Chronic Mild Stress and Deficits in the Rodent Brain: A Preliminary Examination of Neuroinflammation-Induced Cognitive and Behavioral Changes in Rats

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Neurocircuitry of Decison-Making

Introduction/Hypotheses

- **This study was conducted to examine the relationship between stress, neuroinflammation, cognition and behavior.**
- **Past research (Farooq et al., 2012; Jia et al., 2017) has shown that neuroinflammation, induced by the CMS model, induces structural changes in rodent neurochemistry.**
- **These changes may be important to behaviors/cognitions linked with suicidality in humans, such as rumination depression, cognitive rigidity, and anhedonia.**
- **We hypothesized that rats in the CMS condition would fare worse on a battery of tasks when compared with healthy control rats.**

Methods

Subjects: Male and female Sprague Dawley rats weighing 350-400g and 200-250g respectively (3-8 months of age) were used in this study. We utilized two cohorts of rodents, one cohort (COHORT ONE) consisting of 16 rats housed in dyad pairs, and one cohort (COHORT TWO) consisting of 8 rats single-housed two months later.

CMS: Rats were exposed to CMS protocols over six weeks. The four intermittent CMS were:

1. **STROBE – The rats were exposed to a strobe light set on a timer for their night cycle**
2. **WET BED – The rats were exposed to moist bedding for their night cycle**
3. **TILTED CAGES – The rats had their bedding reduced and had their cages tilted at a 30-degree angle during the day cycle**
4. **NOISE – The rats were exposed to a noise machine which was set on a timer during the night cycle.**

Apparatus and Training: Apparatus used was a plus maze with a block used on opposite stem arms (E/W) and a sugar pellet was placed in the high reward arm (N/S). The high reward arm was counterbalanced between rats. Training included an acclimation phase, followed by reward training to acquire a preference for the high reward, after acquisition (80% preference for high reward over 2 days).

Testing Procedure: Rats were run through a battery of tests designed to ascend from least-stressful to most-stressful. Sucrose preference was assessed over three days. The Forced Swim Task was conducted with two successive days of runs, the first lasting 15 minutes and the second lasting 5 minutes in a container 8 inches in diameter and 40 inches tall. Rats were then assessed for exploratory behavior in the Spon. Alt test. Finally, in Reversal Learning rats trained to prefer an arm associated with reward on 80% of trials (high reward) regardless of E/W start arm for 9 out of 10 successive trials OR 160 trials, whichever came first. The high-reward arm was counterbalanced, and the maze rotated 90 degrees every 5 trials. On the following day, the reward was reversed.

After CMS, Rats were run through a battery of tasks, which included maze trials

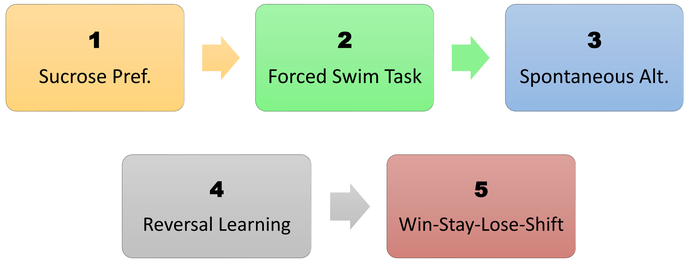

Figure 1: Battery order of tasks.

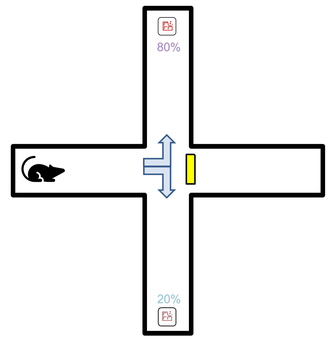

Figure 2: Rats were placed in a particular arm of the maze (East/West) for 3 of the 5 tasks. Typically, a sugar pellet would be placed in the high reward arm 80% of the time, and in the low reward arm 20% of the time. The order of the high reward arm was counterbalanced.

Battery Task Results by Stress Group and Cohort [Stressed v. Unstressed]

Sucrose Preference

We did not find a significant difference in sucrose preference for either cohort.

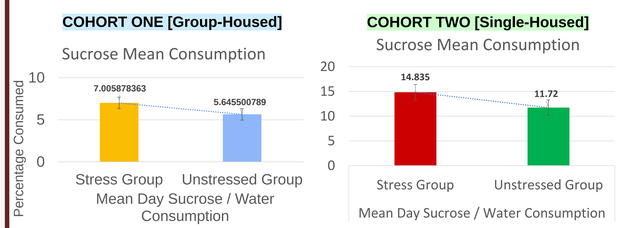

Figure 3: A T-Test could not find a significant difference in means, (t = 0.63, df = 14, p = .54)

Figure 4: A T-Test could not find a significant difference in means, (t =0.58, df = 3, p = .29)

Forced Swim Task

Though stressed rats consistently employed fewer behaviors overall in the FST, we did not find predictable significance in the FST between stress conditions.

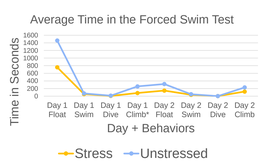

Figure 5: A significant difference emerged between stress groups on climbing behaviors in the first FST (t = -2.77, df = 11, p = .008). However, no other behaviors at either time point were significant (t = [-.86 – 1.12], df = [7 – 14], p = [.14 - .46]

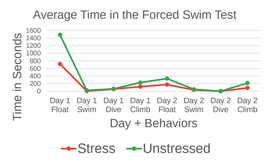

Figure 6: We did not find any significant differences on Forced Swim Task behaviors at either time point, (t = [-1.77 -1.71, df = [4 – 6], p = .07 – 36]

Spontaneous Alternation

We did not find a significant difference for either cohort on the Spontaneous Alternation task.

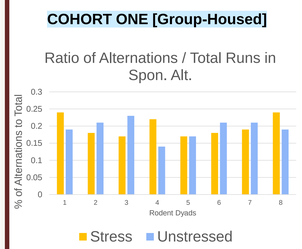

Figure 7: We did not find a significant difference between stressed and unstressed rats in the ratio of alternations to total runs.

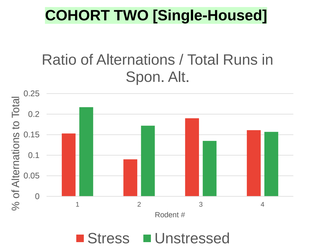

Figure 8: We did not find a significant difference between stressed and unstressed rats in the ratio of alternations to total runs, (t= -.81, df = 6, p = .22)

Win – Stay – Lose – Shift

Win-Stay-Incorrect was significant for both cohorts, indicative of cognitive rigidity. In addition, the socially isolated cohort was significantly higher in Lose-Stay-Incorrect, suggestive of possible negative insensitivity.

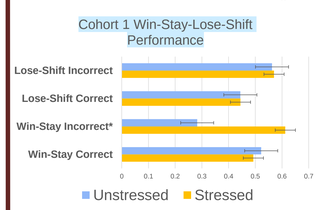

Figure 9: A significant difference emerged on WSI (t = 2.33, df = 13, p = .01). No significant differences were found on WSC (t = -1.01, df = 11, p = .26), LSI (t = .09, df = 14, p = .46) or LSC (t = .003, df = 14, p =.49)

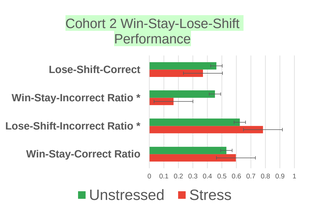

Figure 10: While there was no significant difference between WSC (t = 1.53, df = 6, p = .08) and the LSC (t = -0.48, df = 6, p = .32), a significant difference emerged on WSI (t = -2.09, df = 5, p = .04) and the LSI (t = 2.21, df = 7, p = .03).

Reversal Learning

We did not find significant difference in number of trials or time to acquisition or reversal by stress condition.

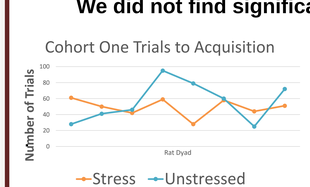

Figure 11: a T-Test did not detect a significant difference in the number of trials a rat took to acquisition, (t = -0.68, df = 10, p = .25). There was also not a significant difference in the Time to Acquisition, (t = .35, df = 12, p = .363)

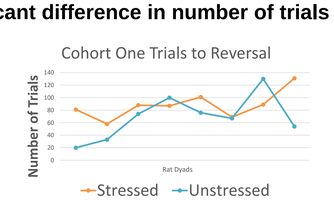

Figure 12: a T-Test did not detect a significant difference in the number of trials a rat took to reversal, (t = 1.28 df = 12, p = .11). There was also not a significant difference in the Time to Reversal, (t = -.06, df = 12, p = .47)

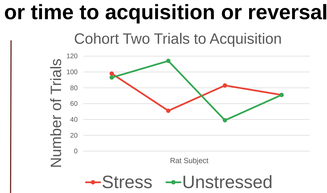

Figure 13: a T-Test did not detect a significant difference in the number of trials a rat took to acquisition, (t = -0.18, df = 5, p = .43). There was also not a significant difference in the Time to Acquisition, (t = .49, df = 6, p = .32)

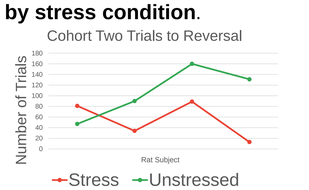

Figure 14: a T-Test did not detect a significant difference in the number of trials a rat took to reversal, (t = -1.71, df = 6, p = .068). There was also not a significant difference in the Time to Reversal, (t = -1.13, df = 5, p = .11)

Differences in Total Weight Gain

A significant & sex-linked difference did emerge in total weight gain through the CMS for both cohorts.

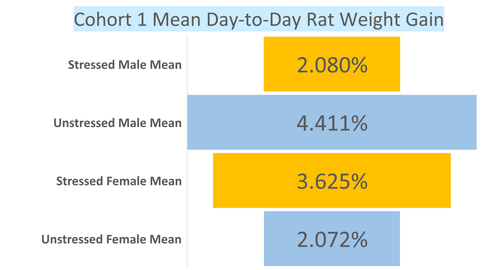

Figure 15 : An ANOVA detected a significant difference between Isolated and Stress Rats, (F(7, 80) = 286, p = <.001). Paired T-Tests found significant differences (all p's = <.003) between all rats except Rat 8 and Rat 9.

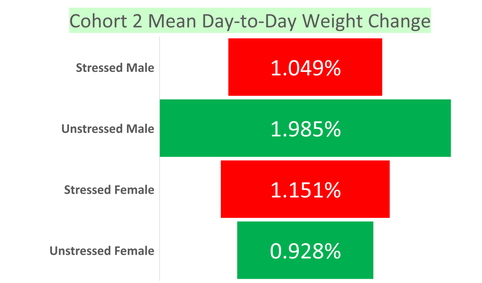

Figure 16: An ANOVA detected a significant difference between Isolated and Stress Rats, (F(15, 208) = 62.2, p = <.001). Females exhibited a greater difference (F(7, 104) = 3.1, p = .005) than males (F(7, 104) = 2.7, p = .01).

Summary and Discussion

- **By and large, the hypothesis was unsupported as rats in the CMS condition did not significantly differ in performance on many of the cognitive tasks.**
- **Significant differences did emerge in regard to sex-linked weight gain during the CMS. In addition, a difference emerged in climbing behaviors in the FST, and in errors in Win-Stay-Lose-Shift, particularly in the Win-Stay-Incorrect ratio, indicating the stress rats may have had a difficult time in shifting strategies after positive reinforcement.**
- **One possible explanation lies in the application of painful stimuli. It is possible that painful or provocative stimuli must be induced in order to detect cognitive changes in rodents. In addition, additional cohorts would be required to fully power all comparisons.**
- **We did also conduct a Resident Intruder task between the Sucrose Preference and Forced Swim tasks for the 2nd cohort, which will be analyzed through machine learning pose estimation using DeepLabCut and SimBA. In addition, we will be conducting a brain slice to assess the presence of microglia in the Lateral Habenula at a later date.**

These authors contributed equally to this work
References immediately available upon request, contact marksj2@spu.edu