

Sex Differences in Pain Sensitization Book of Data

Contents

About

This bookdown project contains R code and statistical outputs, and code to generate analyses for the paper **Inflammatory injury induces pain sensitization that is expressed beyond the site of injury in male (and not in female) mice**.

- Raw data and code to generate the figure panels are available on our github.
- Code to generate the figures and statistical analyses was written by Jennet Baumbach.
- Any questions about these data should be directed to the corresponding author: Loren Martin, Ph.D lj.martin@utoronto.ca

Figure 1 - Homecage Behaviors after CFA in male mice

Published Image

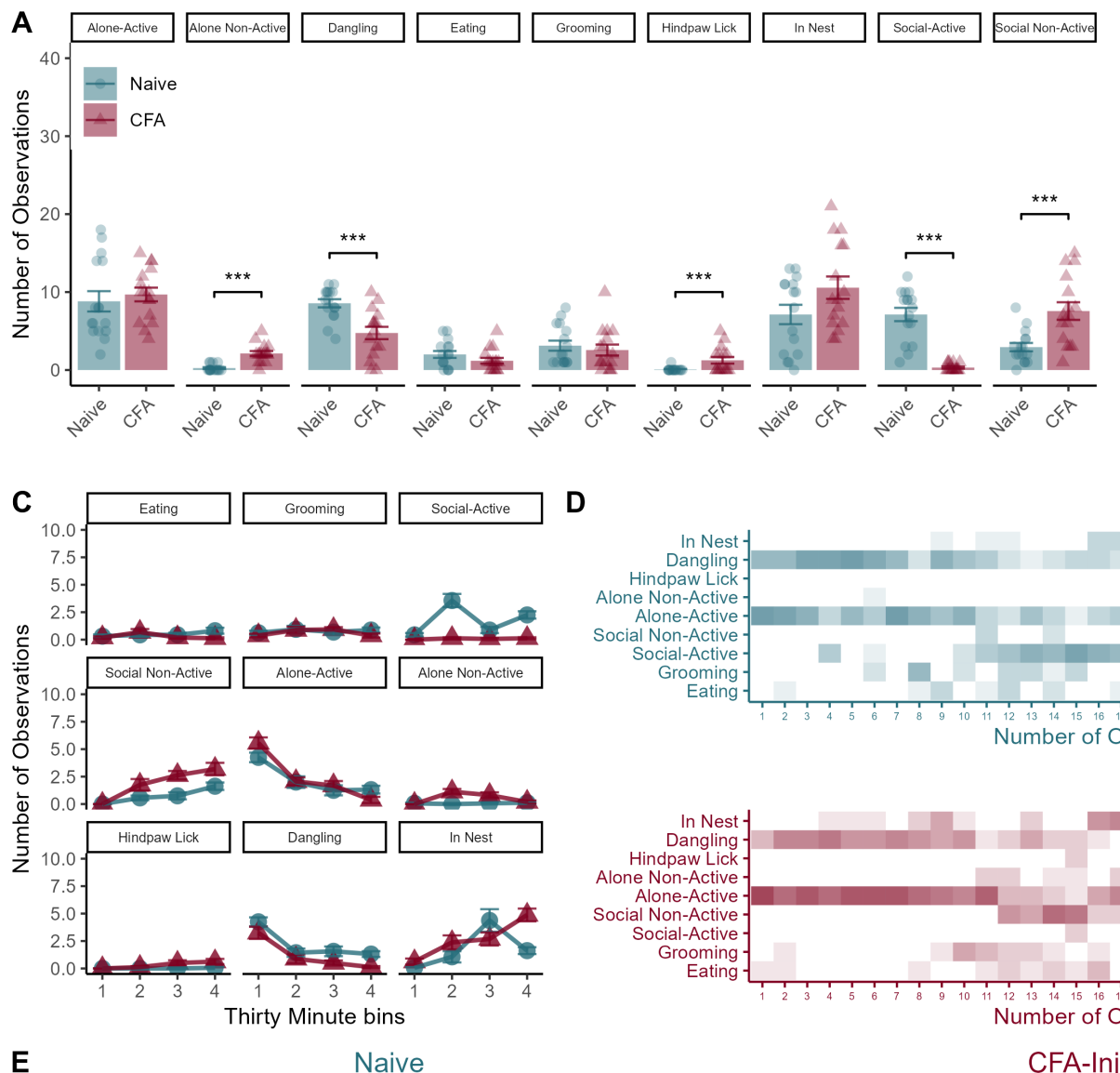


Figure 1. *Homecage behaviors in male mice after an injection of 10 μ l of 50% CFA.* (A) Total number of observations of each behavior category across the two-hour observation period. (B) Donut charts showing the breakdown of average time spent engaging in each behavior for each group. (C) Line charts showcase group differences in changes in behavior across the two-hour long session. (D and E) are qualitative representations of the distribution of behaviors observed across the 40 time points. Data represented as mean value \pm SEM. *** indicates $p < 0.001$.

Statistical Analyses

Overall MANOVA for HC Behavs for males

```
# All behaviours in the model throws an error - it knows that you need to leave one out I suppose
# It is important to leave one behavior out of the MANOVA to allow for a degree of freedom in the
## I thought originally that I would leave time in the nest out, but bc there is a clear sex diff

fit <- manova(cbind(Grooming, `Social-Active`, `Social Non-Active`, `Alone-Active`, `Alone Non-Active`),
summary(fit)

##              Df Pillai approx F num Df den Df              Pr(>F)
## Condition    1  0.536   17.183      8   119 < 0.00000000000000022 ***
## Residuals 126
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- The overall MANOVA for males was significant ($F(1,30) = 43.46$, $p < 0.001$), indicating that 10 μ l of 50% CFA altered patterns of behaviour during the two-hour interval after injection.

Follow up analyses

```
# Prints out the individual ANOVAs for each behaviour
summary.aov(fit)

## Response Grooming :
##              Df Sum Sq Mean Sq F value Pr(>F)
## Condition    1  0.633  0.63281   0.7691 0.3822
```

```

## Residuals    126 103.672 0.82279
##
## Response Social-Active :
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Condition    1  92.82  92.820    50.51 0.00000000007788 ***
## Residuals   126 231.55   1.838
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Response Social Non-Active :
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Condition    1  42.78  42.781    15.729 0.0001219 ***
## Residuals   126 342.72   2.720
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Response Alone-Active :
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Condition    1   1.53   1.5313    0.2959 0.5874
## Residuals   126 651.97   5.1744
##
## Response Alone Non-Active :
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Condition    1   7.031   7.0312    17.83 0.00004588 ***
## Residuals   126 49.688   0.3943
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Response Hindpaw Lick :
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Condition    1  2.820  2.82031    10.231 0.001747 **
## Residuals   126 34.734  0.27567
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Response Dangling :
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Condition    1  29.07  29.0703    8.3725 0.00449 **
## Residuals   126 437.48   3.4721
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Response In Nest :
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Condition    1  23.63  23.6328    3.332 0.07031 .
## Residuals   126 893.67   7.0926

```

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

- Male mice that were injected with CFA exhibited fewer socially-active behaviours ($F(1,30) = 66.62$, $p < 0.001$),
- More socially inactive behaviours ($F(1,30) = 14.55$, $p < 0.001$),
- More hindpaw licks ($F(1,30) = 8.07$, $p = 0.008$),
- And less time dangling ($F(1,30) = 17.19$, $p < 0.001$).

Note that the non-statistically significant results shown above are not reported in the manuscript.

Figure 2 - Female Mice: Homecage Behaviors after CFA

Published Image

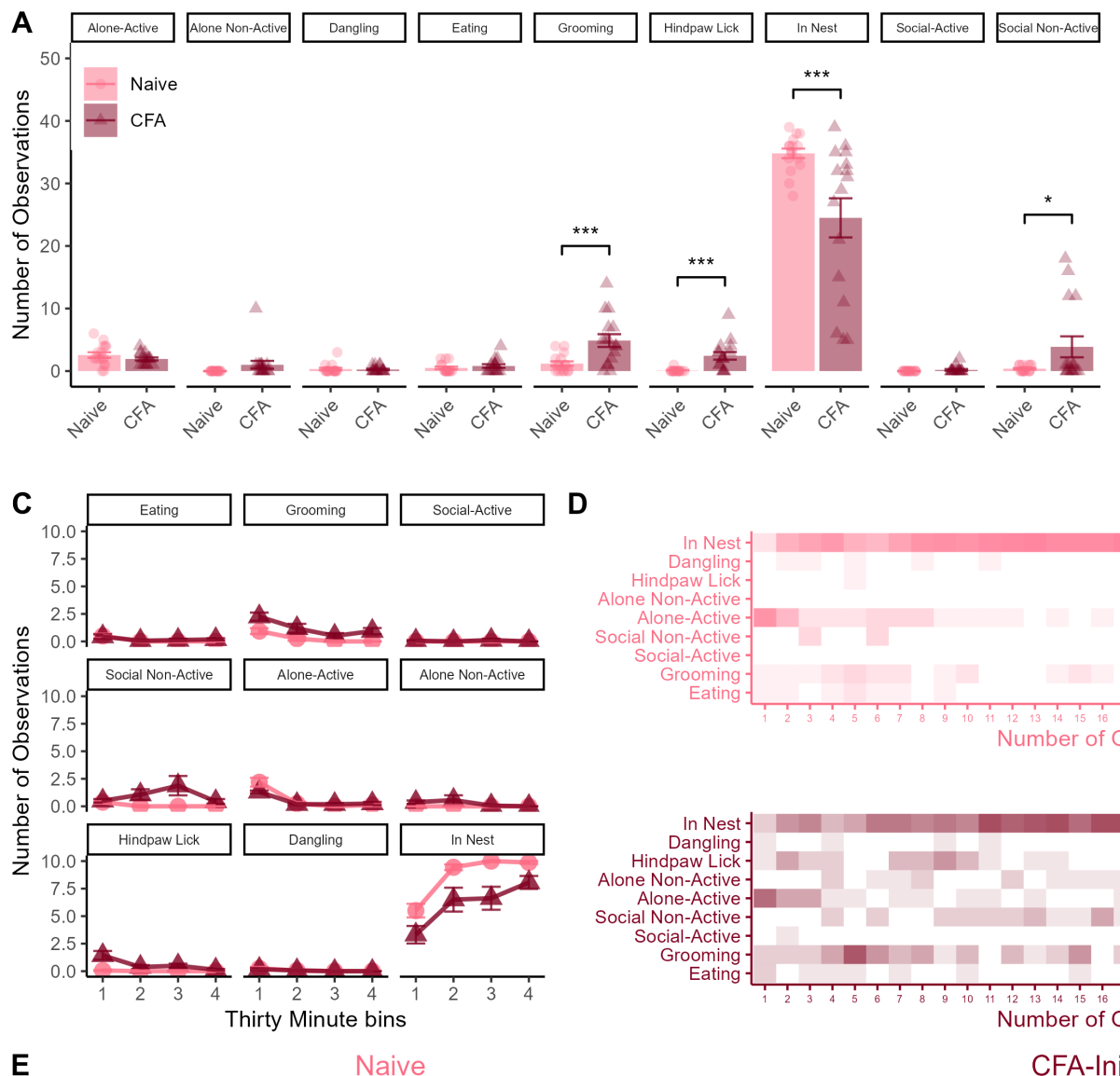


Figure 2. *Homecage behaviors in female mice after injection of 10 μ l of 50% CFA.* (A) Total number of observations of each behavior category across the two-hour observation period. (B) Donut charts showing the breakdown of average time spent engaging in each behavior for each group. (C) Line charts showcase group differences in changes in behavior across the two-hour long session. (D and E) are qualitative representations of the distribution of behaviors observed across the 40 time points. Data represented as mean value \pm SEM. * * * indicates $p < 0.001$.

Statistical Analyses

0.0.1 Overall MANOVA for HC Behavs for females

```
# All behaviours in the model throws an error - it knows that you need to leave one out I suppose
## I thought originally that I would leave time in the nest out, but bc there is a clear sex diff

fit <- manova(cbind(Grooming, `Social-Active`, `Social Non-Active`, `Alone-Active`, `Alone Non-Active`),
summary(fit)
```

```
##              Df Pillai approx F num Df den Df      Pr(>F)
## Condition    1 0.2647    5.355      8    119 0.000009378 ***
## Residuals 126
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- The overall MANOVA for female mice was also significant ($F(1,30) = 3.05$, $p = 0.017$)

0.0.2 Follow up:

```
# Prints out the individual ANOVAs for each behaviour
summary.aov(fit)
```

```
## Response Grooming :
##              Df Sum Sq Mean Sq F value      Pr(>F)
## Condition    1  27.195  27.1953   20.121 0.00001617 ***
## Residuals  126 170.297   1.3516
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```

## Response Social-Active :
##           Df Sum Sq Mean Sq F value Pr(>F)
## Condition  1 0.0703 0.070313  1.8232 0.1794
## Residuals 126 4.8594 0.038566
##
## Response Social Non-Active :
##           Df Sum Sq Mean Sq F value Pr(>F)
## Condition  1 24.50 24.5000 10.742 0.001354 **
## Residuals 126 287.38  2.2808
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Response Alone-Active :
##           Df Sum Sq Mean Sq F value Pr(>F)
## Condition  1  0.781 0.78125  0.7768 0.3798
## Residuals 126 126.719 1.00570
##
## Response Alone Non-Active :
##           Df Sum Sq Mean Sq F value Pr(>F)
## Condition  1      2 2.00000      4.5 0.03585 *
## Residuals 126      56 0.44444
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Response Hindpaw Lick :
##           Df Sum Sq Mean Sq F value Pr(>F)
## Condition  1 11.281 11.2813 19.682 0.00001971 ***
## Residuals 126 72.219  0.5732
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Response Dangling :
##           Df Sum Sq Mean Sq F value Pr(>F)
## Condition  1  0.0078 0.007813  0.095 0.7584
## Residuals 126 10.3594 0.082217
##
## Response In Nest :
##           Df Sum Sq Mean Sq F value Pr(>F)
## Condition  1 212.7 212.695 20.546 0.00001336 ***
## Residuals 126 1304.4 10.352
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

CFA-injected Female mice exhibited:

- Increased grooming during the observation session ($F(1,30) = 12.26$, $p =$

0.0015)

- Increased social inactive behaviour ($F(1,30) = 4.626$, $p = 0.039$)
- More hindpaw licks ($F(1,30) = 15.95$, $p < 0.001$)
- And less observations in the nest ($F(1,30) = 10.93$, $p = 0.002$)

```
knitr::opts_chunk$set(message = FALSE,  
                        warning = FALSE,  
                        echo = FALSE,  
                        fig.align = 'center')  
options(scipen = 999)
```


Figure 3. *CFA injection produces mechanical hypersensitivity that resolves within 7 days in male and female mice.* (A) Timeline of experimental testing. (B) Pain model to induce sensitization. (C) Representative images of Chaplan up-down von Frey measurements after CFA injection. CFA administration produces robust hypersensitivity at the site of injection that persists for at least 3 days and resolves within one week in both male (D, E) and female (H, I) mice. There were no changes in sensitivity of the contralateral (non-injected; right) hind paw during inflammatory pain and recovery from CFA injury in either males (F, G) or females (J, K). Data expressed as mean \pm SEM. *** Indicates between-group difference where $p < 0.001$ and # indicates a within-subject difference from baseline where $p < 0.05$.

Statistical Analyses

```
# Select the left paws
left_paws <- rbind(female_left,male_left)

# Switch to long form
a <- left_paws %>%
  melt(id.vars=c("ID","Sex","CFA"))

# Run RM anova on the 4 days of VF measuremenets
b <- anova_test(data=a, dv=value,wid=ID,between=c(CFA,Sex),within=variable,effect.size="pes")
knitr::kable(get_anova_table(b))
```

Effect	DFn	DFd	F	p	p<.05	pes
CFA	1	60	128.271	0.000	*	0.681
Sex	1	60	1.211	0.275		0.020
variable	3	180	99.726	0.000	*	0.624
CFA:Sex	1	60	1.314	0.256		0.021
CFA:variable	3	180	91.678	0.000	*	0.604
Sex:variable	3	180	2.651	0.050		0.042
CFA:Sex:variable	3	180	3.570	0.015	*	0.056

- Significant main effects of CFA and timepoint.
- Significant interaction between CFA and timepoint ($F(3,180) = 91.67$, $p < 0.001$)
- Significant 3-way interaction between Sex, CFA and timepoint ($F(3,180) = 3.57$, $p = 0.015$)