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Does Chronic Stress Impact Your Brain Activity Levels? A Study into the Effect of Psychosocial Stress on Entorhinal Cortex-hippocampal Circuitry

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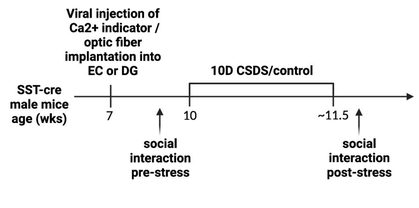
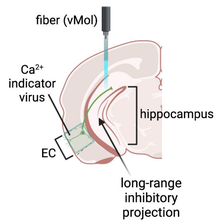
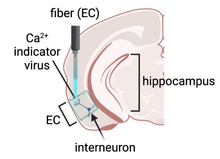
QUESTION

- **Does chronic stress alter entorhinal cortex (EC)-DG long range inhibitory projection neuronal activity (firing frequency and amplitude) during social interaction?**
- **Does chronic stress alter EC interneuron activity during social interaction?**

APPROACH







SST-cre brain

RESULTS

During interaction with conspecific "bully" mice, chronic stress:

- **decreases firing frequency of EC-DG SST+ long-range inhibitory projections**
- **increases firing frequency and amplitude of EC SST+ interneurons**

IMPLICATION

- By detecting chronic stress-induced **abnormal EC-DG circuit and cell types**, our study may enable the development of diagnostic and treatment strategies for MDD patients

ABSTRACT

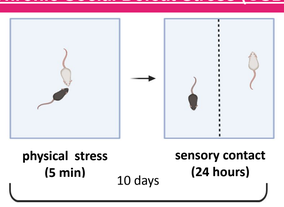
Depression affects millions of people worldwide. Over half relapse and a third are drug-resistant with current antidepressant treatment. Because of this, we need to more accurately target the brain for treatments. It has been found that patients with major depressive disorder (MDD) have decreased brain activity in their hippocampus[4]. MDD is marked by hippocampal dysfunction and current chronic antidepressants ameliorates hippocampal dysfunction. However, direct targeting of hippocampal regions does not show beneficial effects in mood regulation. Here, we look to target cortical brain regions such as the entorhinal cortex since it is easily accessible and preferable to targeting a deeper brain region[2]. Recently we published that stimulation of the excitatory input from the entorhinal cortex to the dentate gyrus (DG) ameliorates depressive-like behavior. However not much is known about the entorhinal cortex inhibitory input's role in chronic stress. Thus, this brought the question of the impact of chronic stress on EC local interneuron activity and EC long range inhibitory neurons that project onto the dentate gyrus. Since stress is a big factor for depression, we study the effects of stress on certain parts of the brain to get a better understanding of what happens when one is depressed. Our preliminary study[5] shows that stimulating the somatostatin (SST+) inhibitory projections from the entorhinal cortex to the DG after chronic stress leads to a decrease in social avoidance caused by stress. Other research has found that depressed patients have hypoactivity in their temporal cortex[1]. Thus, we hypothesize that chronic stress reduces entorhinal cortex interneuron activity, especially EC-DG SST+ long-range projecting neurons. We used fiber photometry to record in vivo SST+ cell activity of the EC and DG inhibitory projections before and after chronic stress by injecting a AAV9-CAG-flex-GCaMP6f virus into the Ent and an optic fiber implant into the EC or DG respectively. When the mice were at 7 weeks old, the optic fiber was implanted and the Ca2+ indicating virus was injected into the entorhinal cortex. At 9 weeks, we performed a pre-stress locomotion test. At 10 weeks, we started chronic social defeat stress (CSDS) for a period of 10 days. When the mice were 12 weeks old and CSDS was over, we performed an elevated plus maze test, and followed it up with another post-stress locomotion test. Results showed that during interaction with conspecific "bully" mice, chronic stress decreases firing frequency of EC-DG SSST+ LRIP and increases the firing frequency and amplitude of EC SST + interneurons. By detecting chronic stress-induced abnormal EC-DG circuit and cell types, our study may enable the development of diagnostic and treatment strategies for MDD patients.

Abnormal brain circuit in MDD: Hippocampus-related circuit

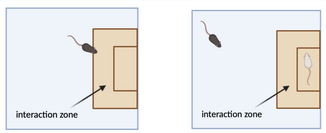
- **Direct target to hippocampus is not effective for MDD treatment[4]**
- **EC provides direct inputs to the hippocampus[2]**
- **EC volume is decreased in treatment-resistant MDD[3]**

Mouse model of chronic stress to induce depressive-like behaviors

Chronic Social Defeat Stress (CSDS):

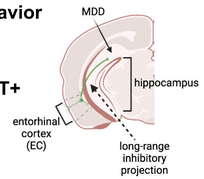


Social Interaction (SI) Test: Stress-induced social avoidance



EC long-range inhibitory projection (LRIP):

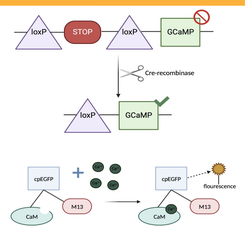
- **Inhibition of somatostatin (SST+) EC-DG LRIP induces depressive-like behavior (unpublished data)**
- **Does chronic stress decrease EC-DG SST+ LRIP activity?**



EC interneurons:

- **Hypoactivity of GABAergic interneurons in the frontal and temporal cortex in MDD[1]**
- **Does chronic stress decrease SST+ EC interneurons activity?**

GCAMP:Ca2+ sensor



HYPOTHESIS

Chronic stress decreases activity of EC-DG SST+ LRIP cells and EC local interneurons during social avoidance test.

Chronic stress decreases firing frequency of EC-DG SST+ LRIP during social interaction with conspecific "bully" mice.

A

B

Social interaction test with CD-1

C

D

E

Cohen's d = 1.14, p-value = 0.112

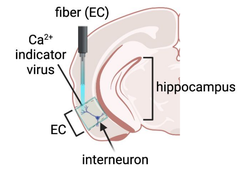
F

Cohen's d = 0.849, p-value = 0.401

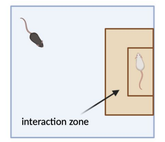
A. Schematics show locations of fiber placement and Ca2+ indicator viral infusion in the DG. **B.** Fiber photometry was performed during the social interaction test w/o and with CD-1. The mice underwent this test before and after being exposed to chronic stress. The stressor was placed in the interaction zone shown in red and data was collected from the mice depending on where they were in the chamber and proximity to the interaction zone. **C-D.** Representative SST+ EC LRIP firing tracing images of fluorescence changes (dF/F) derived from Ca2+ sensor in control mice (**C**) and CSDS mice (**D**) when they were in the presence of the aggressor (CD1). **E-F.** The Cohen's d between Control and CSDS is shown in the Gardner-Altman estimation plot. Both groups are plotted on the left axes; the mean difference is plotted on a floating axes on the right as a bootstrap sampling distribution. The mean difference is depicted as a dot; the 95% confidence interval is indicated by the ends of the vertical error bar. There is a strong effect size (cohen-d: -1.14, p-value: 0.112) in the normalized peak # values when the CSDS mice were in the interaction zone relative to control groups (F). There is a medium effect size (cohen-d: 0.849, p-value: 0.401) in the peak amplitude values when the CSDS mice were in the interaction zone, relative to control group.

Chronic Stress increases firing frequency and amplitude of entorhinal cortex interneuron during social interaction with conspecific "bully" mice.

A

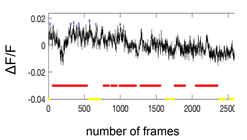


B

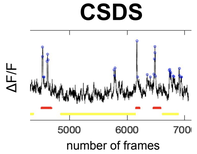


Social interaction test with CD-1

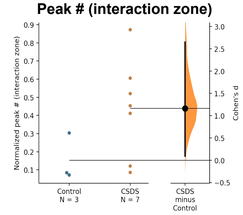
C



D

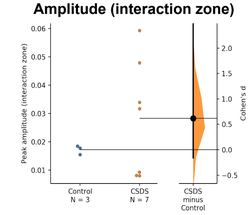


E



Cohen's d = 1.16, p-value = 0.0998

F



Cohen's d = 0.617, p-value = 0.415

A. Schematics show locations of fiber placement and Ca2+ indicator viral infusion in the LEC. **B.** Fiber photometry was performed during the social interaction test w/o and with CD-1. The mice underwent this test before and after being exposed to chronic stress. The stressor was placed in the interaction zone shown in red and data was collected from the mice depending on where they were in the chamber and proximity to the interaction zone. **C-D.** Representative SST+ EC interneuron firing tracing images of fluorescence changes (dF/F) derived from Ca2+ sensor in control mice (**C**) and CSDS mice (**D**) when they were in the presence of the aggressor (CD1). **E-F.** The Cohen's d between Control and CSDS is shown in the Gardner-Altman estimation plot. Both groups are plotted on the left axes; the mean difference is plotted on a floating axes on the right as a bootstrap sampling distribution. The mean difference is depicted as a dot; the 95% confidence interval is indicated by the ends of the vertical error bar. There is a strong effect size (cohen-d: 1.16, p-value: 0.0998) in the normalized peak # values when the CSDS mice were in the interaction zone relative to control groups (F). There is a medium effect size (cohen-d: 0.617, p-value: 0.415) in the peak amplitude values when the CSDS mice were in the interaction zone, relative to control group.

Conclusions/Future Directions

- **Chronic stress**:
  - **decreases EC-DG SST + long-range inhibitory projection activity**
  - **increase entorhinal cortex interneuron activity**
- Confirm the virus and fiber placement in postmortem tissues.
- Analyze the neuronal activity changes from stress susceptible and resilient group
- Within subject analyze neuronal Pre and Post-stress

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