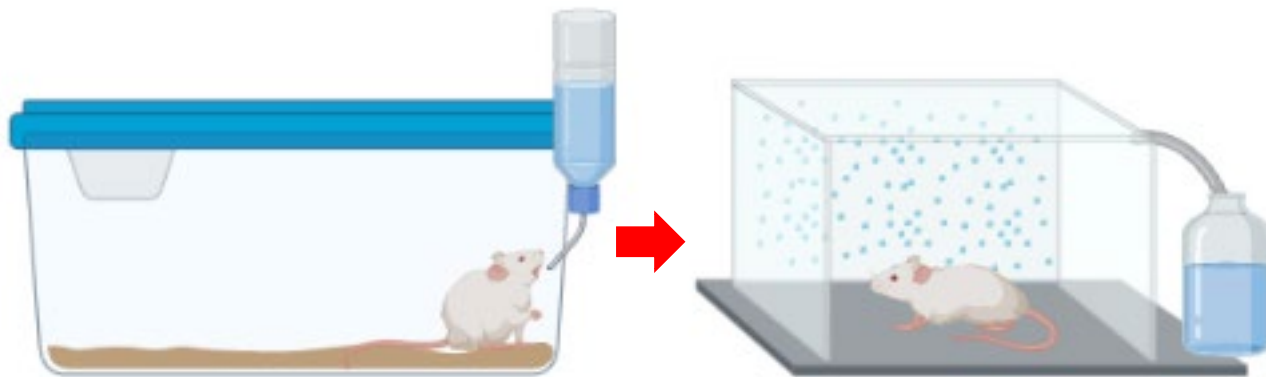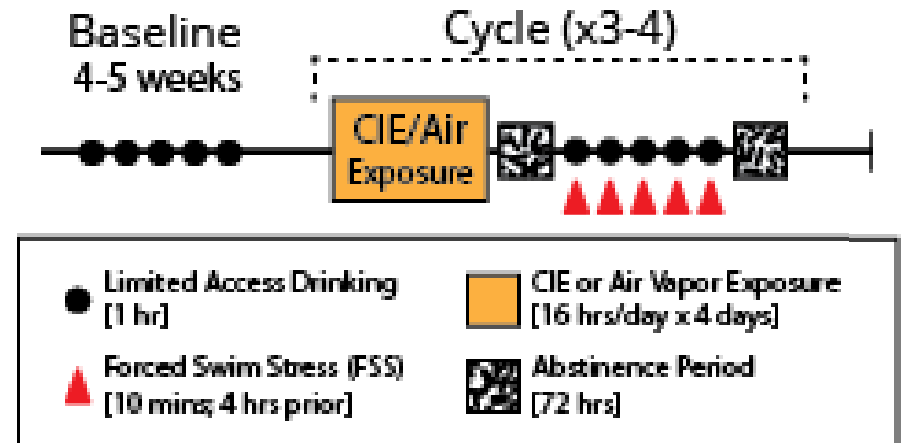
Ellen Rodberg

Background

(Rodberg et al., 2017)

- Alcohol consumption patterns vary by individuals

- Chronic stress and alcohol exposure has been shown to increase volitional alcohol consumption (Becker et al., 2011; Sinha and O'Malley, 1999)

- Previously, in our lab we have found (Rodberg et al., 2017)
    forced swim stress significantly increases alcohol consumption
    chronic ethanol exposure does not
    combined stress and ethanol exposure caused largest increases

- This pilot study aimed to investigate if baseline cognitive performance can predict future alcohol consumption and if stress and alcohol exposure alters volitional consumption

- This presentation will focus on validating our previous findings that stress and ethanol (EtOH) exposure increases volitional EtOH consumption in mice.
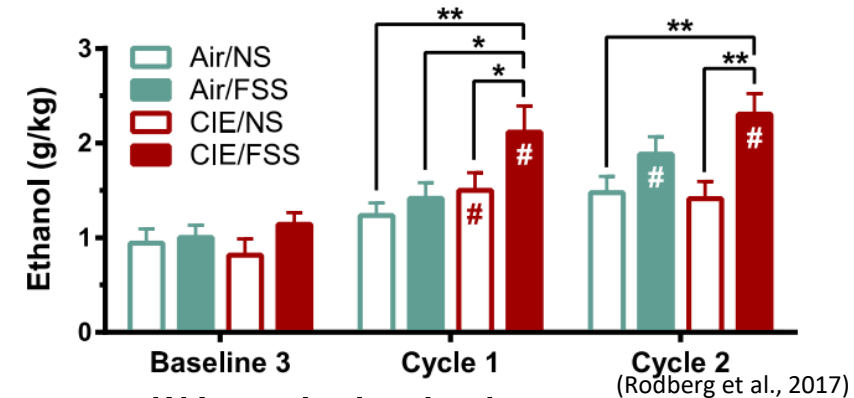
Does exposure to chronic stress or EtOH increase volitional ethanol consumption?

Methods

- 24 mice (11 female, 13 male; n=8 per group)
- Attentional set shifting (we will ignore this)
- Baseline drinking (1hr, 15%)
- Stress and alcohol exposure
- Test drinking (1hr, 15%)

Baseline
4-5 weeks

Cycle (x3-4)

CIE/Air
Exposure

- Limited Access Drinking [1 hr]
- CIE or Air Vapor Exposure [16 hrs/day x 4 days]
- Forced Swim Stress (FSS) [10 mins; 4 hrs prior]
- Abstinence Period [72 hrs]

Baseline Drinking

Chronic Ethanol Exposure

OR

Forced Swim Stress

Test Drinking

Methods

- Drinking was calculated as grams EtOH/kg of bodyweight

- Drinking comparisons were made by averaging daily EtOH consumption across all weeks of baseline & test drinking per animal

- Independent variables:
  - Treatment: none, stress, ethanol exposure
  - Pre/post treatment

- Dependent variables: average g/kg EtOH consumed

- Nuisance variables: sex differences, combined 2 cohorts

- 2 Levels:
  - Treatment – 3 factors: Air/NS, Air/FSS, CIE/NS
  - Stage – 2 factors: Baseline, Test

Treatment Groups	No Stress	Stress
No Chronic EtOH	Air/NS	Air/FSS
Chronic EtOH	CIE/NS	CIE/FSS

A priori power:

Stage

Small effect = 0.1
n=1302/6
n=217 per group

Medium effect = 0.25
n=210/6
n=35 per group

Large effect = 0.4
n=84/6
n= 14 per group

Treatment

Small effect = 0.1
n=1548/6
n=258 per group

Medium effect = 0.25
n=251/6
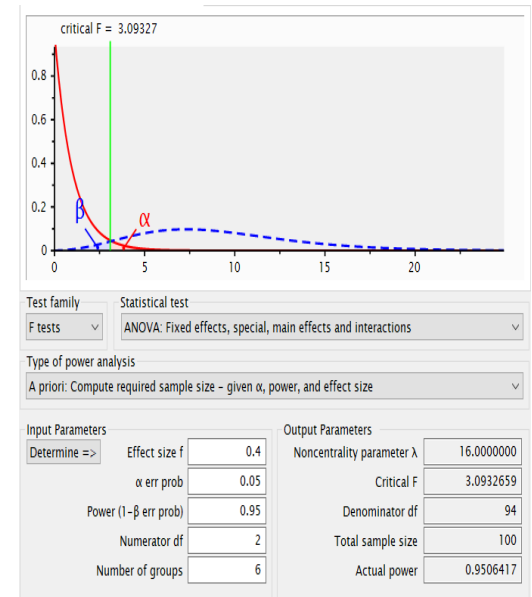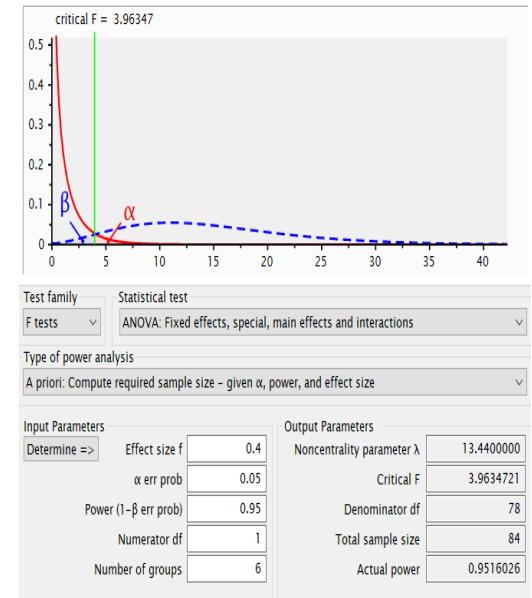n=42 per group

Large effect = 0.4
n=100/6
n= 17 per group

Interaction
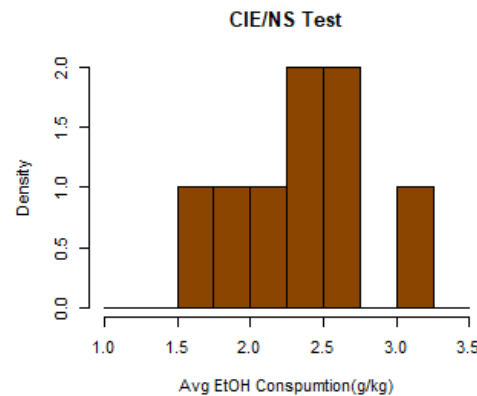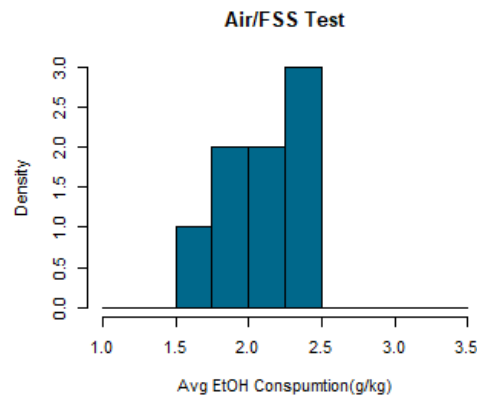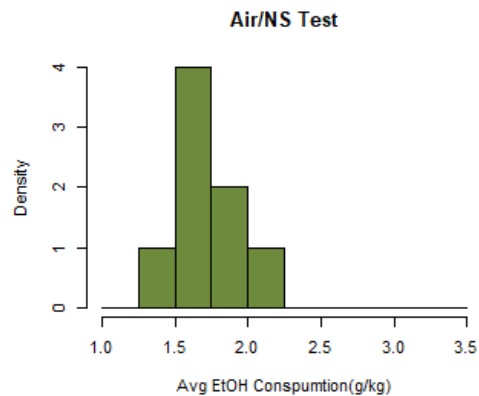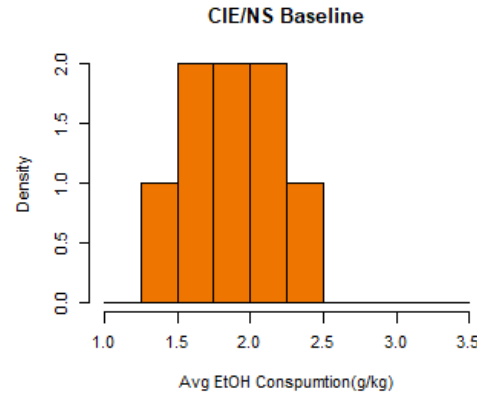
Small effect = 0.1
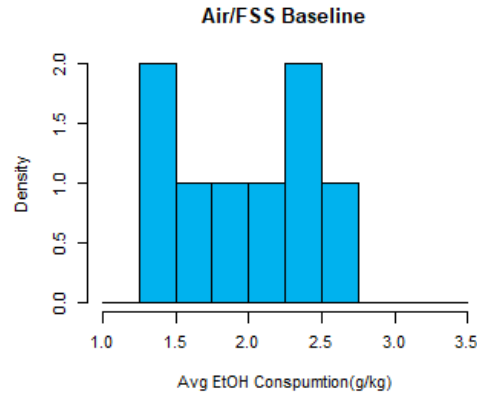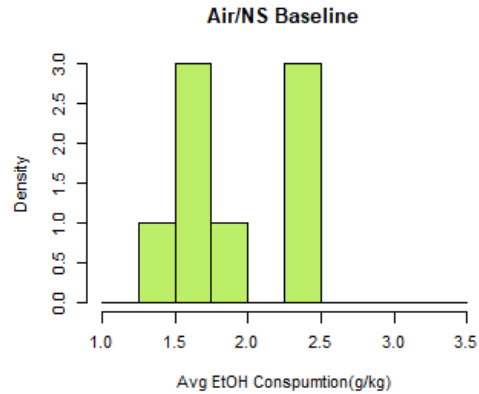n=1548/6
n=258 per group

Medium effect = 0.25
n=251/6
n=42 per group

Large effect = 0.4
n=100/6
n= 17 per group

Even with large a large effect size, this study is underpowered

Assumptions:

Shapiro test for normality
Air/NS baseline: p=0.1567
Air/FSS baseline: p=0.755
CIE/NS baseline: p=0.988
Air/NS test: p=0.8746
Air/FSS test: p=0.2067
CIE/NS test: p=0.9864

-reject the null hypothesis that the data is not normal

Independence:
No- this is a within subject test

HOV: Levene Test
-reject the null hypothesis that there is not homogeneity of variance
p=0.2411

```
> leveneTest(drink$drink~drink$Tx*drink$stage, center='mean')
Levene's Test for Homogeneity of Variance (center = "mean")
      Df F value Pr(>F)
group  5  1.4079 0.2411
      42
```

Drinking Data by treatment group and stage
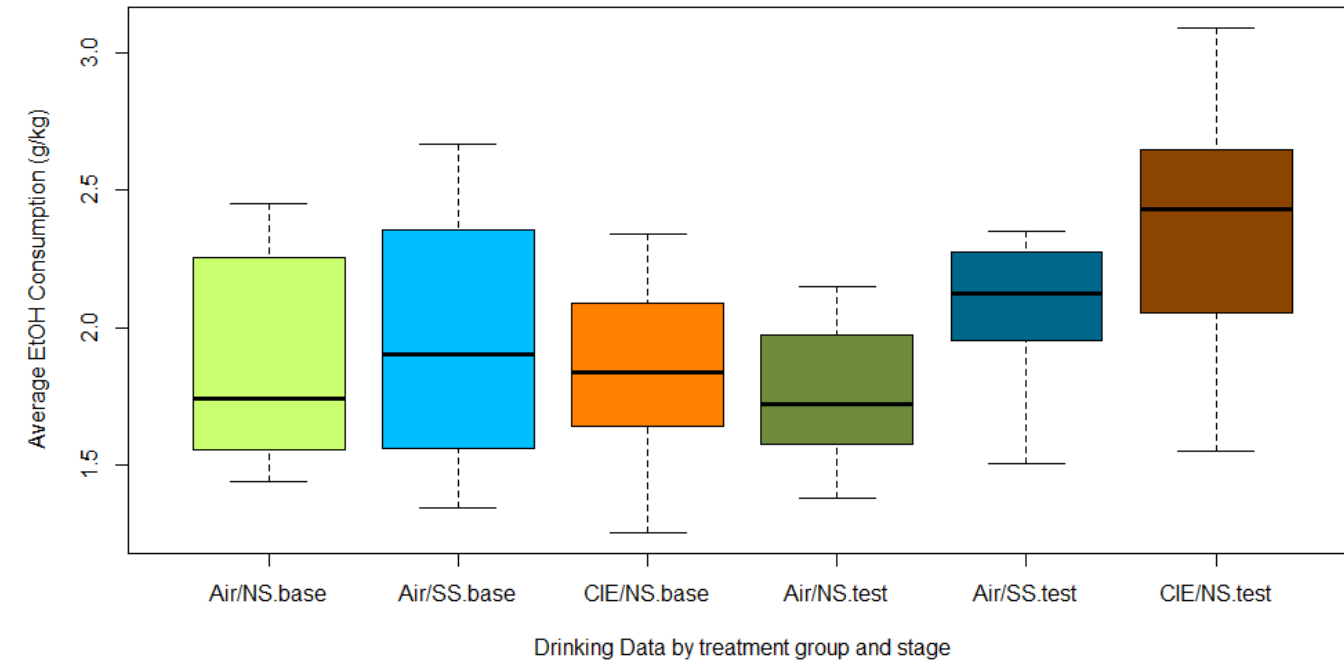
```
> summary(aov(drink$drink~drink$Tx*drink$stage))
                   Df Sum Sq Mean Sq F value Pr(>F)
drink$Tx            2  0.687  0.3437   2.387 0.1043
drink$stage         1  0.356  0.3562   2.474 0.1233
drink$Tx:drink$stage 2  0.840  0.4199   2.917 0.0651 .
Residuals          42  6.047  0.1440
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```
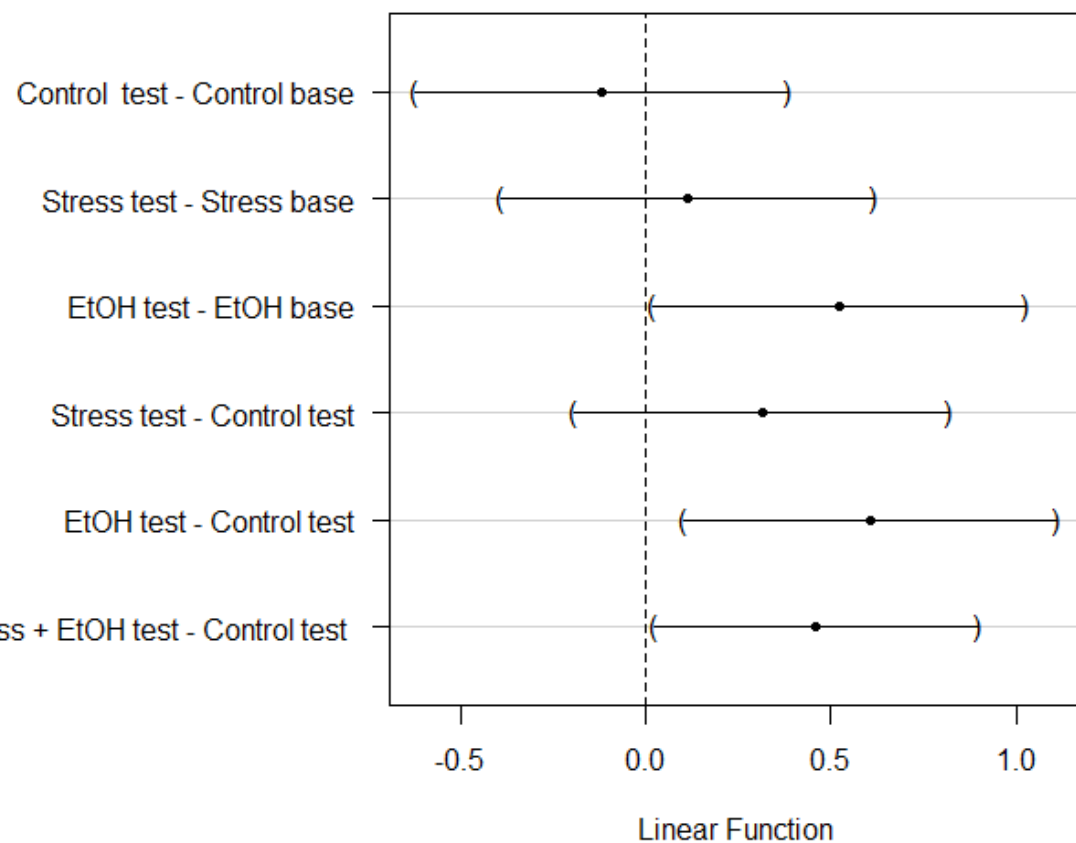
Drinking Data by treatment group

Drinking Data by stage
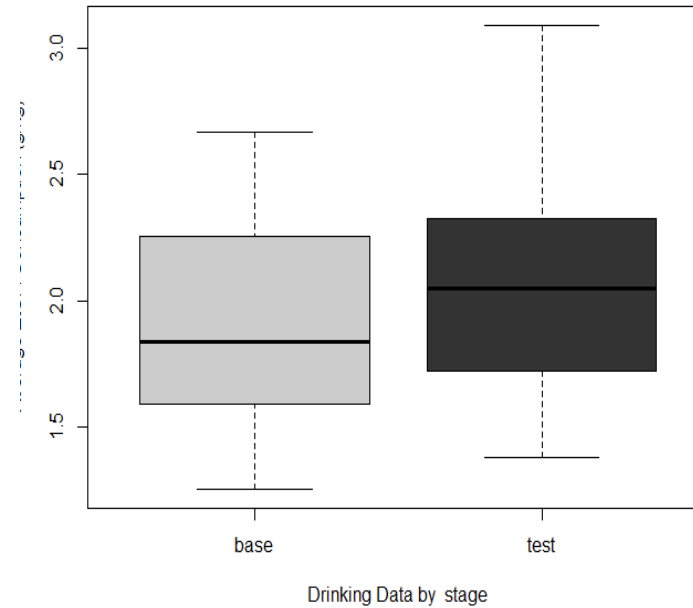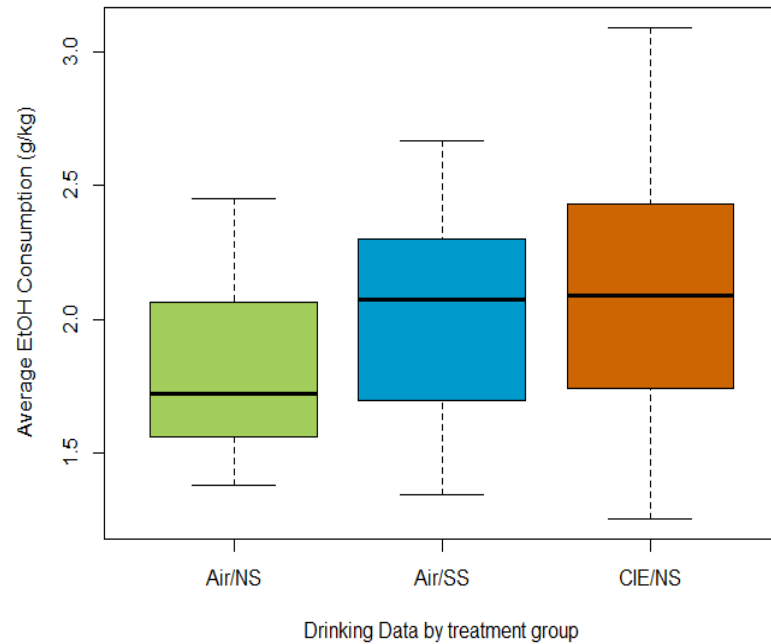
Planned Contrasts

95% family-wise confidence level

	Estimate	Std. Error	t value	Pr(>|t|)
Control test - Control base == 0	-0.1183	0.1897	-0.624	0.9601
Stress test - Stress base == 0	0.1135	0.1897	0.598	0.9661
EtOH test - EtOH base == 0	0.5217	0.1897	2.750	0.0403 *
Stress test - Control test == 0	0.3129	0.1897	1.649	0.3756
EtOH test - Control test == 0	0.6065	0.1897	3.197	0.0127 *
Stress + EtOH test - Control test == 0	0.4597	0.1643	2.798	0.0357 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

Effect Size

Drinking Data by treatment group and stage
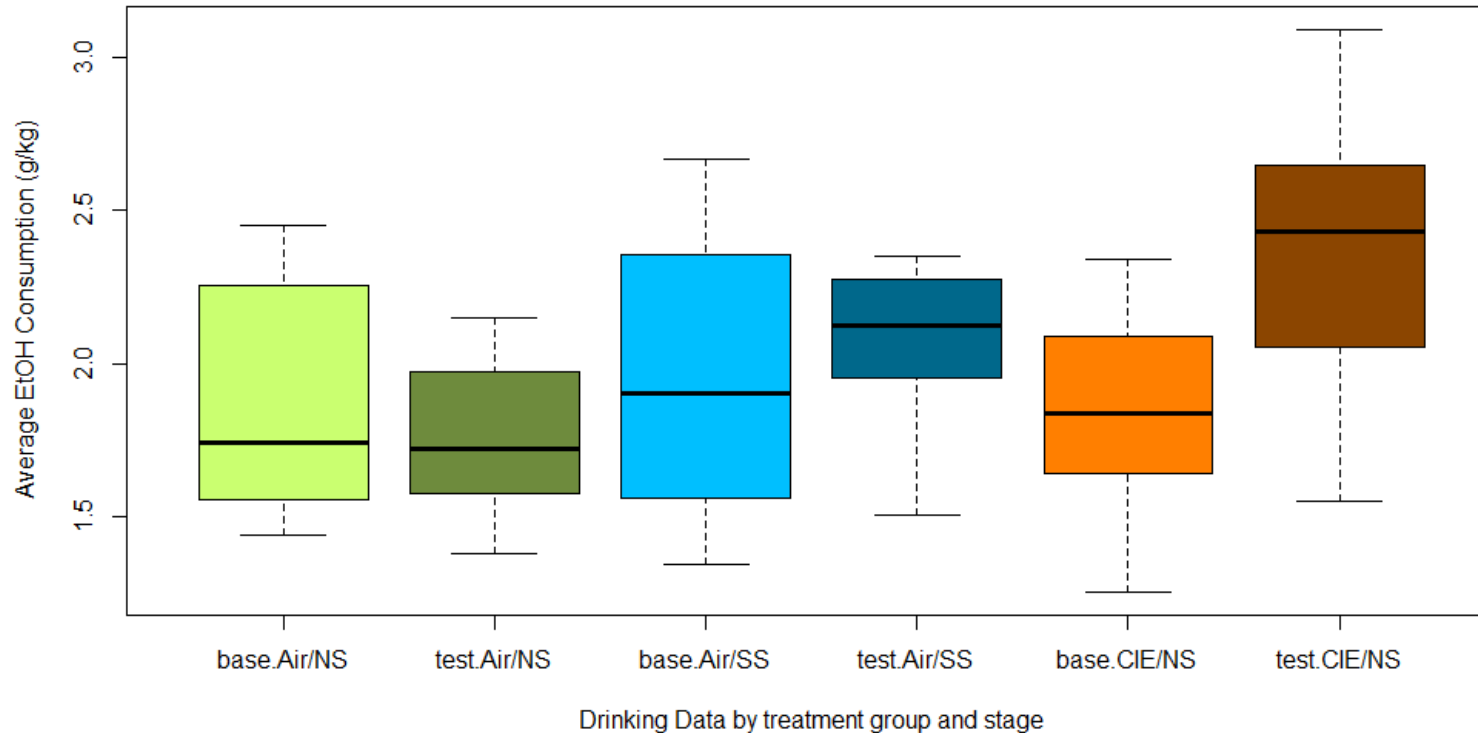
Eta squared:
Main effect of treatment: 0.1020196
Main effect of stage: 0.05559894
Interaction effect: 0.1219689

Omega squared:
Main effect of treatment: 0.0545753
Main effect of stage: 0.02975856
Interaction effect: 0.09148265

Effect Size	Use	Small	Medium	Large
omega-squared	Anova; See Field (2013)	0.01	0.06	0.14
Multivariate eta-squared	one-way MANOVA	0.01	0.06	0.14

(https://imaging.mrc-cbu.cam.ac.uk/statswiki/FAQ/effectSize)

Conclusions

- This study was underpowered
  - Even with large effect size (cohen F=.4) we would need 17 animals/group
  - This study had 8 animals/group
- ANOVA found no main effects of treatment, stage, or interaction
- Planned comparisons found 3 significant contrasts
  - Ethanol group (test – baseline)
  - Ethanol group test – Control group test
  - Ethanol + Stress groups test – Control group test