Project 4: RM ANOVA

02/24/2021

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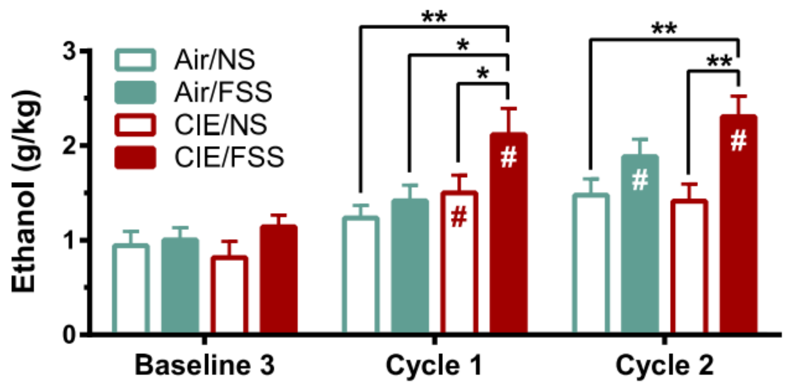
Background



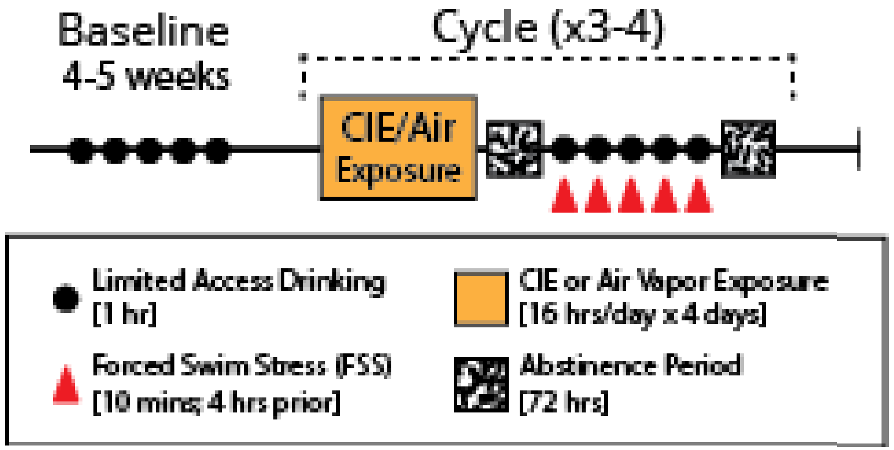
(Rodberg et al., 2017)

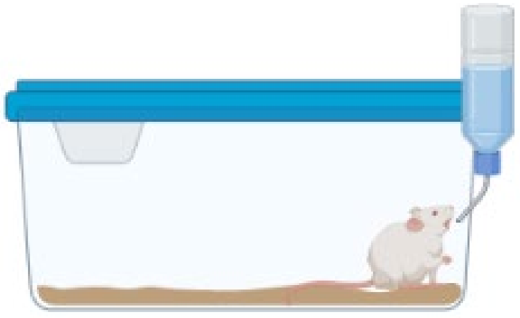
- Alcohol consumption patterns vary by individuals
- Chronic stress and alcohol exposure has been shown to increase volitional alcohol consumption (Becker et al., 2011; Sinha and O'Malley, 1999)
- Previously, in our lab we have found (Rodberg et al., 2017)
  - forced swim stress significantly increases 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.

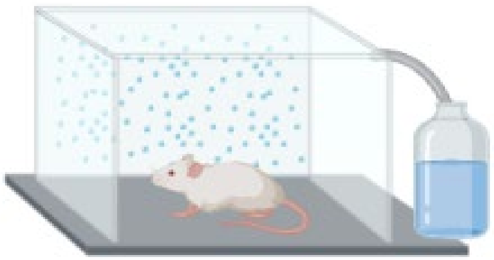
Does exposure to chronic stress and/or EtOH increase volitional ethanol consumption over time?

Methods

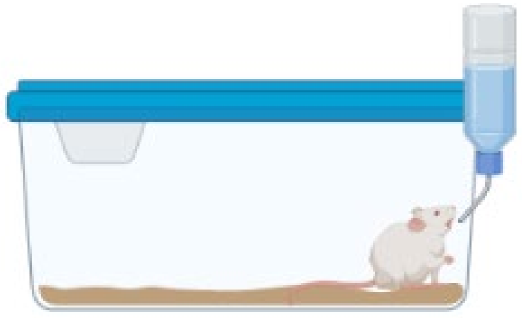
- 16 mice (10 female, 6 male; n=4 per group)
- Attentional set shifting (we will ignore this)
- Baseline drinking (1hr, 15%)
- Stress and/or alcohol exposure
- Test drinking (1hr, 15%)
- Drinking is calculated as grams EtOH/kg of bodyweight
  - Daily EtOH consumption averaged across 1 week at 3 timepoints




Baseline Drinking


Chronic Ethanol Exposure


Forced Swim Stress


Test Drinking

Methods

- Independent variables:
  - Treatment (between subject, fixed):
    - None (Air/NS)
    - Stress (Air/FSS)
    - Ethanol (CIE/NS)
    - Stress and Ethanol (CIE/FSS)
  - Timepoint: (within subjects, random):
    - Week 1
    - Week 2
    - Week 3
- Dependent variables: average g/kg EtOH consumed
- Nuisance variables: sex differences, combined 2 cohorts

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

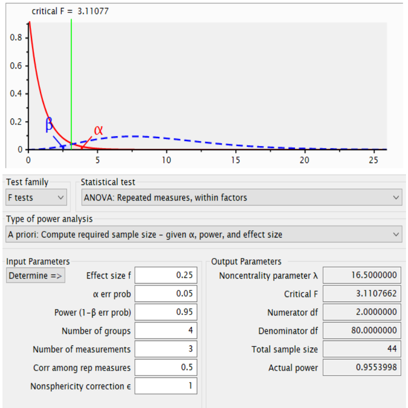
A priori power:

Factor A: Treatment

Small effect = 0.1
n=1152
n= 288 per group

Medium effect = 0.25
n=188
n= 47 per group

Large effect = 0.4
n=76
n= 19 per group

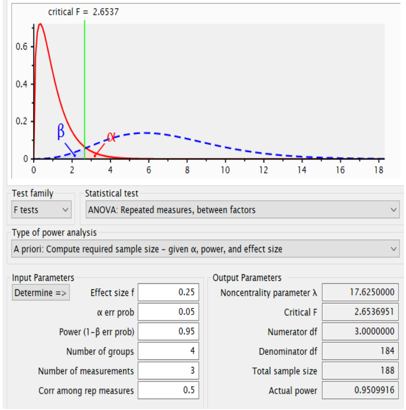


Factor B: Week

Small effect = 0.1
n=260
n= 65 per group

Medium effect = 0.25
n=44
n= 11 per group

Large effect = 0.4
n=20
n= 5 per group

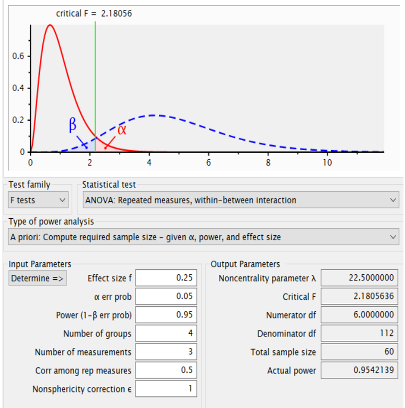


AxB Interaction

Small effect = 0.1
n=352
n= 88 per group

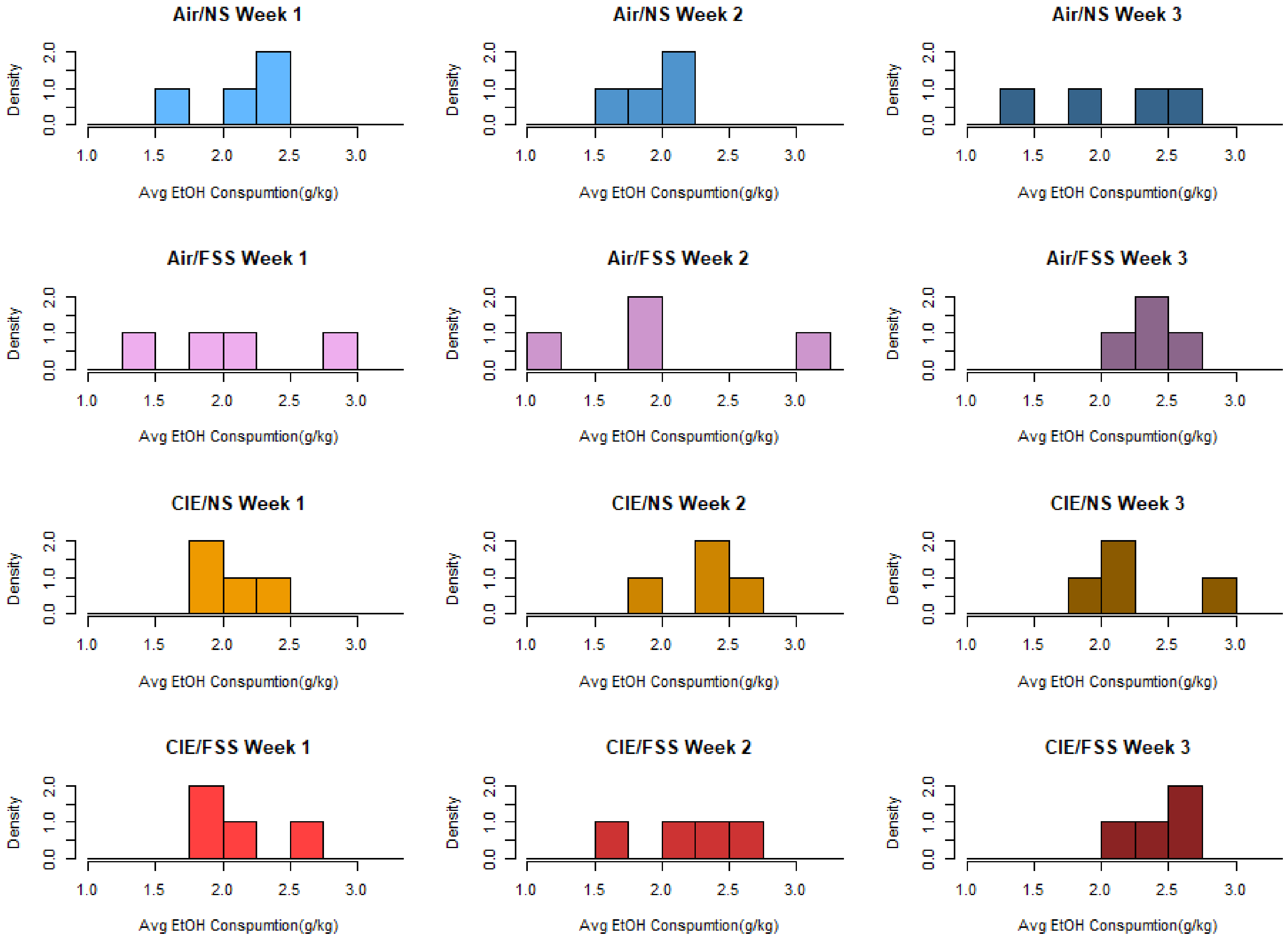
Medium effect = 0.25
n=60
n= 15 per group

Large effect = 0.4
n=28
n= 7 per group



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

Assumptions:



<u>Shapiro test for normality</u>

Air/NS week 1: p=0.2442

Air/FSS week 1: p=0.9829

CIE/NS week 1: p=0.3771

CIE/FSS week 1: p=0.1571

Air/NS week 2: p=0.2601

Air/FSS week 2: p=0.8386

CIE/NS week 2: p=0.8499

CIE/FSS week 2: p=0.7527

Air/NS week 3: p=0.7135

Air/FSS week 3: p=0.7631

CIE/NS week 3: p=0.3613

CIE/FSS week 3: p=0.5714

reject the null hypothesis that the data is not normal

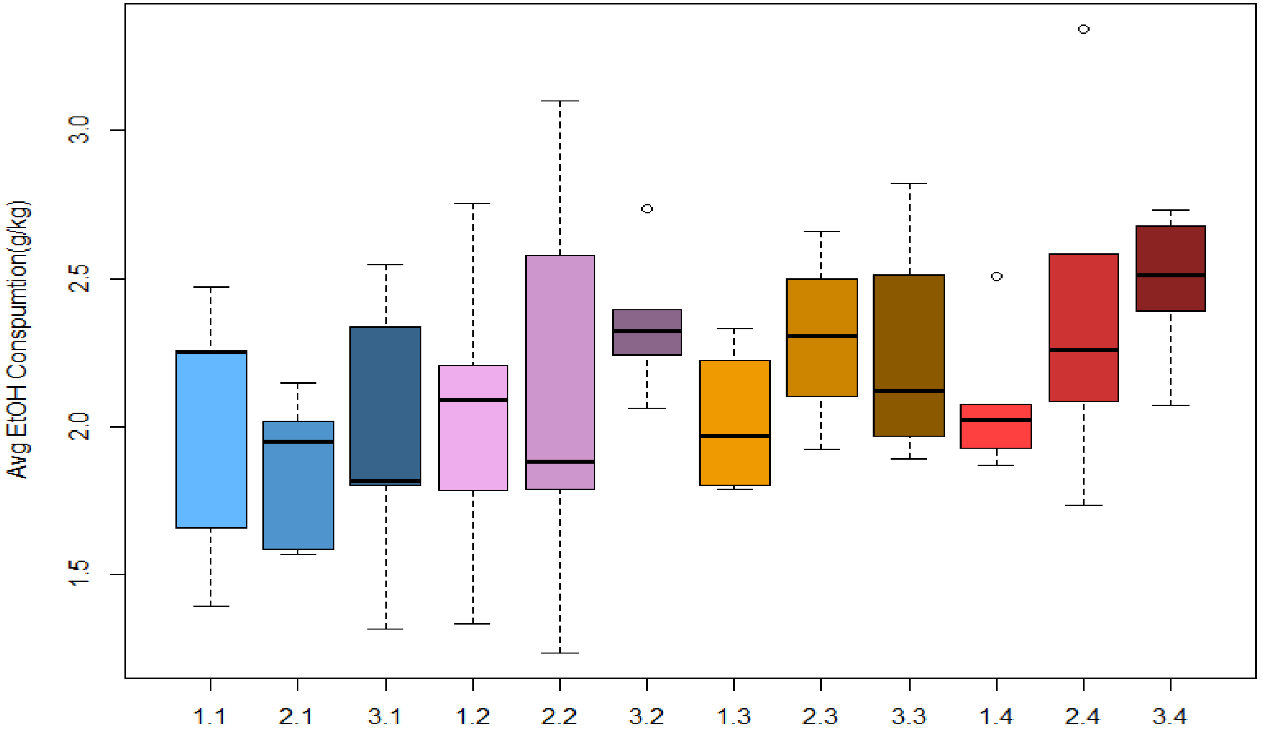
Assumptions:

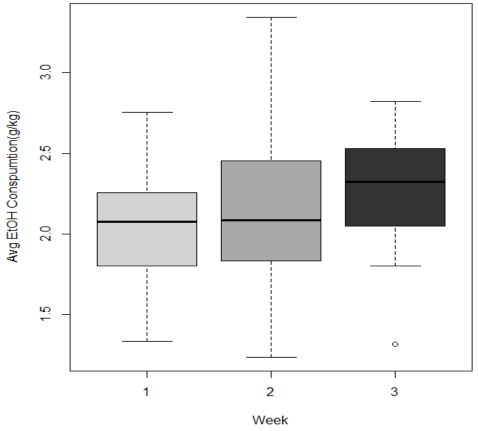
Independence:
We assume independence of between-subject variables hold

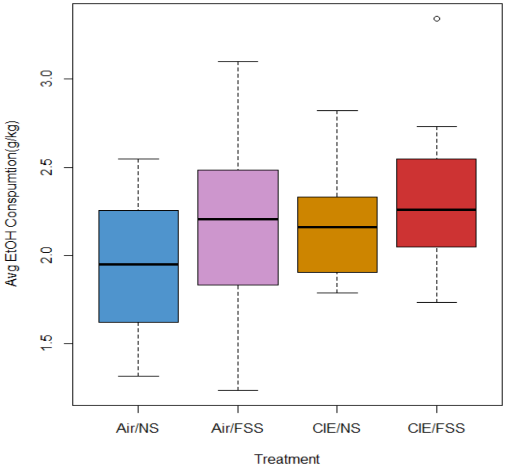
Sphericity:
Sphericity holds
Week: $p=0.119$
Treatment:Week: $p= 0.119$

HOV: Levene Test
Reject the null hypothesis that there is not homogeneity of variance
$p=0.1585$

HOCovariance: Box’s M Test
Homogeneity of covariance is not violated
$p=0.189$







—	df	SS	MS	F	pval
S	63	2.924	0.046	-	-
A	3	1.177	0.392	0.944	0.472
S/A	60	1.747	0.029	-	-
B	2	1.533	0.767	93.739	3.034e-25
AB	6	2.368	0.395	48.256	9.884e-30
B*S/A	120	0.008	0.015	-	-

Expected mean squares

$$EMS(A) = \sigma_e^2 + nb\theta_A^2 + b\sigma_{S/A}^2 + n\sigma_{AB}^2 + \sigma_{BS/A}^2$$
$$EMS(S/A) = \sigma_e^2 + b\sigma_{S/A}^2 + \sigma_{BS/A}^2$$
$$EMS(B) = \sigma_e^2 + na\sigma_B^2 + \sigma_{BS/A}^2$$
$$EMS(AB) = \sigma_e^2 + n\sigma_{AB}^2 + \sigma_{BS/A}^2$$
$$EMS(BS/A) = \sigma_e^2 + \sigma_{BS/A}^2$$

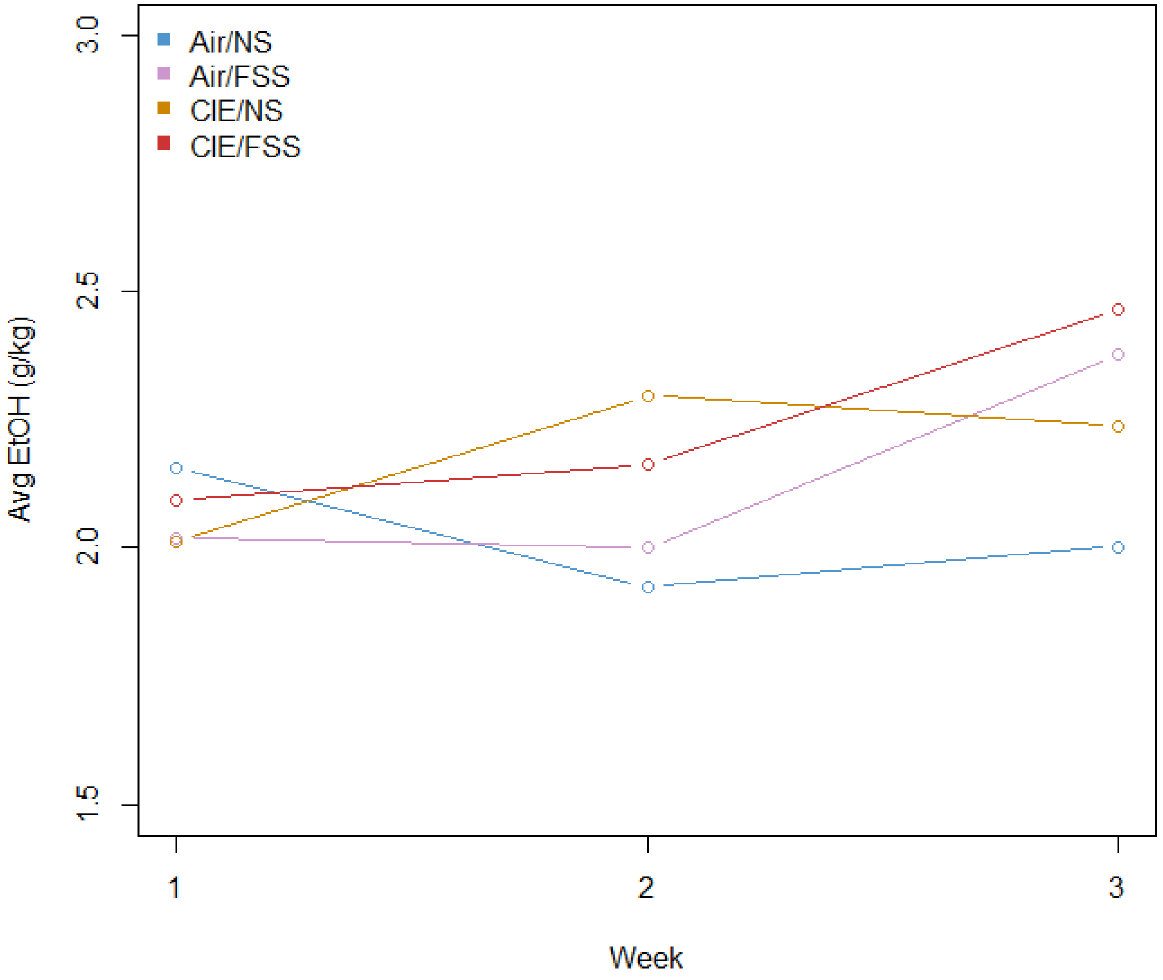
A: Treatment
- Between subjects
- Fixed

B: Week
- Within subjects
- Random

F values

A: MS.A / (MS.AB + MS.SwA - MS.BSwA)
B: MS.B / MS.BSwA
AB: MS.AB/ MS.BSwA

Effect Size

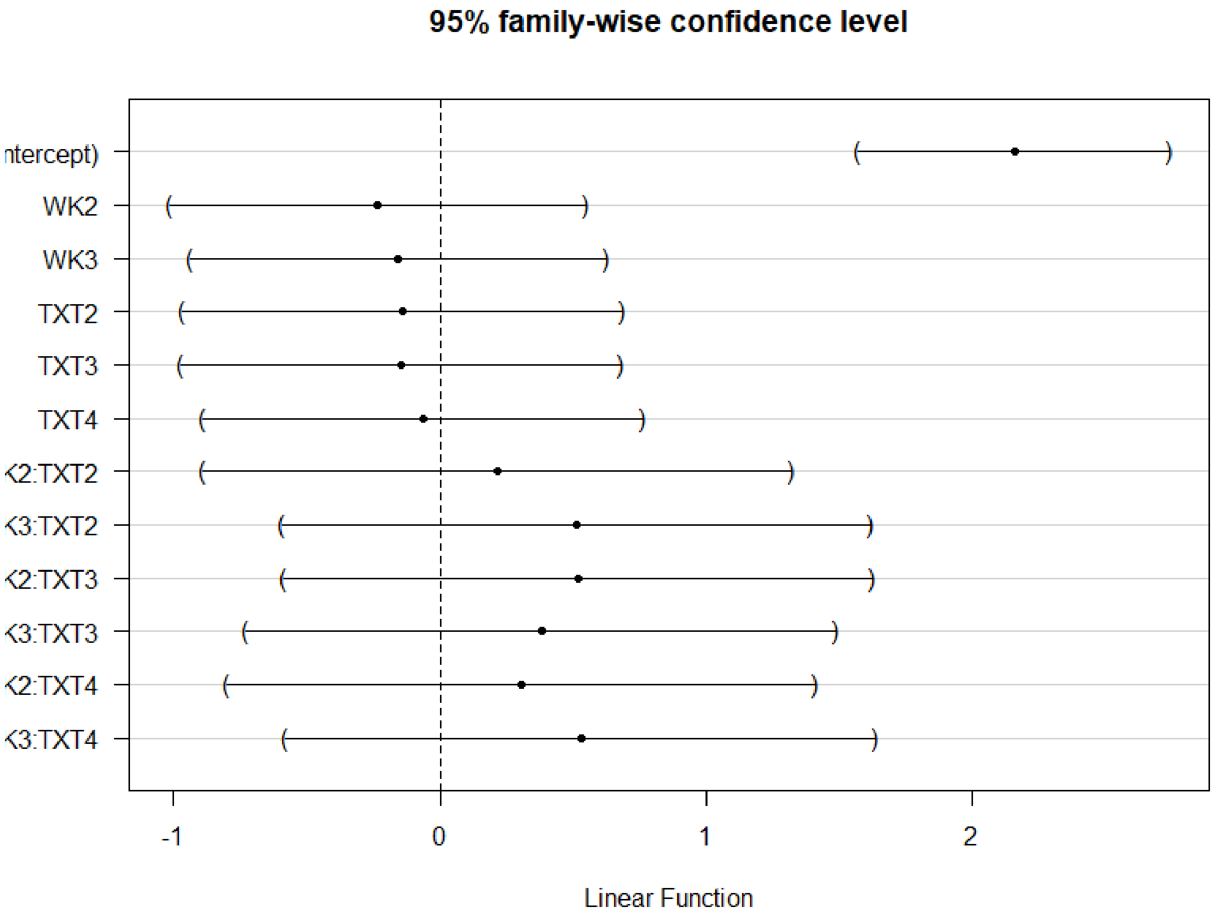


<u>Eta squared:</u>
A: 0.1279528
B: 0.6097274
AB: 0.7069837

<u>Omega squared:</u>
A: 0.0470355

Planned Contrasts

No significant contrasts compared to Week 1 Air/NS

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Linear Hypotheses:
                  Estimate Std. Error z value Pr(>|z|)
(Intercept) == 0   2.15795    0.21308  10.127   <2e-16 ***
WK2 == 0          -0.23395    0.28533  -0.820        1
WK3 == 0          -0.15507    0.28533  -0.543        1
TXT2 == 0         -0.13957    0.30134  -0.463        1
TXT3 == 0         -0.14592    0.30134  -0.484        1
TXT4 == 0         -0.06475    0.30134  -0.215        1
WK2:TXT2 == 0      0.21607    0.40352   0.535        1
WK3:TXT2 == 0      0.51519    0.40352   1.277        1
WK2:TXT3 == 0      0.51992    0.40352   1.288        1
WK3:TXT3 == 0      0.38105    0.40352   0.944        1
WK2:TXT4 == 0      0.30475    0.40352   0.755        1
WK3:TXT4 == 0      0.52938    0.40352   1.312        1
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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- bonferroni method)
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Conclusions

- This study was underpowered
  - Even with large effect size (cohen F=.4) we would need
    - 19 animals/group to find effect of treatment
    - 5 animals/group to find effect of week
    - 7 animals/group to find interaction effect
  - This study had 4 animals/group
- ANOVA found main effect of
  - Week
  - Interaction of treatment and week
- Planned comparisons found no significant contrasts from week 1 Air/NS