ADOLESCENT STRESS INDUCES LONG-LASTING LOSS OF EXCITATORY AND INHIBITORY SYNAPSES FROM THE POSTERIOR PARIETAL CORTEX

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1. Abstract

In humans, adolescent stress is known to induce enduring effects in the posterior parietal cortex (PPC). Stress effects have also been shown to be sex specific. In experiments done in rodents, stress leads to a decrease in the number of synapses in several brain regions. Yet, the precise mechanistic details of these persistent stress effects have remained elusive. To better understand the long-lasting effects of adolescent stress, we quantified the density of excitatory and inhibitory synapses in the PPC of male mice directly after stress and following a month-long recovery period. We concurrently tested depression, anxiety and cognitive behaviors. We found that stress persistently reduced the number of excitatory synapses in the PPC. The number of inhibitory synapses were reduced directly after stress, however the after a month there was no longer a difference. Furthermore, we observed impaired y-maze performance at both time points, decreased neophobia, and the absence of a learned helplessness phenotype in stressed mice. These experiments are being done in female animals to see if these differences are observed in the opposite sex as well.

2. Introduction

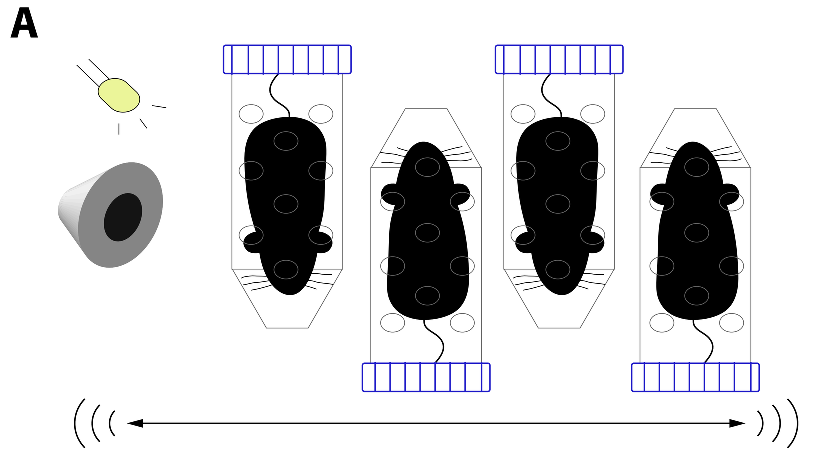
~Stress has a severe impact on human adolescent brain development, however, the mechanisms underlying these effects remain elusive (Gould et al. 2012)
~Stress effects males and females in different ways (Golfarb et al., 2019)
~Neonatal stress in rodents can have long-lasting behavioral effects, but there is little known about how persistent the imapct of adolescent stress exposure is (Romeo et al., 2003)
~It is still unknown whether the adverse effects of adolescent stress on the PPC's connectivity and function in mice persists into adulthood (Libovner et al., 2020)

Main Question: *Does adolescent stress cause a loss of excitatory/inhibitory synapses in the PPC that persists into early adulthood? What are the behavioral consequences?*

3. Methods- Stress Paradigm and Timeline

Figure 1. Overview of RMS paradigm and project timeline

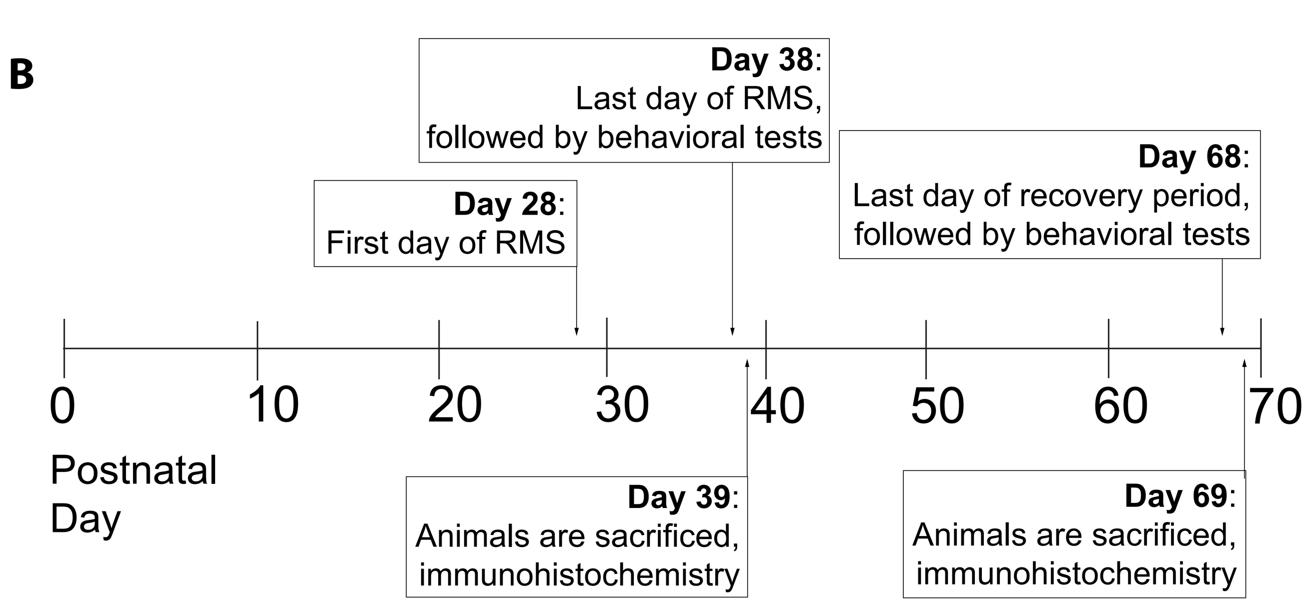
A



male C57BL/J mice

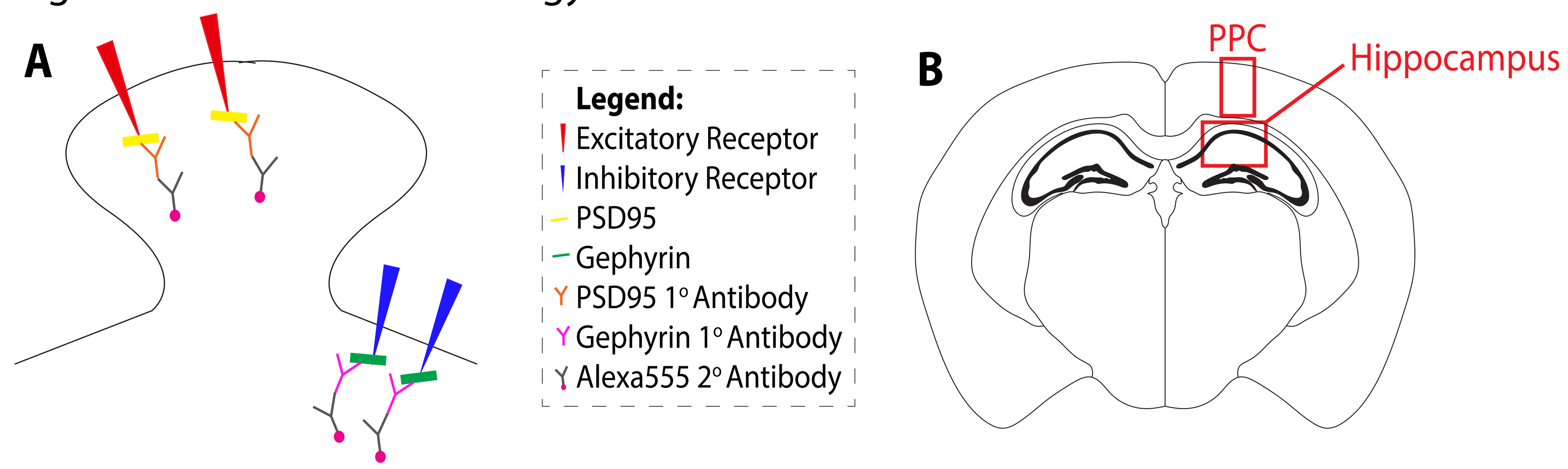
Repeated multi-concurrent stress paradigm (RMS) includes:
~light
~loud noise
~rocking back and forth
~restraint
~stressed in groups of 5-8

B



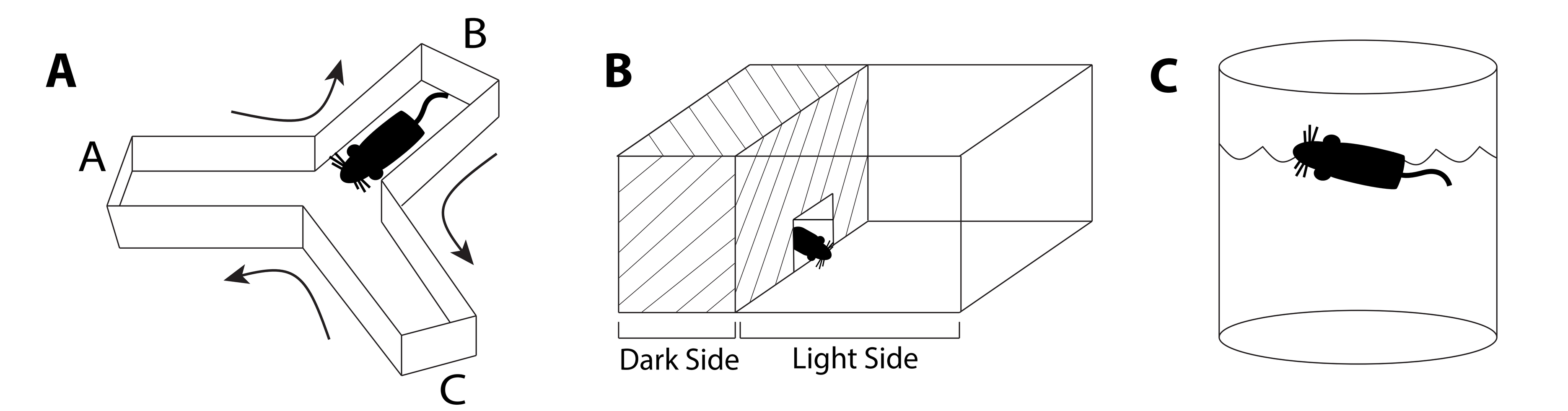
4. Methods- Histology and Behavioral Tests

Figure 2. Overview of Histology



A, Schematic of immunostaining. PSD95 was used to visualize excitatory synapses and Gephyrin was used to visualize inhibitory synapses. **B**, Drawing of a coronal brain slice. Synapse density was quantified from the PPC and hippocampus.

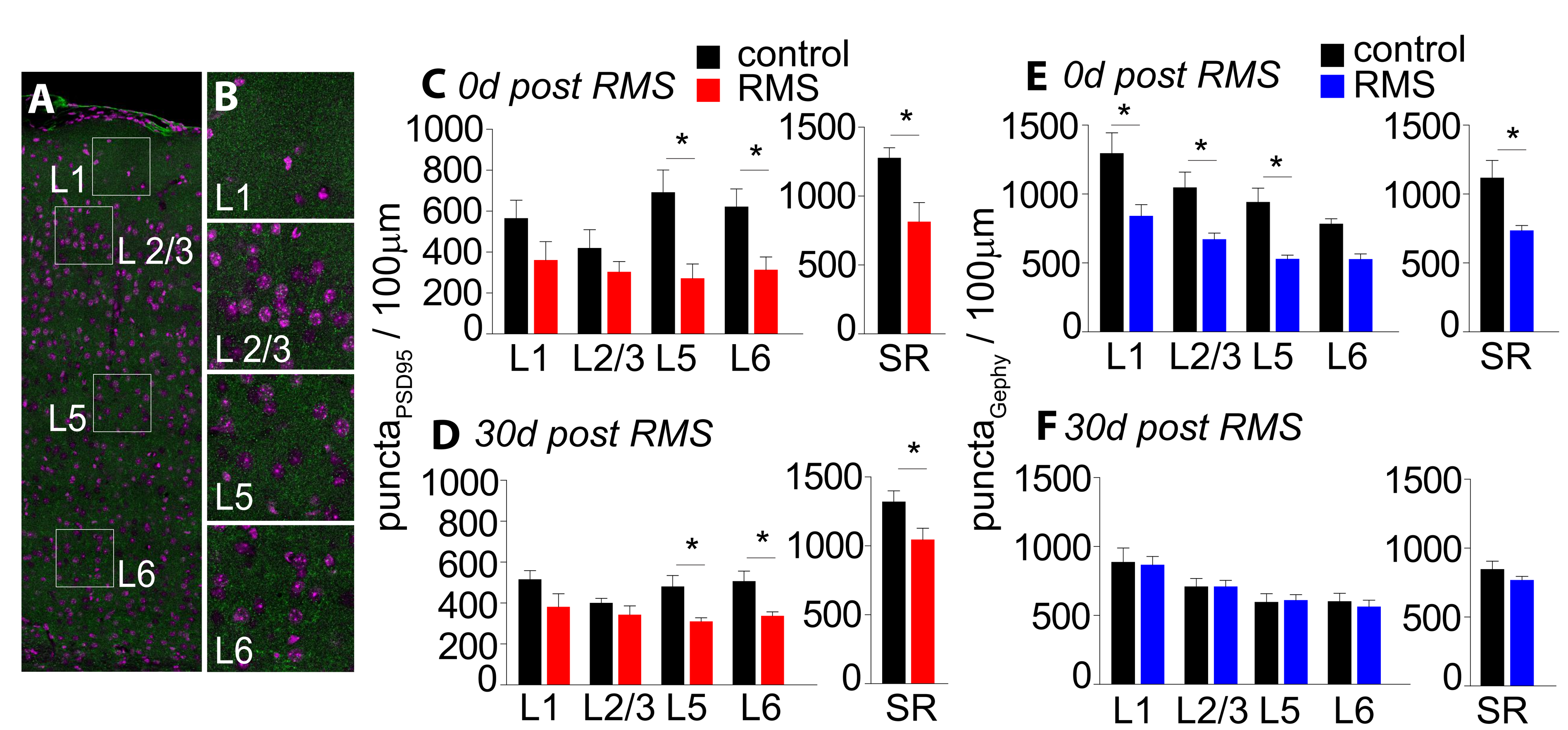
Figure 3. Overview of Behavioral Tests



A, Schematic of Y-maze test. Spontaneous alternations and arm entries were quantified for 8 minutes of exploration time. **B**, Visual representation of light/dark box test apparatus. Animals started in the dark side and were recorded for 5 minutes. **C**, Drawing of forced swim test. Mice were placed in a cylinder filled with water (23C+/-1) so that their tails could not reach the bottom and were recorded for 6 minutes. The behavior from the last 4 minutes was quantified.

5. RMS Causes a Persistent Loss of Excitatory Synapses and Short Term Loss of Inhibitory Synapses in the PPC

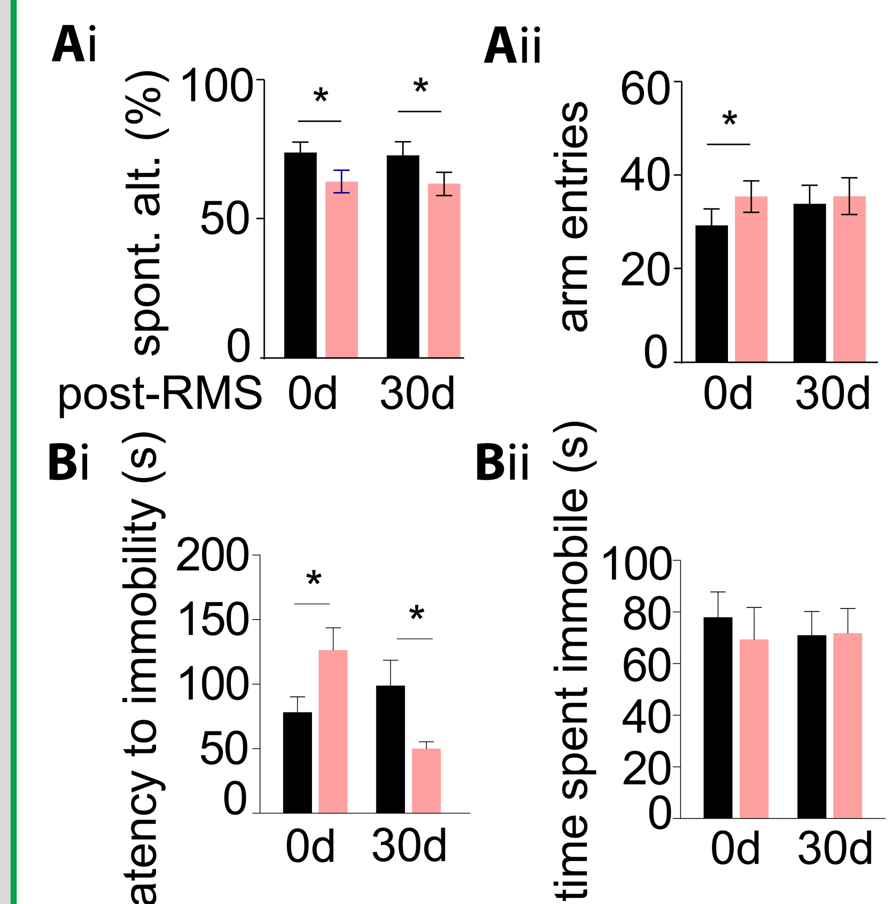
Figure 4. RMS causes excitatory and inhibitory synapse loss in the PPC



A, Example image of PSD-95 (green) and DAPI (magenta)-stained PPC section. **B**, Representative crops for layers 1, 2/3, 5, and 6 of the PPC. PSD-95 puncta densities quantified directly after (**C**), and 30 days after (**D**), 10 days of RMS. Gephyrin puncta densities quantified directly after (**E**), and 30 days after (**F**), 10 days of RMS. Bar graphs represent the mean (+/- SEM). *p<0.05, Student's t-test for hippocampal data, one-way ANOVA with Bonferroni's multiple comparison test for PPC data.

6. RMS effects PPC Linked Behavior, but Doesn't Induce Depression or Anxiety-like Phenotypes

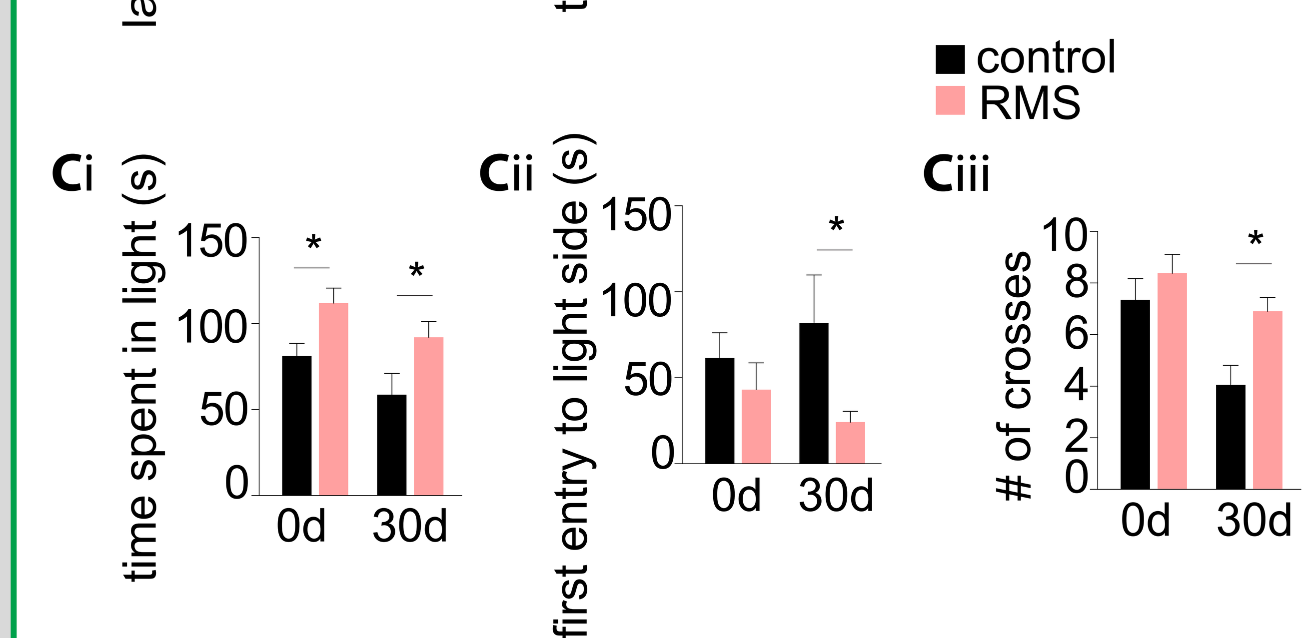
Figure 5. RMS causes specific behavioral deficits



A, **Y-maze data**. **Ai**, Spontaneous alternation rate. **Aii**, Number of arm entries.

B, **Forced swim test data. Bi**, Latency to immobility in seconds. **Bii**, Time spent immobile in seconds.

C, **Light/dark box test data**. **Ci**, Time spent in light in seconds. **Cii**, Time in seconds until the first entry to the light side. **Ciii**, Number of crosses. 0d and 30d signify data collected 0 days and 30 days after RMS. Bar graphs indicate the mean (+/-SEM). *p<0.05, Student's t-test.



7. Conclusions

Histology:
~RMS causes a loss of excitatory synapses in the PPC directly after stress that persists until early adulthood
~RMS induces an immediate loss of inhibitory synapses from the PPC that recovers during the 30-day rest period, which is possibly due to pruning of inhibitory synapses during adolescence in controls

Behavior:
~Y-maze data showed that PPC related spatial navigation behavior remained impaired during both time points
~Anxiety was not observed from light/dark box and learned helplessness was not observed from forced swim test

Next Steps:
~Investigating whether these effects are sex specific

8. References

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