A novel prefrontal neurocircuit regulating fear-associated defensive behaviors relevant to PTSD

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Introduction

- PTSD (Post Traumatic Stress Disorder) is debilitating disease.
- Treatments for are quite limited despite its prevalence.
- PTSD is a fear-associated disorder with dysregulated threat responding and persistent trauma memories due to deficits in fear extinction.
- Most studies investigating the mechanisms underlying PTSD focus on threat responses to physical, external stressors in animal models, such as a foot shock.
- Mounting evidence shows that PTSD is also associated with dysregulated responses to threats to internal homeostasis, such as low-dose CO2 inhalation.

Relevance of CO2 inhalation and SFO- IL circuitry

- The subfornical organ or SFO, is a part of the brain with a leaky blood-brain barrier.
- The SFO has access to the cerebrospinal fluid and is important in body to brain signaling for regulating behavior.
- The SFO has projections to the infralimbic cortex or IL, a region within the prefrontal cortex that is affected in PTSD and plays an important role in fear extinction.
- Therefore, the purpose of this study is to investigate the role of SFO to IL projections in mediating threat responses to internally and externally evoked threats relevant to PTSD
- CO2 inhalation (non-hypoxic) induces acidosis, an interoceptive threat that is reliably used to induce panic attacks in PD (Papp et al. 1993)
- Recent evidence suggests PTSD patients have increased sensitivity to CO2 inhalation (Muhtz et al., 2011). Prior sensitivity to CO2 also associates with later symptoms of PTSD (Telch et al., 2012), suggesting CO2 sensitivity may predict vulnerability to PTSD. Thus, CO2 inhalation is relevant to PD and PTSD pathophysiology.
- We recently reported an association of CO2 inhalation with later development of fear extinction deficits in male mice (McMurray et al., 2020), providing a useful paradigm for mechanistic studies on PD-PTSD.
- Collectively, this evidence supports a possible role of SFO-IL circuit in CO2-enhanced conditioned fear delayed fear-extinction deficits relevant to PTSD.

Hypothesis

Inhibiting SFO to IL projections during CO2 inhalation will reduce CO2 evoked fear and CO2-enhanced conditioned fear and fear extinction deficits one week later

Methods

Subjects: Male mice (BALB/C)

DREADDs: (Designer Receptors Exclusively Activated by Designer Drugs) are exogenous receptors that are only activated by exogenous ligands, such as CNO. This allows us to specifically inhibit activation of neurons expressing the inhibitory DREADD receptor only in mice that receive CNO. Male mice received an infusion of a Cre-dependent DREADD virus into the SFO and bilateral infusions of a retrogradely transported Cre virus into the IL. This limited the expression of DREADD receptors to SFO neurons projecting to IL because SFO neurons were only able to express DREADD receptors if they also expressed Cre. A control group received Cre-virus within the IL, but a Cre-dependent mCherry "sham" virus within SFO. Within this group SFO neurons projecting to IL expressed mCherry, but not DREADD receptors.

CO2 inhalation paradigm: On Day 0, mice acclimated to the CO2 chamber for 7 minutes. The next day, mice received a single injection of either vehicle (saline) or 3 mg/kg CNO 30 minutes before being exposed to 5%, non-hypoxic CO2 for 10 minutes. The following day, the mice were re-exposed to the context in which they received CO2 for five minutes, but were not exposed to CO2.

Contextual Fear Conditioning: On day 1, they habituated to a novel context for 5 minutes then received 3, 0.5mA shocks 1 minute apart. Mice were then returned to same context in which they received foot shocks for 5 days in a row to assess extinction learning.

Data Analysis: Within both the CO2 and fear conditioning paradigms, rearing and freezing were quantified as measures of active and passive coping responses to threatful stimuli.

Results

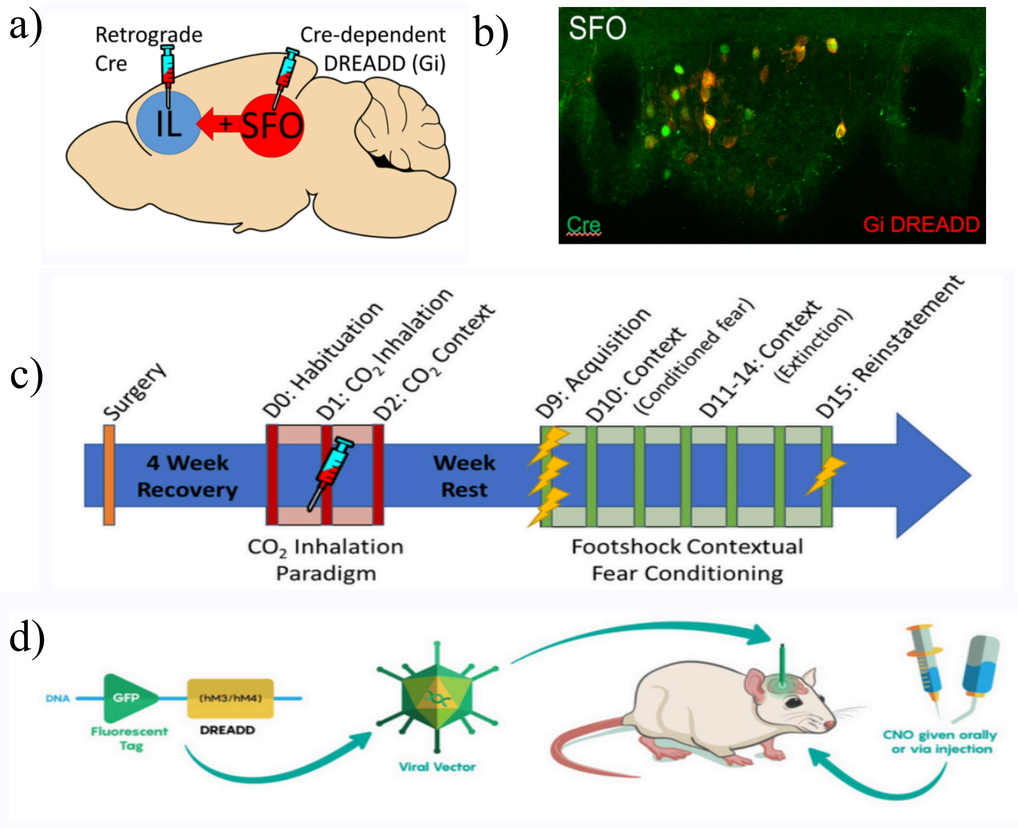

Fig. 1. Experimental Approach (a) Illustration of DREADD strategy used to inhibit SFO-IL circuits via infusion of a retrogradely transported Cre-virus into the infralimbic (IL) cortex and a Cre-dependent inhibitory DREADD (Gi) or sham virus (mCherry) into the subfornical organ (SFO). (b) Representative image showing selective G- DREADD expression within SFO neurons that co-express Cre. (c) Experimental timeline: After a four-week recovery period after surgery, mice underwent the CO2-inhalation paradigm (see methods for details) receiving a single dose of CNO or VEH , 30 minutes before undergoing CO2-inhalation. The mice were allowed a week rest, after which they underwent the foots hock contextual fear conditioning paradigm (see methods for details). (d) Illustration of DREADD chemogeentic approach (Ju et al., 2018)

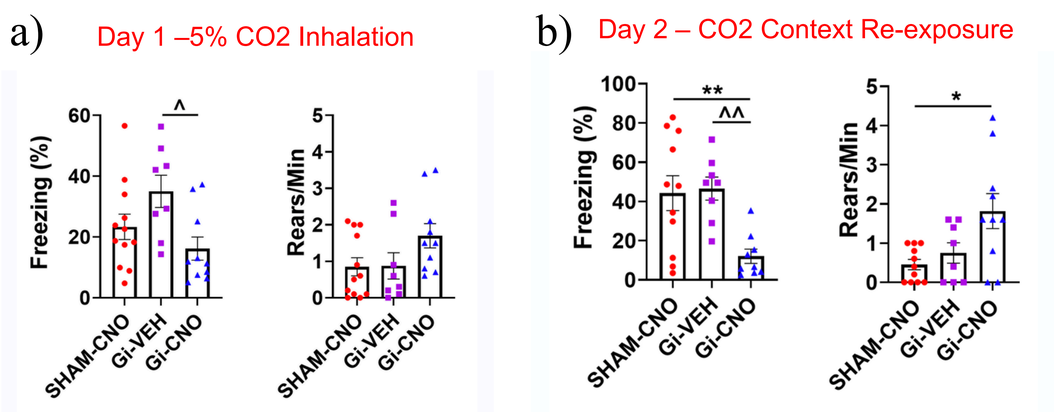

Fig. 2. SFO-Infralimbic cortex (IL) projections mediate CO2-evoked conditioned fear. (a) SFO-IL inhibition reduced CO2-evoked freezing, but not rearing, which suggests that in the presence of this threatening stimulus, SFO-IL inhibition only affected passive coping responses. (b) SFO-IL inhibition reduced freezing and increased rearing. This suggests the circuit affected both active and passive coping during a conditioned fear response.

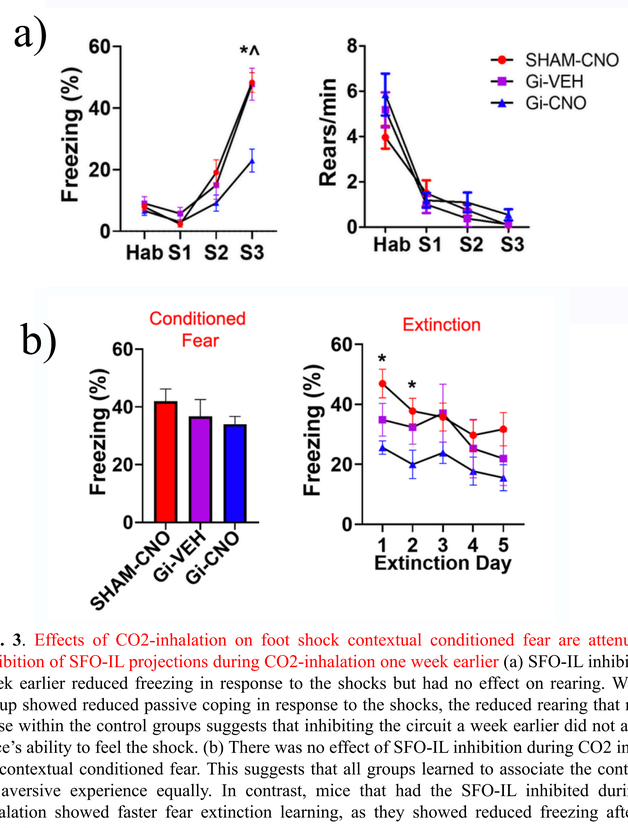

Fig. 3. Effects of CO2-inhalation on foot shock contextual conditioned fear are attenuated by inhibition of SFO-IL projections during CO2-inhalation one week earlier (a) SFO-IL inhibition one week earlier reduced freezing in response to the shocks but had no effect on rearing. While this group showed reduced passive coping in response to the shocks, the reduced rearing that mirrored those within the control groups suggests that inhibiting the circuit a week earlier did not affect the mice's ability to feel the shock. (b) There was no effect of SFO-IL inhibition during CO2 inhalation on contextual conditioned fear. This suggests that all groups learned to associate the context with an aversive experience equally. In contrast, mice that had the SFO-IL inhibited during CO2 inhalation showed faster fear extinction learning, as they showed reduced freezing after fewer context re-exposures.

Conclusions/Future Directions

- Using a recently developed CO-fear conditioning paradigm by our lab, we report a novel SFO to PFC circuit that regulates fear to homeostatic-interoceptive threats and external stressors.
- We found that inhibition of SFO-IL circuits during CO2 inhalation led to reduced CO2-contexr conditioned defensive behaviors.
- We also found that reduced foot shock evoked fear acquisition, but not context conditioned fear.
- Lastly, inhibition of SFO-IL circuits led to accelerated contextual fear extinction but had no effect on reinstatement.
- Our studies provide important mechanistic information on PFC dysfunction and extinction deficits in PTSD.
- Future studies will investigate the specific cell-types mediating these effects within the SFO that will reveal novel therapeutic targets.

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