Modulation of Anxiety and Fear via Distinct Intra-Hippocampal Projections to Ventral CA1

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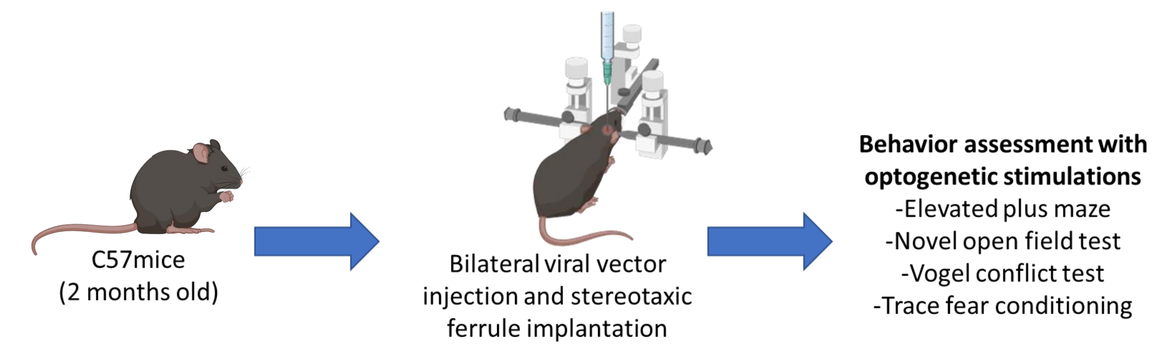
INTRODUCTION

- Anxiety and fear are distinct emotional states triggered by different factors.
- Increased anxiety arises from the anticipation of potential threats, resulting in heightened alertness, while fear is evoked by harmful stimuli, leading to defensive or retreat behaviors.
- The ventral hippocampus is known to be involved in the modulation of anxiety- and fear-related behaviors.
- Previously, we showed that inhibiting dentate gyrus and CA3 principal neurons through α2-containing $GABA_A$ receptors (α2-$GABA_A$Rs) is necessary for the reduction of anxiety by diazepam, whereas inhibition of CA1 pyramidal neurons via α2-$GABA_A$Rs is necessary for diazepam-induced suppression of fear responses.
- In this study, we wanted to test the hypothesis that while the CA3 to CA1 projection would modulate anxiety-related behavior, the direct projection from EC to CA1 would modulate fear-related behavior.
- To test this hypothesis, we used optogenetics to modulate ventral intrahippocampal projections bidirectionally. Adult C57BL/6J mice were subjected to bilateral stereotaxic injection of a viral vector expressing channelrhodopsin [AAV-CamKIIa-hChR2(H134R)-EYFP] or halorhodopsion [AAV-CamKIIa-eNpHR3.0-EYFP or AAV-CamKIIa-EYFP] into vCA3 or into layers II-III of entorhinal cortex, followed by bilateral implantation of fiberoptic ferrules into vCA1.

MATERIALS AND METHODS

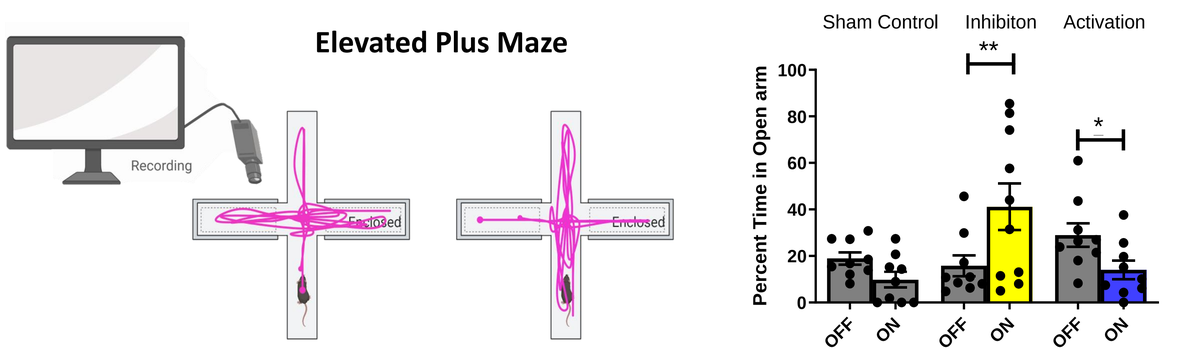
- C57BL/6J mice were purchased from Jackson laboratory at one month of age. Animals were maintained in a reverse 12-hr light/dark cycle with food and water ad libitum. All procedures of handling animals were in accordance with the guidelines from National Research Council and approved by the UIUC IACUC.
- Surgical implantation of fiber optic ferrules and injection of viral vectors for opsins were performed in a single stereotaxic surgery. Separate coordinates were standardized for ventral vCa1, vCa3 and entorhinal cortex 2/3.
- Behavioral studies were performed 3 weeks after surgery. Both male and female mice were used in this study. The tests for anxiety-like behavior were elevated plus maze, novel open field test and Vogel conflict test. We used fear conditioning with freezing detection for fear assessment.
- The total duration was 9 minutes for the elevated plus maze test and the novel open field test with 3 minutes of laser stimulation with before and after a 3 minutes laser off period. For the Vogel conflict test, the test sessions were performed twice, once with laser stimulation and once without laser stimulation (counterbalanced). For fear conditioning, the context fear test and cued fear test sessions were performed with laser stimulations.
- Bilateral optogenetic stimulation. Three-week after viral injection and fiber-optic implantation, mice were handled and habituated to fiber-optic adapter cables for 2 days prior to beginning behavioral experiments. For AAV-CamKII-hChR2(H134R)-EYFP stimulation, 10-15 mW power of light trains at 20 Hz, 5 ms pulses of blue light generated by a 50 mW 473 nm DPSS laser (OEM Laser Systems, UT) were delivered bilaterally via an optical fiber and laser output was manipulated with an optic shutter controller (Thorlabs, Newton, NJ). For AAV-CamKII-eNpHR3.0-EYFP stimulations, constant yellow light with 10 mW power were generated by a 100 mW 593.5 nm DPSS Laser (OEM Laser Systems, Draper, UT) and delivered bilaterally via an optical fiber.
- For all stimulations, the laser was split equally by a 1x2 Fiber-optic Rotary Joints (Doric Lenses, Canada) and delivered bilaterally through two implanted optic fibers connected to the optic patch cords using ceramic or metal sleeves (Thorlabs, Newton, NJ). EthoVision XT 15 software (Noldus, VA) was used to record live tracking of mice during behavioral experiments. Following the behavioral experiments, animals were used to assess the stimulation induced c-fos levels using immunohistochemical staining (not shown). BioRender was used for figure schematics.
- Data are presented as mean ± standard error of the mean (SEM). The statistical differences were analyzed using the unpaired or paired Student's *t*-test, or one way or two-way ANOVA followed by Bonferroni's posthoc test as required for different data sets and significance was set at $p<0.05$.

EXPERIMENTAL DESIGN



RESULTS

Role of Ventral CA3-Ventral CA1 (vCA3-vCA1) Projection on Anxiety-like Behavior



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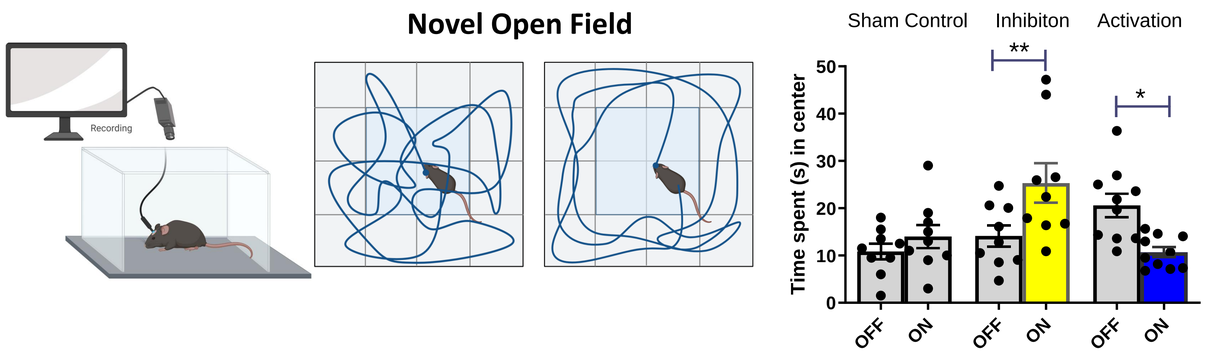
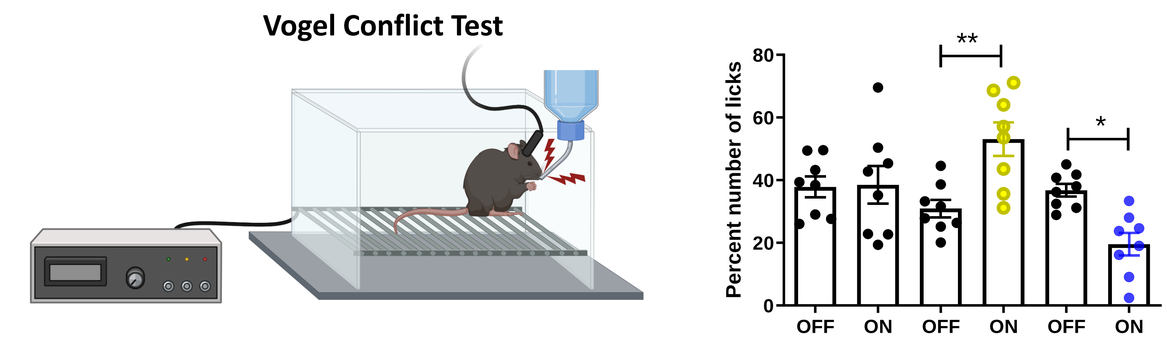
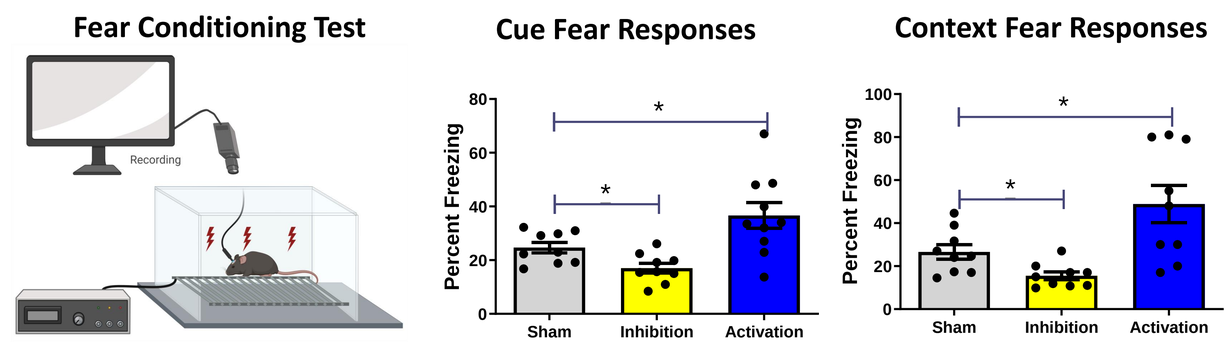
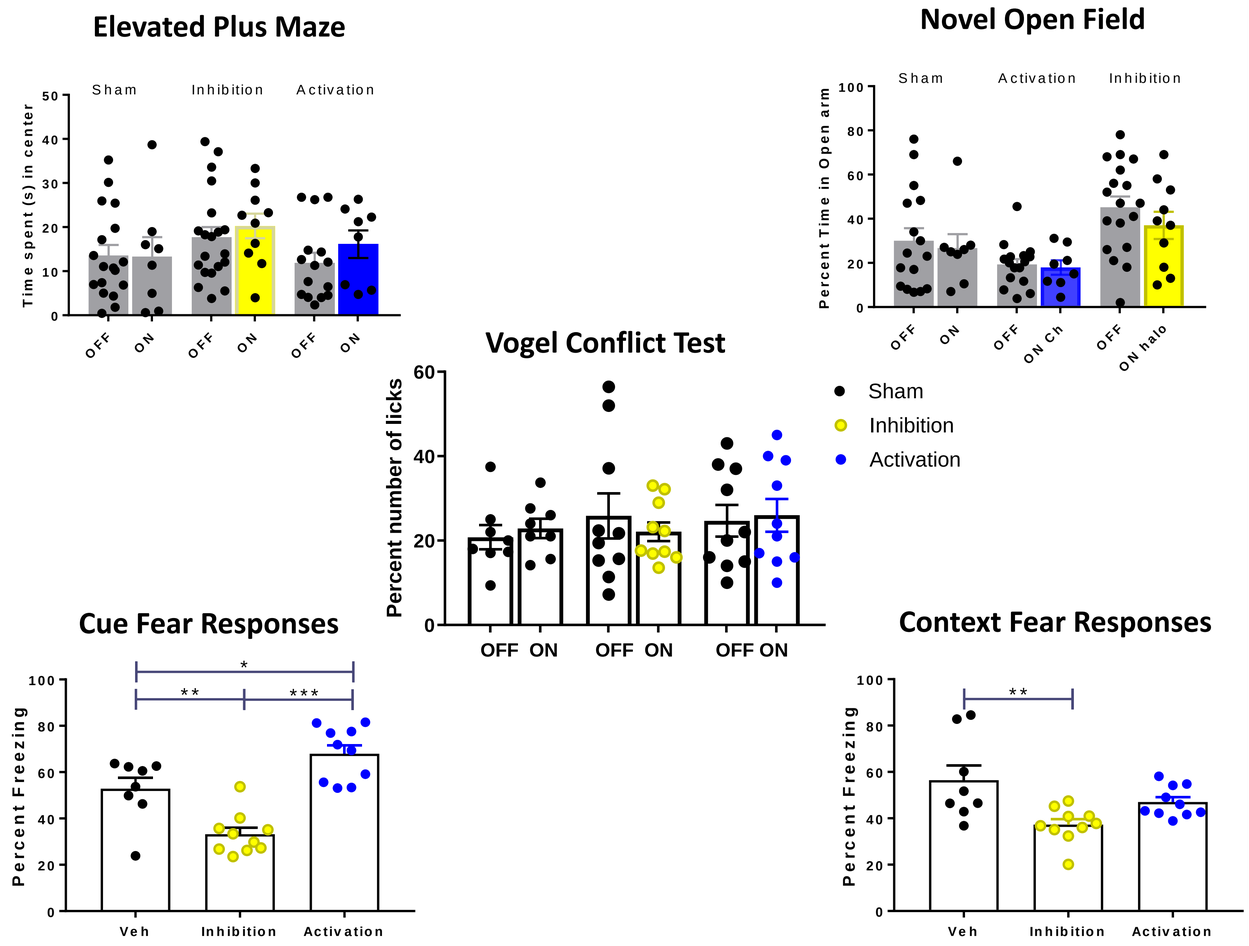




Role of Ventral CA3-Ventral CA1 (vCA3-vCA1) Projection on Fear Responses



Role of Entorhinal Cortex-Ventral CA1 (EC-CA1) Projection on Anxiety-like Behavior and Fear Responses



SUMMARY

- Activation of the vCA3 to vCA1 projection with a blue laser (473 nm, activating channelrhodopsin) increased anxiety-like and increased fear-related behavior, while inhibition of this projection with a yellow laser (593.5 nm, activating halorhodopsin) decreased anxiety-like and decreased fear-related behavior.
- Optogenetic activation or inhibition of the EC to vCA1 projection did not affect anxiety-like behavior. In contrast, optogenetic activation of the EC to vCA1 projection increased, and optogenetic inhibition of the EC to vCA1 projection decreased fear-related behavior.
- In the Vogel conflict test, optogenetic activation or inhibition of the EC to vCA1 projection, but not of the EC to vCA1 projection decreases or increases modified "punished" responding, which is in line with an increased or decreased anxiety-like behavior, respectively, and different from results in fear conditioning experiments.
- These results suggest that vCA3 pyramidal projections to vCA1, but not the EC projections to vCA1, modulate anxiety-like behavior, while both vCA3 to vCA1 projections and entorhinal cortical projections to vCA1 play a role in modulating fear responses.
- Thus, while fear-related behavior is modulated by both inputs to vCA1, modulation of anxiety-related behavior is input-specific for the vCA3 to vCA1 projection.

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