GP_LONG

JLB

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Contents

About

The purpose of this document is to synthesize data generated for the “girly pops project” by JLB, CM & FZT in October / November 2023.

Project Overview

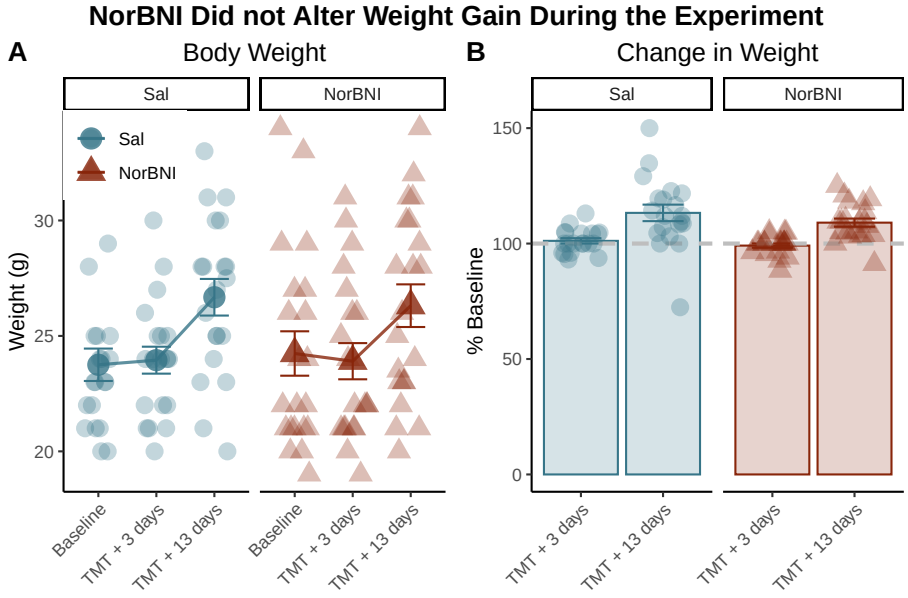
- Extra mice, leftover from Rosha's project were used in this experiment.
- Mice were used weeks after Rosha's manipulations ended, thus, we ignored the mice's experimental history.
- The purpose of these experiments was to generate data in such a way that we would be able to publish a stand-alone paper from this single cohort of animals.

Chapter 1

Weights

1.1 Purpose

- We weighted the mice repeatedly throughout the experiment
- Body weights is one of the best indications of "how they're doing"
- We were interested in investigating effects of TMT exposure, NorBNI injection, or a possible interaction between these two variables.



1.2 Graph changes in body weight

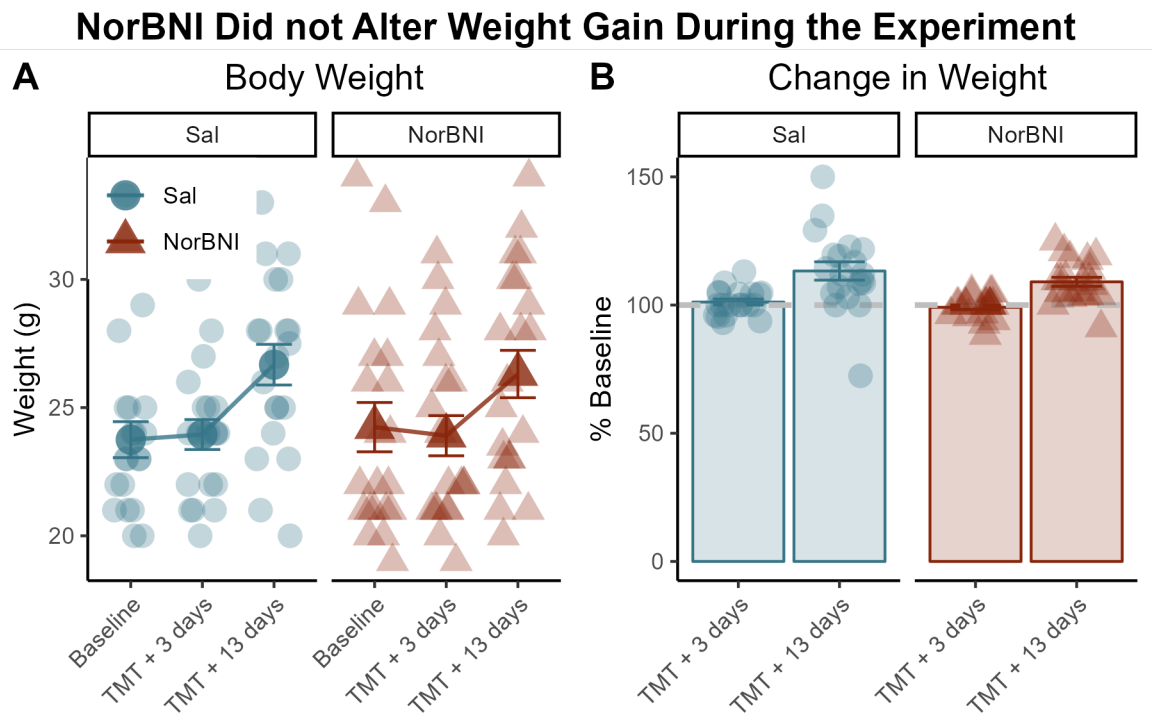


Figure 1.1: (A) Raw weight measurements at each timepoint. (B) Weight data converted into a percentage of each mouse's individual baseline measruement.

1.3 Analyze changes in body weight

```
# Turn off scientific notation
options(scipen=999)

# Run a one-way ANOVA on raw weights
c <- aov(value~variable*Drug, data=a)

# Print out the result
summary(c)
```

```
##                Df Sum Sq Mean Sq F value  Pr(>F)
## variable        2  174.3   87.15   6.867 0.00151 **
## Drug            1    0.0    0.02   0.002 0.96805
```

```
## variable:Drug   2    3.8    1.90   0.150 0.86079
## Residuals     117 1484.7   12.69
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Run a one-way ANOVA on raw weights
c <- aov(value~variable+Drug, data=a)

# Run post-hoc comparisons to investigate which timepoints are different
TukeyHSD(c)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = value ~ variable + Drug, data = a)
##
## $variable
##                        diff        lwr      upr     p adj
## TMT_3d-BL_wt   -0.07317073 -1.9271087 1.780767 0.9951743
## TMT_13D-BL_wt   2.48780488  0.6338669 4.341743 0.0052191
## TMT_13D-TMT_3d  2.56097561  0.7070376 4.414914 0.0038854
##
## $Drug
##                   diff       lwr      upr    p adj
## NorBNI-Sal 0.02579365 -1.237474 1.289061 0.967818
```

1.4 Interpret changes in body weight

- The main effect of day indicated that mice gained weight across the experiment ($F(2,119) = 6.97$, $p = 0.001$).
- Post-hoc analyses indicated that mice gained weight between the baseline measurement and the 13-days post TMT measurement ($p = 0.005$), but not between the baseline and the 3D timepoints ($p = 0.99$).
- NorBNI did not interact with change in weight gain across the timecourse ($p = 0.97$).

Chapter 2

Freezing Behaviour During TMT Presentation

2.1 Statistical Analyses

2.1.1 Two-way ANOVA on Freezing behaivour

```
a <- aov(Frz_Tm ~ Drug * Task, data=train_data)
summary(a)
```

```
##             Df Sum Sq Mean Sq F value               Pr(>F)
## Drug         1    340     340   0.555              0.45835
## Task         1  95925   95925 156.610 < 0.0000000000000002 ***
## Drug:Task    1   6511    6511  10.631              0.00165 **
## Residuals   78  47776     613
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

We quantified freezing behaviour both during the neutral pairing and the TMT exposure to investigate whether Nor BNI administration affected basal or stress-induced levels of freezing.

We computed a 2x2 ANOVA with Drug (saline or Nor BNI) as the between-groups factor and timepoint (Neutral vs TMT) as the within-subjects variable. The omnibus test for this model indicated a significant main effect of timepoint ($F(1,39) = 79.09$, $p < 0.001$) and a timepoint * Drug interaction ($F(1,39) = 11.41$, $p < 0.001$). Follow- up comparisons of the significant interaction indicated that NorBNI-injected mice froze more than did controls during the Neutral exposure ($p < 0.001$) and that Nor-BNI-injected mice spent less time freezing than controls during the TMT exposure ($p = 0.042$). Nevertheless, both groups

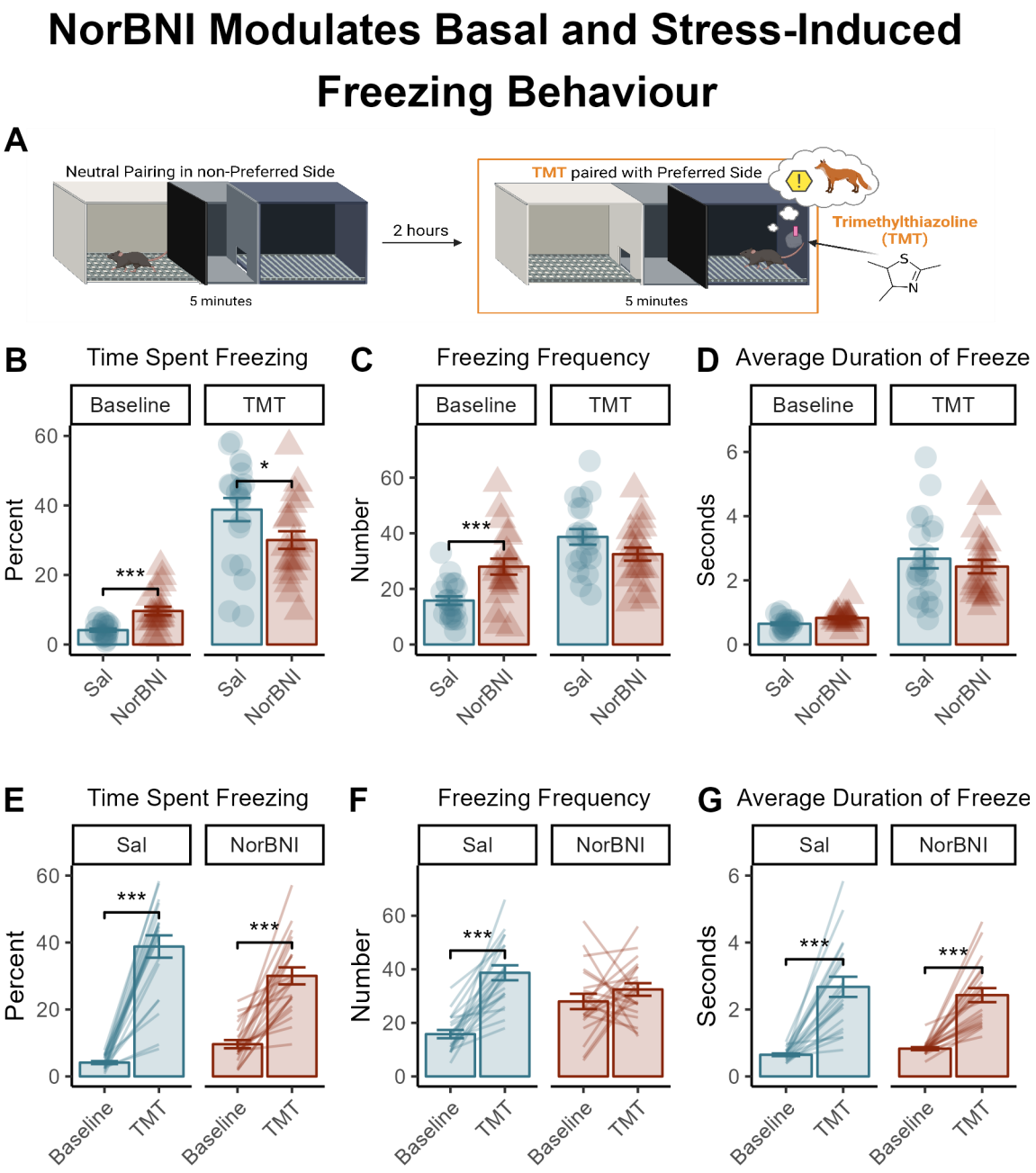


Figure 2.1: Kappa-opioid receptor antagonism modulates basal and stress-induced freezing. (A) CPP was conducted such that each mouse was exposed to TMT for 5-minutes on their preferred side of the behavioural testing apparatus. (B) NorBNI administration 24hours before testing increases basal freezing and decreases freezing during TMT presentation. (C) The number of freezing episodes was increased at Neutral by NorBNI administration. (D) NorBNI did not produce any changes in the average duration of freezing episode under basal conditions or during TMT exposure. All mice exhibit an increase in the % Freezing between the Neutral and TMT sessions (E), but only the saline group increased the number of freezing episodes between the two sessions (F). NorBNI administration did not affect the increase in average duration of freezing episode observed between the Neutral and TMT sessions (G). Data pressented as mean +/- SEM. * Indicates $p < 0.05$, *** indicates $p < 0.001$

spent more time freeing during TMT presentation than they had at Neutral (both $p < 0.001$), suggesting that both groups exhibited an innate defensive response during the single 5-minute TMT session.

```
a <- anova_test(data=train_data, dv=Frz_Tm, wid=ID, within = Task, between = Drug)
knitr::kable(get_anova_table(a))
```

Effect	DFn	DFd	F	p	p<.05	ges
Drug	1	39	0.522	0.474		0.007
Task	1	39	169.279	0.000	*	0.670
Drug:Task	1	39	11.353	0.002	*	0.120

```
b <- train_data %>%
  group_by(Drug)%>%
  pairwise_t_test(
  Frz_Tm ~ Task, paired = TRUE,
  p.adjust.method = "bonferroni"
  )

knitr::kable(b)
```

Drug	.y.	group1	group2	n1	n2	statistic	df	p	p.adj	p.adj.signif
Saline	Frz_Tm	Neutral	TMT	20	20	-10.722043	19	0.0000000	0.0000000	****
Nor-BNI	Frz_Tm	Neutral	TMT	21	21	-7.409239	20	0.0000004	0.0000004	****

```
c <- train_data %>%
  group_by(Task)%>%
  pairwise_t_test(
  Frz_Tm ~ Drug, paired = FALSE,
  p.adjust.method = "bonferroni"
  )

knitr::kable(c)
```

Task	.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
Neutral	Frz_Tm	Saline	Nor-BNI	20	21	0.000182	***	0.000182	***
TMT	Frz_Tm	Saline	Nor-BNI	20	21	0.042000	*	0.042000	*

We also computed ANOVA with the number of freezing episodes during each session entered as the dependent variable. This model also indicated a Drug * Timepoint interaction ($F(1,39) = 13.17$, $p < 0.001$). Saline-injected mice froze more often during the TMT session than the Neutral session ($p < 0.001$) whereas there was no difference in freezing frequency between the two sessions for NorBNI-injected mice ($p = 0.28$). Moreover, NorBNI-injected mice froze more often than did saline controls during the neutral exposure ($p < 0.001$), but there was no difference in freezing frequency between the groups during the TMT session ($p = 0.09$).

```
a <- anova_test(data=train_data, dv=Frz_Freq, wid=ID, within = Task, between = Drug)
get_anova_table(a)
```

```
## ANOVA Table (type III tests)
##
##      Effect DFn DFd      F          p p<.05   ges
## 1      Drug   1  39  1.602 0.21300000       0.019
## 2      Task   1  39 29.078 0.00000361     * 0.286
## 3 Drug:Task   1  39 13.170 0.00081500     * 0.153
```

```
b <- train_data %>%
  group_by(Drug)%>%
  pairwise_t_test(
  Frz_Freq ~ Task, paired = TRUE,
  p.adjust.method = "bonferroni"
  )

knitr::kable(b)
```

Drug	.y.	group1	group2	n1	n2	statistic	df	p	p.adj	p.adj
Saline	Frz_Freq	Neutral	TMT	20	20	-7.644742	19	0.0000003	0.0000003	****
Nor-BNI	Frz_Freq	Neutral	TMT	21	21	-1.105378	20	0.2820000	0.2820000	ns

```
c <- train_data %>%
  group_by(Task)%>%
  pairwise_t_test(
  Frz_Freq ~ Drug, paired = FALSE,
  p.adjust.method = "bonferroni"
  )

knitr::kable(c)
```

Task	.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
Neutral	Frz_Freq	Saline	Nor-BNI	20	21	0.00065	***	0.00065	***
TMT	Frz_Freq	Saline	Nor-BNI	20	21	0.09460	ns	0.09460	ns

ANOVA on the average duration of freezing episode indicated a significant main effect of timepoint ($F(1,39) = 103.45$, $p < 0.001$) that did not interact with drug treatment ($p = 0.24$). The average duration of freezing episode was increased by TMT.

```
a <- anova_test(data=train_data, dv=Av_Dur, wid=ID, within = Task, between = Drug)
get_anova_table(a)
```

```
## ANOVA Table (type III tests)
##
##      Effect DFn DFd       F                 p p<.05      ges
## 1      Drug   1  39   0.029 0.86600000000000       0.000396
```

```
## 2      Task   1  39 103.456 0.00000000000158     * 0.553000
## 3 Drug:Task   1  39   1.452 0.23500000000000       0.017000
```

```
b <- train_data %>%
  group_by(Drug)%>%
  pairwise_t_test(
  Av_Dur ~ Task, paired = TRUE,
  p.adjust.method = "bonferroni"
  )

knitr::kable(b)
```

Drug	.y.	group1	group2	n1	n2	statistic	df	p	p.adj	p.adj.signif
Saline	Av_Dur	Neutral	TMT	20	20	-6.971897	19	0.0000012	0.0000012	****
Nor-BNI	Av_Dur	Neutral	TMT	21	21	-7.589131	20	0.0000003	0.0000003	****

```
c <- train_data %>%
  group_by(Task)%>%
  pairwise_t_test(
  Av_Dur ~ Drug, paired = FALSE,
  p.adjust.method = "bonferroni"
  )

knitr::kable(c)
```

Task	.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
Neutral	Av_Dur	Saline	Nor-BNI	20	21	0.00381	**	0.00381	**
TMT	Av_Dur	Saline	Nor-BNI	20	21	0.50100	ns	0.50100	ns

Taken together, these findings indicate that NorBNI administration increases time spent freezing at Neutral by increasing the frequency of bouts of freezing. TMT exposure increases both the frequency and average duration of bouts of freezing among naive mice, and the magnitude of the change in freezing behaviour is reduced among NorBNI treated mice.

2.1.2 Regressions

```
##
## Call:
## lm(formula = Perc ~ Frz_Freq * Drug, data = BL_data)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -2.8267 -0.7702 -0.1686  0.3901  8.3884
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)           0.35976    1.03281   0.348 0.729567
```

```
## Frz_Freq              0.23889    0.06017   3.970 0.000318 ***
## DrugNor-BNI          -1.78723    1.40227  -1.275 0.210422
## Frz_Freq:DrugNor-BNI  0.15621    0.06761   2.311 0.026535 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.804 on 37 degrees of freedom
## Multiple R-squared:  0.8815, Adjusted R-squared:  0.8719
## F-statistic: 91.78 on 3 and 37 DF,  p-value: < 0.00000000000000022
```

```
##
## Call:
## lm(formula = Perc ~ Frz_Freq * Drug, data = b)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -2.8267 -0.7702 -0.1686  0.3901  8.3884
##
## Coefficients:
##                      Estimate Std. Error t value            Pr(>|t|)
## (Intercept)         -1.42747    0.94851  -1.505              0.1408
## Frz_Freq             0.39510    0.03082  12.820 0.00000000000000351 ***
## DrugSaline           1.78723    1.40227   1.275              0.2104
## Frz_Freq:DrugSaline -0.15621    0.06761  -2.311              0.0265 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.804 on 37 degrees of freedom
## Multiple R-squared:  0.8815, Adjusted R-squared:  0.8719
## F-statistic: 91.78 on 3 and 37 DF,  p-value: < 0.00000000000000022
```

A regression model with Percent freezing during the Neutral session entered as the dependent variable and freezing frequency entered as the independent variable accounted for 87% of the variability in freezing ($F(3,37) = 91.78$, Adjusted $R\hat{}2 = 0.87$, $p < 0.001$). Simple effects for this model indicated a significant main effect of Freezing Frequency ($t = 3.97$, $p < 0.001$) and a significant Frequency * Drug interaction ($t = 2.33$, $p = 0.026$). Evaluation of the simple slopes for each drug condition indicated that for saline-treated mice, a 10-unit increase in freezing frequency was associated with a 2.3% increase in total time freezing. Among NorBNI-treated mice, a 10-unit increase in freezing was associated with a 3.9% increase in total time freezing.

```
##
## Call:
## lm(formula = Perc ~ Frz_Freq * Drug, data = TMTs)
##
## Residuals:
```

```
##      Min       1Q   Median       3Q      Max
## -26.5697  -7.2100   0.5262   9.3678  23.8668
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)           26.5527     9.5304   2.786  0.00837 **
## Frz_Freq               0.3165     0.2349   1.347  0.18607
## DrugNor-BNI          -12.8500    13.1699  -0.976  0.33554
## Frz_Freq:DrugNor-BNI   0.1866     0.3551   0.525  0.60249
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 12.79 on 37 degrees of freedom
## Multiple R-squared:  0.2159, Adjusted R-squared:  0.1523
## F-statistic: 3.395 on 3 and 37 DF,  p-value: 0.02779
```

```
# Model 2: X=Av_Dur, y=Perc
a <- lm(Perc~Av_Dur * Drug, data=BL_data)
summary(a)
```

```
##
## Call:
## lm(formula = Perc ~ Av_Dur * Drug, data = BL_data)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -7.8633 -1.6178 -0.8294  1.2113 10.2455
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)         -0.1638     2.8444  -0.058   0.9544
## Av_Dur               6.6422     4.2461   1.564   0.1263
## DrugNor-BNI         -5.6693     4.2286  -1.341   0.1882
## Av_Dur:DrugNor-BNI  12.0048     5.6131   2.139   0.0391 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.292 on 37 degrees of freedom
## Multiple R-squared:  0.6058, Adjusted R-squared:  0.5738
## F-statistic: 18.95 on 3 and 37 DF,  p-value: 0.00000013
```

```
# Re-order the "drug" variable to get the intercept for the NorBNI group.
b <- BL_data
b$Drug <- as.character(b$Drug)
b$Drug <- factor(b$Drug,levels = c("Nor-BNI","Saline"))

# A regression model on Neutral Freezing
```

```r
a <- lm(Perc~Av_Dur * Drug, data=b)
summary(a)
```

```
##
## Call:
## lm(formula = Perc ~ Av_Dur * Drug, data = b)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -7.8633 -1.6178 -0.8294  1.2113 10.2455
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)         -5.833      3.129  -1.864   0.0702 .
## Av_Dur              18.647      3.671   5.079 0.000011 ***
## DrugSaline           5.669      4.229   1.341   0.1882
## Av_Dur:DrugSaline  -12.005      5.613  -2.139   0.0391 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.292 on 37 degrees of freedom
## Multiple R-squared:  0.6058, Adjusted R-squared:  0.5738
## F-statistic: 18.95 on 3 and 37 DF,  p-value: 0.00000013
```

Chapter 3

Conditioned Place Aversion to TMT

3.1 Statistical Analyses

We conducted an initial preference test to evaluate each mouse's preferred side of the CPP apparatus. Time spent in each of the 3 compartments was scored manually. We found that overall, mice preferred the black side of the apparatus over the white side (paired $t(40) = 8.53$, $p < 0.001$).

```
##
##  Paired t-test
##
## data:  BL_black_time and BL_white_time
## t = 8.5259, df = 40, p-value = 0.0000000001553
## alternative hypothesis: true mean difference is not equal to 0
## 95 percent confidence interval:
##  48.93262 79.33958
## sample estimates:
## mean difference
##         64.1361
```

Indeed, 38 / 41 mice tested in these experiments spent more time on the black side of the apparatus than the white side during the baseline test. Each mouse was exposed to TMT on the side that it exhibited a preference for during the baseline test (i.e. 38 of 41 mice were exposed to TMT on the black side of the apparatus; see figure XXX).

but did not exhibit a change in time spent in the neutral (non-preferred) compartment ($p = 0.88$).

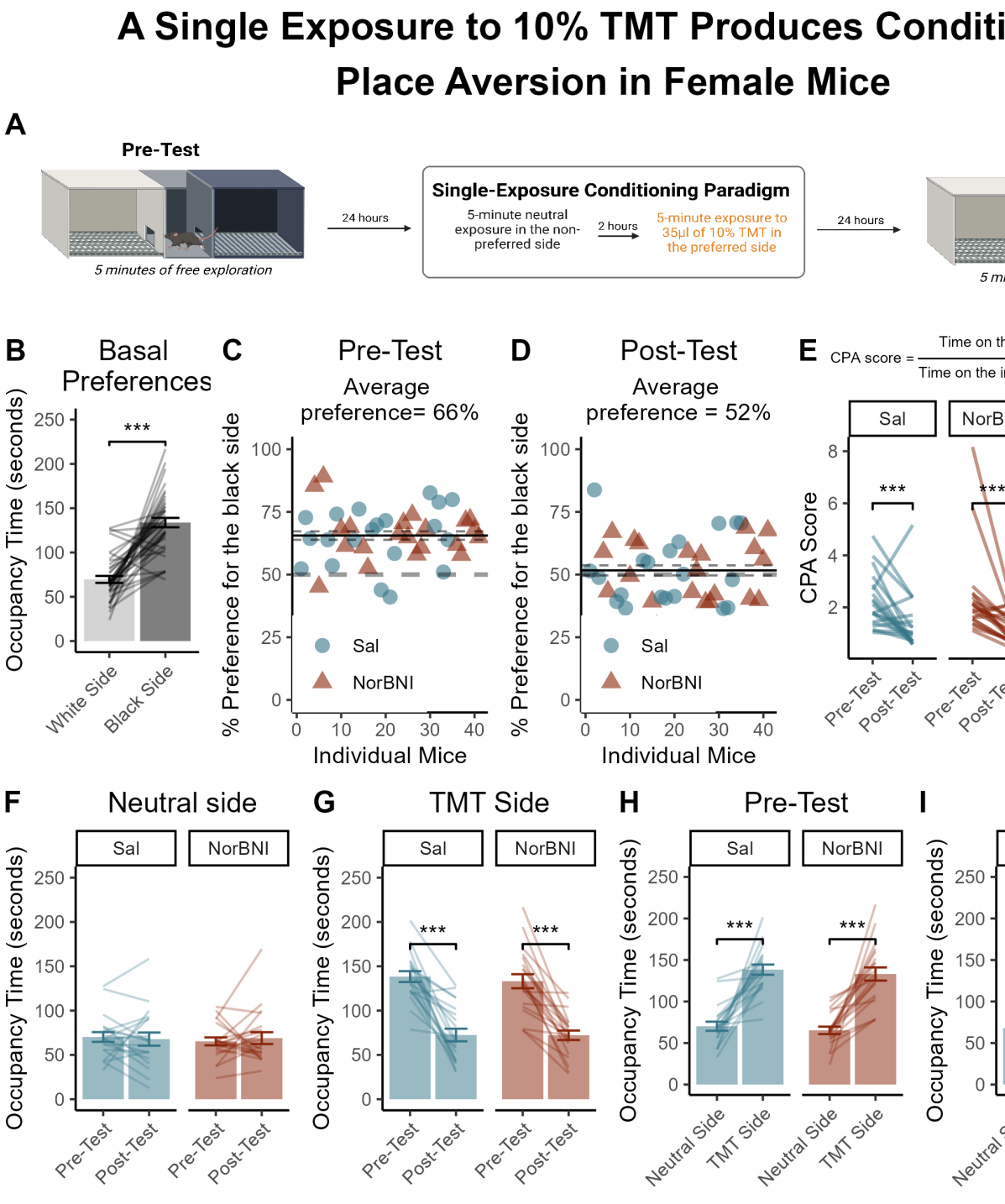


Figure 3.1: Timeline of experimental proceedings for Conditioned Place Aversion (A). The Conditioning apparatus used in these epxeriments was biased, such that mice exhibit an overall preference for the black compartment over the white compartment under basal conditions (B,C). The preference for the black compartment was reduced after the training session with TMT (D). Mice exhibited conditioned place aversion between the pre-test and the post-test (E) Which was not altered by NorBNI (F). Between the pre-test and the post-test, mice reduced time spent on the TMT-paried side (G) but did not exhibit a change in time spent on the netutral side (H). The strong side preferences that

```
##
##  Paired t-test
##
## data:  Baseline_tm_NON_PREF and Post_tm_NON_PREF
## t = -0.15062, df = 40, p-value = 0.881
## alternative hypothesis: true mean difference is not equal to 0
## 95 percent confidence interval:
##  -10.352845   8.916748
## sample estimates:
## mean difference
##      -0.7180488
```

To investigate chagnes in occupancy time between the Pre-test and the Post-test, computed a 3-way mixed model ANOVA with Task (pre-test or post-test) and Side (preferred or non-preferred) entered as the within-subjects variables, and Drug condition (Sailne or NorBNI) entered as a between-groups factor. The model indicated a significant Task * Side interaction ($F(1,39) = 76.52$, $p < 0.001$).

Effect	DFn	DFd	F	p	p<.05	ges
Drug	1	39	0.214	0.646		0.0020000
Task	1	39	59.793	0.000	*	0.2350000
Side	1	39	48.177	0.000	*	0.2870000
Drug:Task	1	39	0.461	0.501		0.0020000
Drug:Side	1	39	0.007	0.936		0.0000544
Task:Side	1	39	76.520	0.000	*	0.2420000
Drug:Task:Side	1	39	0.006	0.939		0.0000250

Follow-up analyses of the significant interaction indicated that mice exhibited robust preferences during the baseline session ($t(41) = 10.27$, $p < 0.001$) and that the preferences were not present during the post-test session ($p = 0.51$). Moreover, Mice decreased the amount of time spent on the initially prefrred side of the apparatus between the pre-test and the post-test ($t(41) = 10.57$, $p < 0.001$) but there was no change in occupancy time on the non-preferred side between the two sessions ($p = 0.88$). The 3-way interaction between Drug, Task and Side was non-significant ($p = 0.94$), indicating that kappa opioid antagonism did not alter the induction or the expression of conditioned place aversion to TMT in female mice.

Task	.y.	group1	group2	n1	n2	statistic	df	p	p.adj	p.adj.signif
Post	value	Preferred	Non-Preferred	41	41	0.6629701	40	0.511	0.511	ns
Pre	value	Preferred	Non-Preferred	41	41	10.2746859	40	0.000	0.000	****

Side	.y.	group1	group2	n1	n2	statistic	df	p	p.adj	p.adj.signif
Preferred	value	Post	Pre	41	41	-10.5721883	40	0.000	0.000	****
Non-Preferred	value	Post	Pre	41	41	0.1506239	40	0.881	0.881	ns

We also computed "CPA Scores" which represent the *ratio* of time on the pre-

ferred side to the non-preferred side:

$$CPA\ score = \frac{time\ on\ the\ preferred\ side}{time\ on\ the\ non-preferred\ side}$$

We found a significant effect of timepoint on CPA scores such that the ratio decreased between the pre-test and the post-test ($F(1,39) = 22.67$, $p < 0.001$)

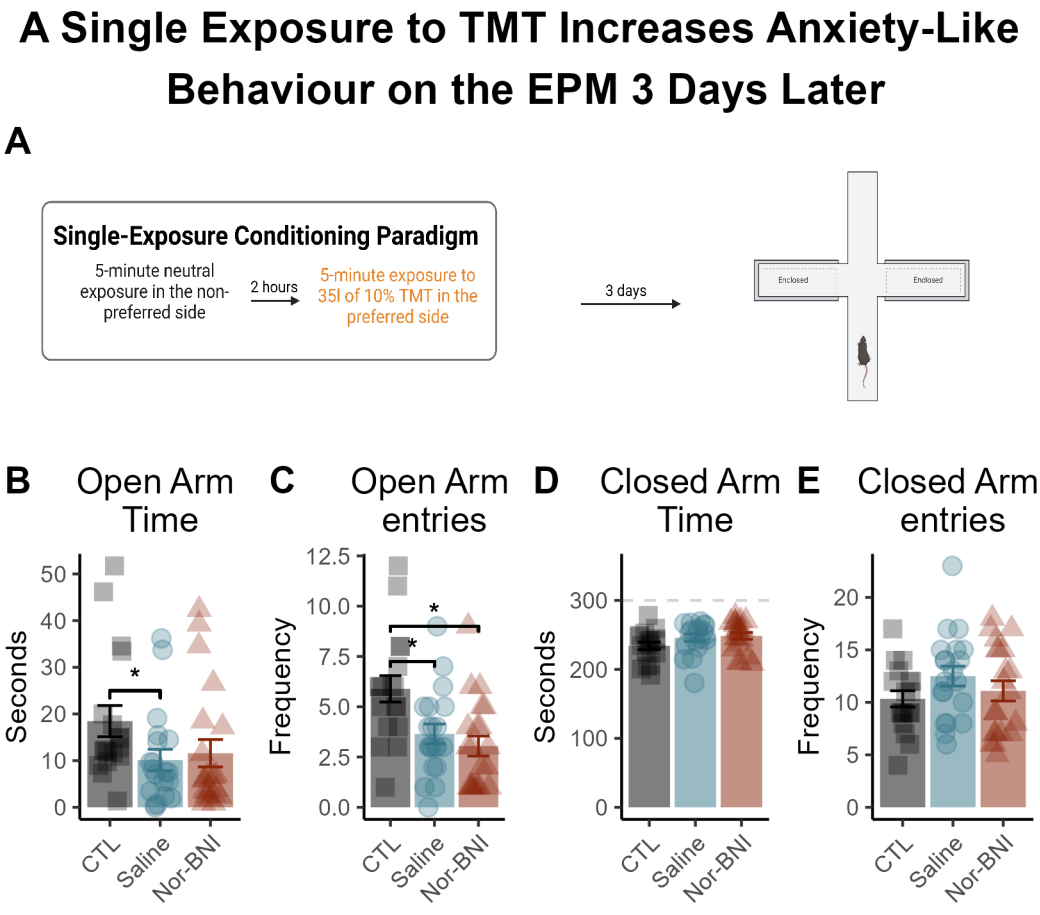
Effect	DFn	DFd	F	p	p<.05	ges
Drug	1	39	0.007	0.9330000		0.000115
variable	1	39	22.666	0.0000266	*	0.178000
Drug:variable	1	39	0.498	0.4850000		0.005000

The magnitude of the change in CPA score was not different for NorBNI-treated mice ($p = 0.48$).

```
##
##  Welch Two Sample t-test
##
## data:  CPA_change_score by Drug
## t = 0.70831, df = 38.579, p-value = 0.483
## alternative hypothesis: true difference in means between group Sal and group NorBNI
## 95 percent confidence interval:
##  -0.5736782  1.1916416
## sample estimates:
##    mean in group Sal mean in group NorBNI
##           -0.8879249           -1.1969066
```

Chapter 4

Elevated Plus Maze



4.1 Statistical Analyses

One-way ANOVA on time spent in the open arm indicated a significant effect of group ($F(1,57) = 4.78$, $p = 0.03$).

```
##
##  One-way analysis of means
##
## data:  Open_Tm and TMT
## F = 4.7778, num df = 1, denom df = 57, p-value = 0.03295
```

.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
Open_Tm	CTL	Saline	18	20	0.0428	*	0.128	ns
Open_Tm	CTL	Nor-BNI	18	21	0.0889	ns	0.267	ns
Open_Tm	Saline	Nor-BNI	20	21	0.7080	ns	1.000	ns

One-way ANOVA on time the number of entries into the open arm indicated a significant effect of group ($F(1,57) = 14.78$, $p < 0.001$)

```
##
##  One-way analysis of means
##
## data:  Open_Freq and TMT
## F = 14.782, num df = 1, denom df = 57, p-value = 0.0003064
```

```
##
##  One-way analysis of means
##
## data:  Open_Tm and TMT
## F = 4.7778, num df = 1, denom df = 57, p-value = 0.03295
```

.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
Open_Freq	CTL	Saline	18	20	0.004870	**	0.01460	*
Open_Freq	CTL	Nor-BNI	18	21	0.000403	***	0.00121	**
Open_Freq	Saline	Nor-BNI	20	21	0.415000	ns	1.00000	ns

Follow-up pairwise comparisons indicated that CTL mice made more entries into the open arms than TMT+Saline ($p = 0.005$) or TMT+NorBNI ($p < 0.001$) mice, and that there was no effect of NorBNI treatment among TMT-exposed mice ($p = 0.41$)

There were no differences in time spent in the closed arms or number of enteries into the closed arms.

```
##
##  One-way analysis of means
##
## data:  Closed_Tm and TMT
## F = 1.2163, num df = 1, denom df = 57, p-value = 0.2747
```

```
##
##  One-way analysis of means
##
## data:  Open_Tm and TMT
## F = 4.7778, num df = 1, denom df = 57, p-value = 0.03295
```

.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
Closed_Tm	CTL	Saline	18	20	0.677	ns	1.000	ns
Closed_Tm	CTL	Nor-BNI	18	21	0.141	ns	0.422	ns
Closed_Tm	Saline	Nor-BNI	20	21	0.276	ns	0.827	ns

```
##
##  One-way analysis of means
##
## data:  Closed_Freq and TMT
## F = 1.691, num df = 1, denom df = 57, p-value = 0.1987
```

```
##
##  One-way analysis of means
##
## data:  Open_Tm and TMT
## F = 4.7778, num df = 1, denom df = 57, p-value = 0.03295
```

.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
Closed_Freq	CTL	Saline	18	20	0.0949	ns	0.285	ns
Closed_Freq	CTL	Nor-BNI	18	21	0.5480	ns	1.000	ns
Closed_Freq	Saline	Nor-BNI	20	21	0.2570	ns	0.771	ns

```
##
##  One-way analysis of means
##
## data:  exp_index and TMT
## F = 11.698, num df = 1, denom df = 57, p-value = 0.001163
```

```
##
##  One-way analysis of means
##
## data:  Open_Tm and TMT
## F = 4.7778, num df = 1, denom df = 57, p-value = 0.03295
```

.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
exp_index	CTL	Saline	18	20	0.00474	**	0.0142	*
exp_index	CTL	Nor-BNI	18	21	0.00411	**	0.0123	*
exp_index	Saline	Nor-BNI	20	21	0.98600	ns	1.0000	ns

Chapter 5

Basal Freezing Long After TMT

5.1 Statistical Analyses

5.1.1 Time Spent Freezing

We computed a 2x2 ANOVA with side (Neutral or TMT-paired) as the within subjects factor and Drug condition (Saline or NorBNI) as the between groups factor. This model indicated a significant side * Drug interaction ($F(1,38) = 5.69$, $p = 0.02$)

Effect	DFn	DFd	F	p	p<.05	ges
Drug	1	38	11.071	0.002	*	0.195
variable	1	38	2.542	0.119		0.011
Drug:variable	1	38	5.693	0.022	*	0.025

Follow-up comparisons of the significant interaction indicated that the difference in time spent freezing between Saline and Nor-BNI treated mice was not significant on the neutral size ($p = 0.059$), but Nor-BNI injected mice spent significantly more time freezing in the the TMT-paired compartment ($p < 0.001$).

variable	.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
Neutral side	value	Saline	Nor-BNI	19	21	0.05780	ns	0.05780	ns
TMT side	value	Saline	Nor-BNI	19	21	0.00015	***	0.00015	***

Saline-treated mice exhibited a decrease in time spent freezing between the first and the second session of the long after test ($p = 0.003$) whereas TMT-treated mice did not decrease freezing between the two sessions ($p = 0.62$).

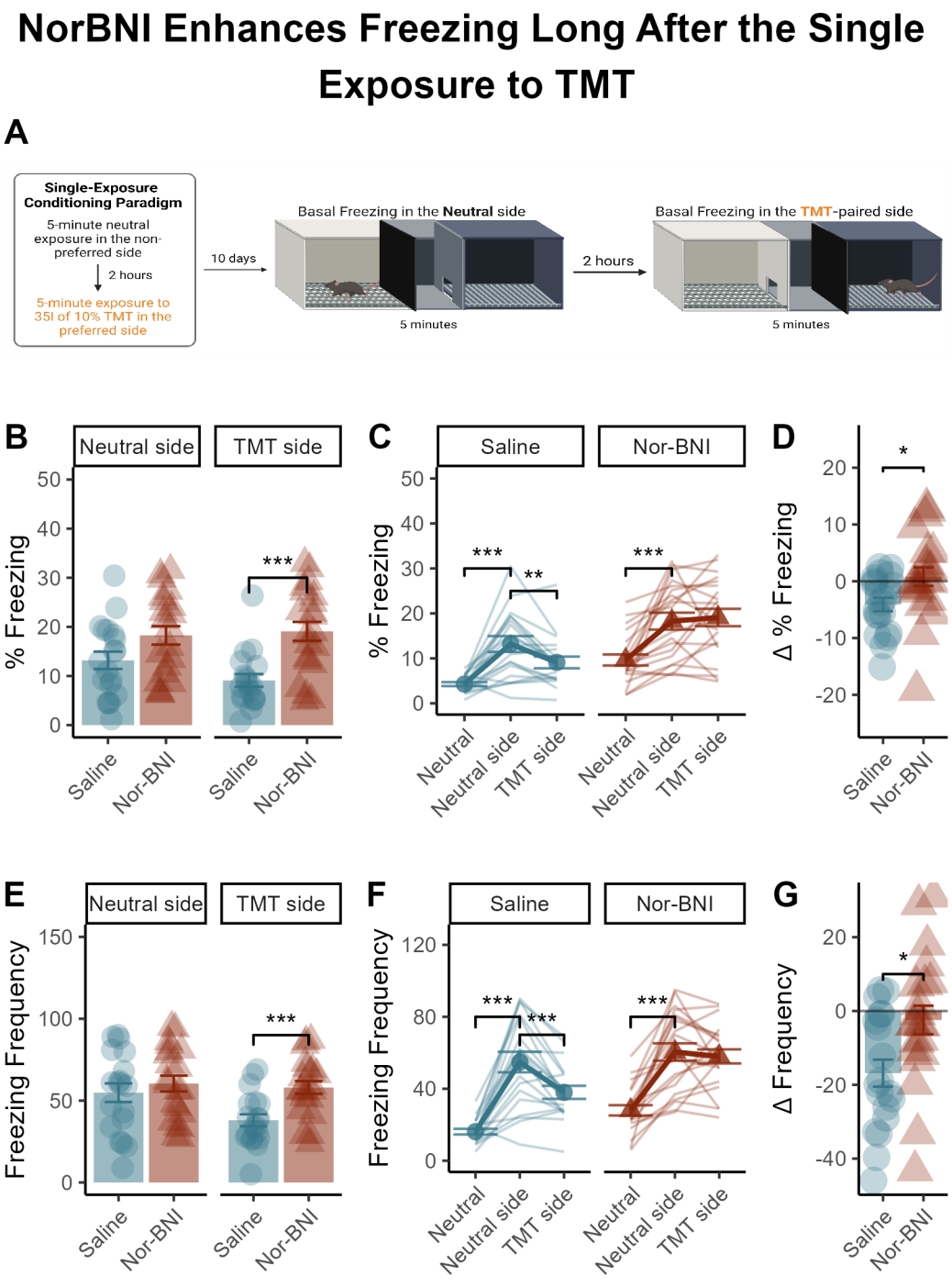


Figure 5.1: Timeline of experimental proceedings: 10 days after the single 5-minute exposure to 10% TMT, mice were placed in each side of the conditioning apparatus for 5 minutes and basal levels of freezing were measured (A). Nor-BNI injected mice froze more than saline mice on the TMT-paired side, but not on the neutral side (B). Both drug conditions exhibited a robust increase in freezing relative to their baseline data from 10 days earlier (C). Saline mice decreased freezing between the two sessions whereas NorBNI-treated mice did not (C). The change in freezing behaviour between the two sessions was significantly reduced by NorBNI administration (D). The frequency of freezing was significantly higher for NorBNI injected mice on the TMT-paried side, but not

Drug	.y.	group1	group2	n1	n2	statistic	df	p	p.adj	p.adj.signif
Saline	value	Neutral side	TMT side	19	19	3.4548388	18	0.003	0.003	**
Nor-BNI	value	Neutral side	TMT side	21	21	-0.4979122	20	0.624	0.624	ns

Additionally, the change in freezing behaviour between the two sessions was significantly greater for Saline-treated mice than for NorBNI ($t(38) = 2.43$, $p = 0.01$)

```
##
##  Welch Two Sample t-test
##
## data:  change by Drug
## t = -2.4301, df = 35.605, p-value = 0.02027
## alternative hypothesis: true difference in means between group Saline and group Nor-BNI is not
## 95 percent confidence interval:
##  -27.018872  -2.430953
## sample estimates:
##  mean in group Saline mean in group Nor-BNI
##            -12.281579              2.443333
```

5.1.2 Freezing Frequency

We also computed a 2x2 ANOVA on freezing frequency, which also indicated a Drug*Timepoint interaction ($F(1,38) = 7.26$, $p = 0.01$).

Effect	DFn	DFd	F	p	p<.05	ges
Drug	1	38	4.708	0.036000	*	0.093
variable	1	38	12.972	0.000902	*	0.055
Drug:variable	1	38	7.257	0.010000	*	0.032

Follow-up comparisons of the significant interaction indicated that there was no effect of Drug treatment on the frequency of freezing on the neutral size ($p = 0.45$), but Nor-BNI injected mice spent significantly more time freezing than saline-treated mice in the the TMT-paired compartment ($p < 0.001$).

variable	.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
Neutral side	value	Saline	Nor-BNI	19	21	0.45600	ns	0.45600	ns
TMT side	value	Saline	Nor-BNI	19	21	0.00063	***	0.00063	***

Saline-treated mice exhibited a decrease in the frequency of freezing between the first and the second session of the long after test ($p = <0.001$) whereas TMT-treated mice did not decrease freezing between the two sessions ($p = 0.53$).

Drug	.y.	group1	group2	n1	n2	statistic	df	p	p.adj	p.adj.signif
Saline	value	Neutral side	TMT side	19	19	4.6050714	18	0.00022	0.00022	***
Nor-BNI	value	Neutral side	TMT side	21	21	0.6281951	20	0.53700	0.53700	ns

Additionally, the change in freezing behaviour between the two sessions was

significantly greater for Saline-treated mice than for NorBNI (t(38) = 2.71, p = 0.01)

```
##
##  Welch Two Sample t-test
##
## data:  change by Drug
## t = -2.7084, df = 38, p-value = 0.01008
## alternative hypothesis: true difference in means between group Saline and group Nor
## 95 percent confidence interval:
##  -25.186925  -3.640142
## sample estimates:
##  mean in group Saline mean in group Nor-BNI
##            -16.842105             -2.428571
```

5.1.3 Average Duration of Freeze

Repeated measures ANOVA on the average duration of freezing episode indicated a main effect of drug only (F (1,38) = 15.71, p < 0.001).

Effect	DFn	DFd	F	p	p<.05	ges
Drug	1	38	15.713	0.000314	*	0.252
variable	1	38	1.561	0.219000		0.007
Drug:variable	1	38	1.716	0.198000		0.008

Nor-BNI treated mice exhibited a significantly higher average duration of freezing episode than did saline-injected mice on both the neutral (p = 0.001) and the TMT-paired (p < 0.001) sides of the CPA apparatus.

variable	.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
Neutral side	value	Saline	Nor-BNI	19	21	0.00107	**	0.00107	**
TMT side	value	Saline	Nor-BNI	19	21	0.00074	***	0.00074	***

The within-subjects change in the average duration of freezing episode between the two sessions was not significant for either NorBNI-injected (p = 0.13) or saline-injected (p = 0.96) mice.

Drug	.y.	group1	group2	n1	n2	statistic	df	p	p.adj	p.adj.signif
Saline	value	Neutral side	TMT side	19	19	0.055716	18	0.956	0.956	ns
Nor-BNI	value	Neutral side	TMT side	21	21	-1.572287	20	0.132	0.132	ns

5.1.4 Regressions

To investigate how each the frequency and the average duration of freezing contribute to the total amount of freezing during the session, we computed regression models with either Frequency or Average duration entered as the predictor and % Freezing entered as the dependent variable.

```r
# A regression model
a <- lm(Perc~Frz_Freq * Drug + Task, data=TwoWeeksFrz_data)
summary(a)
```

```
##
## Call:
## lm(formula = Perc ~ Frz_Freq * Drug + Task, data = TwoWeeksFrz_data)
##
## Residuals:
##    Min     1Q Median     3Q    Max
## -5.871 -2.318 -0.202  1.202 11.596
##
## Coefficients:
##                       Estimate Std. Error t value            Pr(>|t|)
## (Intercept)           -3.55899    1.40642  -2.531              0.0135 *
## Frz_Freq               0.30168    0.02485  12.141 <0.0000000000000002 ***
## DrugNor-BNI           -1.82331    2.02100  -0.902              0.3698
## TaskTMT side           1.39949    0.75210   1.861              0.0667 .
## Frz_Freq:DrugNor-BNI   0.09288    0.03535   2.628              0.0104 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.24 on 75 degrees of freedom
## Multiple R-squared:  0.8685, Adjusted R-squared:  0.8615
## F-statistic: 123.9 on 4 and 75 DF,  p-value: < 0.00000000000000022
```

```r
# Re-order the "drug" variable to get the intercept for the NorBNI group.
b <- TwoWeeksFrz_data
b$Drug <- as.character(b$Drug)
b$Drug <- factor(b$Drug,levels = c("Nor-BNI","Saline"))

# A different version of the same regression model
a <- lm(Perc~Frz_Freq * Drug + Task, data=b)
summary(a)
```

```
##
## Call:
## lm(formula = Perc ~ Frz_Freq * Drug + Task, data = b)
##
## Residuals:
##    Min     1Q Median     3Q    Max
## -5.871 -2.318 -0.202  1.202 11.596
##
## Coefficients:
##                     Estimate Std. Error t value            Pr(>|t|)
## (Intercept)         -5.38230    1.64750  -3.267             0.00164 **
```

```
## Frz_Freq             0.39456    0.02545  15.506 < 0.0000000000000002 ***
## DrugSaline           1.82331    2.02100   0.902              0.36985
## TaskTMT side         1.39949    0.75210   1.861              0.06669 .
## Frz_Freq:DrugSaline -0.09288    0.03535  -2.628              0.01042 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.24 on 75 degrees of freedom
## Multiple R-squared:  0.8685, Adjusted R-squared:  0.8615
## F-statistic: 123.9 on 4 and 75 DF,  p-value: < 0.00000000000000022
```

The first model accounted for 86% of the variability in time spent freezing ($F(4,75) = 123.9$, Adjusted R^2 $= 0.86$, $p < 0.001$), and indicated that Freezing frequency was a strong independent predictor of total time freezing ($t(76) = 12.14$, $p < 0.001$). The model also indicated a significant Frequency * Drug interaction ($t(76) = 2.63$, $p = 0.01$). We evaluated the simple effects to further contextualize the significant interaction. Among saline-treated mice, a 10-unit increase in freezing frequency was associated with a 3% increase in total time spent freezing whereas for NorBNI-injected mice, a 10-unit increase in frequency was associated with a 3.9% increase in freezing among Nor-BNI treated mice. In other words, there was a 30% increase in the estimated magnitude of the relationship between the frequency of freezing and total time spent freezing for mice injected with NorBNI.

```
# Model 2: X=Av_Dur, y=Perc
a <- lm(Perc~Av_Dur * Drug + Task, data=TwoWeeksFrz_data)
summary(a)
```

```
##
## Call:
## lm(formula = Perc ~ Av_Dur * Drug + Task, data = TwoWeeksFrz_data)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -11.1228  -3.0015  -0.0514   3.2502   8.8207
##
## Coefficients:
##                    Estimate Std. Error t value       Pr(>|t|)
## (Intercept)         -10.528      3.295  -3.195        0.00205 **
## Av_Dur               33.903      4.666   7.266 0.000000000293 ***
## DrugNor-BNI           2.655      4.384   0.606        0.54667
## TaskTMT side         -2.629      1.044  -2.518        0.01393 *
## Av_Dur:DrugNor-BNI   -3.244      5.623  -0.577        0.56572
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.64 on 75 degrees of freedom
```

```
## Multiple R-squared:  0.7304, Adjusted R-squared:  0.716
## F-statistic: 50.79 on 4 and 75 DF,  p-value: < 0.00000000000000022
```

The second model accounted for 70% of the variability in time spent freezing (Adjusted R^2 = 0.70). The simple effects found that although average duration of freezing episode was a strong independent predictor of freezing time ($t(76) = 7.03$, $p < 0.001$), there was no interaction between this predictor and drug treatment ($p = 0.56$). A 0.1-second increase in the average duration of freezing episode was associated with a 3% increase in time freezing.