Ventral Pallidum GABA Neuron Inhibition Augments Context-Appropriate Defensive Responses to Learned Threat Cues

Erica M. Ramirez*[1], Maricela X. Martinez*[1], Ryan K. Rokerya[1], Vanessa Alizo Vera[1], Christina M. Ruiz[1], Mitchell R. Farrell[1], Shreeya A. Walawalkar[1], Grace J. Kollman[2], and Stephen V. Mahler[1]

[1]Department of Neurobiology & Behavior, University of California, Irvine. 1203 McGaugh Hall, Irvine, CA 92697, USA
949-824-4727

[2]Department of Neurosciences, University of California, 9375 Gilman Dr, San Diego, CA 92161, USA

Corresponding Author: Erica M. Ramirez ericamr1@uci.edu

Other Authors: maricexm@uci.edu, ryanrokerya@gmail.com, valizove@uci.edu, cruiz1@uci.edu, Mitchell.Farrell.phd@gmail.com, swalawal@uci.edu, gracekollman@gmail.com, mahlers@uci.edu

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Abstract

The ventral pallidum (VP) is embedded within the brain circuits controlling motivated behavior, which are heavily implicated in addiction and other psychiatric disorders. Prior work showed that VP GABAergic neurons (VP^{GABA}) promote reward approach and seeking, while intermixed populations of VP glutamate neurons instead promote avoidance and aversion. Some have thus suggested a functional dichotomy between these VP subpopulations in reward versus threat. We test this hypothesis by asking how inhibiting VP^{GABA} impacts active and passive defensive responses to learned threat cues. We taught GAD1:Cre rats with inhibitory VP^{GABA} DREADDs (or WT littermates without DREADDs) that a metal-wrapped probe delivers shock, or that a 20sec auditory cue precedes footshocks. These threats elicit active defensive burying, or passive freezing responses, respectively. We found that VP^{GABA} inhibition markedly increased stimulus-appropriate defensive responses to both types of threats, but failed to alter new learning about threat—suggesting VP^{GABA} mediates aversive motivation but not memory formation. VP^{GABA} inhibition also altered threat-related c-Fos expression patterns within VP cell populations, and in their efferent target region LHb—pointing to underlying circuit mechanisms of these conditioned defensive responses. Results indicate that VP^{GABA} neurons not only promote reward seeking as previously reported, but that they also actively inhibit defensive responses to threats that might otherwise limit reward seeking. This refines our understanding of subcortical valanced motivation circuits, and may suggest new targets for intervening in disorders like addiction and depression.

1. Introduction

The ventral pallidum (VP) is best known for its role in reward, especially via its strong inputs from nucleus accumbens, and prominent projections to ventral tegmental area (VTA) (Heimer et al., 1991; Kalivas et al., 1993; Mahler et al., 2014; Ottenheimer et al., 2018; Pardo-Garcia et al., 2019; Smith & Berridge, 2007; Zahm, 1989). However, like other nearby hypothalamic and extended amygdala regions (Barker et al., 2017; Jennings et al., 2013a; Lammel et al., 2012; Nieh et al., 2016), VP also contains glutamatergic populations that instead mediate aversion, in part via their direct projections to lateral habenula (LHb) (Haber et al. 1985; Zahm et al. 1996; Faget et al., 2018, 2024; Stephenson-Jones et al., 2020; Tooley et al., 2018; Wang et al., 2020). Yet VP sends both GABA and glutamate projections to LHb, and also to VTA (Soares-Cunha & Heinsbroek, 2023)—suggesting unresolved complexity in the specific behavioral roles of these motivation circuits. Since adaptive decision making depends upon proper balance between these motivational pathways, understanding how they function normally, as well as how they may become imbalanced in psychiatric disorders, is essential.

Cell-type specific manipulation and observation studies in mice suggest that VP^{GABA} neurons selectively mediate reward and approach, while VP glutamate neurons instead mediate aversion and avoidance (Heinsbroek et al., 2020; Stephenson-Jones et al., 2020; Tooley et al., 2018; Wulff et al., 2019, Faget et al., 2018, 2024). In rats, we and others found that VP^{GABA} neurons also impacted high-stakes decision making by mediating motivation in pursuit of highly salient rewards, especially when triggered by learned reward cues (Farrell et al., 2021, 2022; Scott et al., 2023, 2025; Prasad et. al. 2020). In contrast, inhibiting VP^{GABA} neurons had few effects on affective responses to unpleasant stimuli like foot shocks in rats, and we saw only minor effects on operant avoidance of shock (Farrell et al., 2021, 2022), further supporting the idea of a highly specific role for VP^{GABA} in appetitive motivation in particular.

This said, we still wondered if VP^{GABA} neurons might play a role in aversive motivation under certain circumstances. VP^{GABA} neurons may drive appetitive motivation, while also constraining defensive response, both functions that likely come into conflict when animals are faced with competing stimuli. For example, reward-related behavioral effects of VP^{GABA} manipulations are often clearest when motivation is elicited by conditioned cues (Scott et al., 2023; Farrell et. al., 2022), but few studies had interrogated VP^{GABA} involvement in conditioned *aversive* motivation. Might manipulations of VP^{GABA} alter adaptive active or passive defensive responses to cues signifying threat, as they do cues paired with reward?

We asked how chemogenetically inhibiting VP^{GABA} neurons affects defensive responses to perceived threats when encountered in the absence of reward or reward cues. We tested both a spatially-localized threat (a shock-delivering metal-wrapped rod), which normally elicits active defensive burying of the rod, and a temporally-predictive threat (a 20sec auditory cue predicting foot shock), which normally elicits passive defensive freezing. Inhibiting VP^{GABA} neurons augmented the context-appropriate defensive response to the threat that was present (be it active defense or passive freezing), without impacting other behaviors including initial learning about the threats. In addition, inhibiting VP^{GABA} neurons increased Fos expression in neighboring VP cells (likely VP glutamate), as well as in the downstream LHb—suggesting disinhibition of both these aversion-related populations. This report is thus the first to show a clear behavioral role for VP^{GABA} neurons in aversive motivation, and it supports the idea that VP^{GABA} circuits may not only promote reward seeking directly, but also concurrently suppress aversive processes which might otherwise inhibit reward pursuit. We thus provide novel insights into the context-dependent neural mechanisms underlying adaptive responses to salient, affectively-valanced stimuli, and how they may go awry in stress-related disorders.

2. Materials and Methods

2.1 Subjects

Long Evans male and female GAD1:Cre rats (n=84) and their Cre-negative wildtype (WT) littermates (n=17) were bred in-house, and housed in pairs or trios in ventilated tub cages with corncob bedding and *ad libitum* chow and water, with experiments conducted during the dark phase of a reversed 12:12hr light cycle. Rats were 75+ days old at experiment start. Procedures were approved by the UCI Institutional Animal Care and Use Committee, and followed the NIH Guide for the Care and Use of Laboratory Animals.

2.2 Stereotaxic Surgery

Rats were anesthetized with ketamine (56.5mg/kg), xylazine (8.7mg/kg), and meloxicam (1mg/kg). An AAV2 vector containing a Cre-dependent, mCherry-tagged inhibitory DREADD (hSyn-DIO-hM4Di-mCherry; titer: 5×10^{12}vg/mL; Addgene Cat. #:44362-AAV2) was injected bilaterally into VP of both GAD1:Cre and WT rats (~300 nl/hemisphere with a glass pipette); at bregma-relative coordinates (mm): AP0.0, ML±2.0, DV−8.0, as previously described (Martinez et al., 2023).

2.3 CNO Preparation and Treatment

Clozapine-N-oxide (CNO) was obtained from the NIDA Drug Supply Program, stored at 4°C in powder aliquots with desiccant, and protected from light. CNO (5mg/kg/ml) was dissolved daily for IP injection in 5% dimethyl sulfoxide, then diluted in 0.9% saline to dose. For all tests, vehicle (VEH) or CNO was injected IP, 30min prior to commencement of the test (Farrell et al., 2019, 2021, 2022; Lawson et al., 2021, 2023; Mahler et al., 2014; Martinez et al., 2024).

2.4 Group Comparisons and VEH/CNO Treatment

To determine effects of VP^{GABA} inhibition on responses to a previously-learned probe threat, rats received VEH or CNO in counterbalanced order before each of two fear expression tests. Other experiments compared CNO effects in rats with or without VP^{GABA} DREADDs, thus controlling for potential non-specific effects of CNO (Bonaventura et al., 2019; Gomez et al., 2017; Jann et al., 1994; Mahler & Aston-Jones, 2018). In shock probe fear acquisition, and all auditory cue fear expression experiments, Control groups consisted of a mixture of GAD1:Cre rats with DREADDs given VEH (n=27), and WT rats without DREADDs given CNO (n=23); no behavioral differences were seen between these subgroups in either task (ts<1.522, ps>0.05), so they were combined. For Fos experiments, rats were given VEH (n=8; half of which were previously shocked) or CNO (n=4, all previously shocked) prior to test.

2.5 Experimental Timeline

At least 4 weeks after surgery, rats were handled for 5min on 5 consecutive days, then underwent behavioral training. Some rats (n=40) first underwent testing of expression of fear for a probe that previously delivered shock, then they were tested on expression of fear elicited by an auditory cue previously paired with 0.75mA footshock. Others (n=36) instead underwent testing for expression of auditory fear conditioning first (0.3mA shock), then they were used to determine how inhibiting VP^{GABA} during shock probe training impacts *acquisition* of shock probe threat learning. Additional rats (n=12) were used for examination of Fos in VP and LHb following exposure to a probe that previously either delivered or did not deliver shock.

2.6 Behavioral Testing

2.6.1 Shock Probe Defensive Burying Test

Rats were habituated twice for 30min to a specific plexiglass tub cage filled with corncob bedding (5cm height from cage bottom). On fear acquisition day, a copper-wrapped electrified

probe delivering a 1.5mA shock when touched (Coulbourn Precision Animal Shocker) was introduced to the chamber through a 1cm diameter hole in the wall, located 8cm above the cage floor (Fucich & Morilak, 2018, Berridge et al., 1988; Craft et al., 1988; Pinel & Treit, 1978). Rats were placed in the cage on the opposite end from the shock probe, and the session continued for 20min after the first rat-initiated shock.

Probe Fear Expression Test: On the following day, rats were injected with VEH or CNO, and 30min later they were reintroduced to the chamber, including the now-inactive shock probe for 20min—the first "probe fear expression" test. 48h later, rats underwent a short (5min) fear "re-acquisition" session in the chamber with a re-electrified probe, and all rats were shocked again on this session at least once. The next day, they underwent a second probe fear expression test, conducted identically to the first other than that they received the alternative injection (VEH/CNO). Behavior was video recorded for later quantification by blinded observers, as detailed in supplemental methods. "Probe fear expression," as defined by defensive burying behaviors, was statistically equivalent in rats that received VEH on the first versus second probe fear expression test (Mann-Whitney U=162, p=0.6072), and rats received VEH/CNO treatments in counterbalanced order, so data from both tests were hereafter combined for repeated measures statistical analysis. One rat was excluded from analyses because it never touched the probe or received a shock during the acquisition session, and 3 others were excluded for never emitting treading/digging on either of the two fear expression tests (VEH or CNO days).

Probe Fear Acquisition Test: Separate rats underwent the same shock probe fear acquisition procedure described above, except that this group received VEH or CNO prior to this training session featuring the electrified probe. They then underwent the "probe fear expression" test 48h later, without further injections.

2.6.2 Auditory Cue-Elicited Freezing Test

Cued fear conditioning took place in Med-Associates rat operant conditioning chambers, housed within sound-attenuating boxes, with standard metal bars as flooring (30.5x24x21cm; St. Albans, VT), and a peppermint scent (McCormick). Behaviors were video recorded for later analysis, as described in supplemental methods. On the acquisition day, there was a 3min baseline in which only the house light was illuminated, and then a 20sec auditory cue (continuous white noise: 80dB) was played, followed immediately by either a low (0.3mA) or high (0.75mA) intensity foot shock (2sec). Three total cue/shock pairings were made during this acquisition session, with 90sec elapsing between each.

Auditory Cue-Elicited Fear Expression Test: 48hrs later, rats received VEH or CNO 30min prior to the start of fear expression test, which took place in a different Med Associates chamber with a distinct metal grid floor, and an orange scent (McCormick). After a 3min baseline, the same 20sec white noise cue was delivered a total of 24 times, each separated by 90sec. No shocks were delivered during this session.

2.6.3 Examining Threat-Related Neural Activation in VP and LHb

We also examined neural activation (c-Fos expression) elicited by the threatening shock probe, and determined how this is impacted by VP^{GABA} neuron inhibition. A group of male rats that previously underwent a medium-intensity (0.5mA) cued fear conditioning and expression tests were used for this experiment (behavioral data from this pilot experiment not shown). Two weeks later, rats were handled and habituated to the shock probe chamber as described in section 2.6.1, then exposed to an electrified probe (n=8) or a non-electrified probe (n=4) during

a probe fear acquisition session. On the following day, all unshocked rats (n=4) and half of the shocked rats (n=4) were VEH-injected, and the other half of the shocked rats (n=4) received CNO. 30min later they underwent a 20min probe fear expression test (with unelectrified probe), and 90min after the test they were perfused, and brains prepared for Fos staining and analyses.

2.7 Histology, Immunohistochemistry, and Microscopy

2.7.1 Preparation of Slices for Staining

All rats were perfused transcardially with chilled 0.9% saline and 4% paraformaldehyde, brains were postfixed in 4% paraformaldehyde for ~16hr, cryoprotected in 20% sucrose-azide, and sectioned coronally at 40µm into PBS-azide.

2.7.2 Visualizing & Quantifying VP Virus Expression

Cre-dependent virus expression was verified in each rat by analyzing the zone of immunohistochemically-amplified mCherry expression, relative to the Substance P-defined borders of the VP (Haber & Nauta, 1983; Zahm & Heimer, 1988, 1990). No mCherry expression was observed in WT Control rats.

2.7.3 Quantifying Neural Activation in VP Cell Populations and LHb

To examine Fos in VP mCherry+ (previously confirmed to be ~91% GABAergic (Farrell et al., 2021) or VP mCherry- (putative non-GABA) neurons, sections near the center of virus injections were fluorescently stained for Fos and mCherry. To explore the identity of Fos+ VP cells that were mCherry-, we stained additional adjacent sections for Fos and choline acetyltransferase (ChAT) using dual-color 3,3′-Diaminobenzidine (DAB) immunohistochemistry, and counted co-localized cells. A DAB-based Fos staining protocol was also used to examine neural activity in LHb.

2.8 Statistical Analyses

Statistical analyses were conducted in Graphpad Prism. ANOVAs and t-tests compared GAD1:Cre and Control rats in normally-distributed behavioral datasets. When data violated normality assumptions (assessed by D'Agostino & Pearson tests), non-parametric tests were used: Wilcoxon matched-pairs tests for within-subjects comparisons and Mann-Whitney U tests for independent samples. In the within-subjects shock probe fear expression test, data were log-normalized to meet ANOVA assumptions, then interactions between DREADD Group (GAD1:Cre/Control), treatment (VEH/CNO), and sex were examined. One-way ANOVA (and Holm-Šídák posthoc) was used to compare behavioral groups in Fos experiments. ANOVAs with between-subjects DREADD Group and Sex factors, and a repeated measures Trial factor (24 trials), determined main effects and interactions for auditory fear conditioning extinction sessions. All tests were 2-tailed, with significance criterion set at $p<0.05$.

3 Results

3.1 Analysis of VP DREADD Expression

The previously-validated (Farrell et al., 2021, 2022), Cre-dependent hM4Di DREADD/mCherry vector caused robust expression in VP^{GABA} neurons of GAD1:Cre rats (n=70)**,** but not in their Cre- wildtype littermates (n=17; **Fig. 1A,D**). Somatic mCherry expression was localized predominantly within Substance P-defined VP borders of GAD1:Cre rats (**Fig. 1B**), as well as in these neurons' axons, which were present as expected in VP efferent targets including LHb (Groenewegen & Berendse, 1990; Haber et al., 1993; Root et al., 2015; Zahm et al., 1987) (**Fig. 1C**). Some GAD1:Cre rats were excluded from behavioral analyses for significant DREADD expression observed outside VP borders (n=5) or lack of expected mCherry expression (n=9).

3.2 VP^{GABA} Inhibition Enhances Active Defensive Responses to a Localized Threat

We first asked whether inhibiting VP^{GABA} neurons impacts defensive burying behaviors directed at a localized, previously-learned threat—a wire-wrapped shock probe that delivered an electric shock during training, but which was inactive during counterbalanced VEH and CNO fear expression tests **(Fig. 2A)**.

3.2.1 Effects of VP^{GABA} Inhibition on Probe Fear Expression

Inhibiting VP^{GABA} neurons markedly increased expression of defensive responses to the previously-learned shock probe. Relative to VEH day, CNO in GAD1:Cre rats (n=24; 13F) increased time animals spent actively spent burying the shock probe, (Wilcoxon W=210, p=0.002, **Fig. 2B**), bouts of burying (W= 221, p=0.001, **Fig. 2C**), and height (cm) of bedding piles (W=160, p=0.008, **Fig. 2D**). CNO also decreased latency to begin burying (W=-148, p=0.023, **Fig. 2E**). In contrast to these active defensive responses to the probe, inhibiting VP^{GABA} did not alter other behaviors less linked to active defense, such as the number of probe investigations (W=-6, p=0.944, **Fig. 2F**), escape attempts (W=-7, p=0.933), or time spent freezing (W=-13, p=0.969), which was uncommon in this active fear expression task. Results thus indicate that inhibiting VP^{GABA} neurons robustly and selectively increased active defensive responses directed at a previously-learned localized threat.

Effects of CNO in GAD1:Cre rats are attributable to the chemogenetic manipulation of VP^{GABA} neurons, as no equivalent effects of CNO vs VEH were observed in Control rats (n=12; 6F). Defensive burying duration (W=-8, p=0.765, **Fig. 2G**), bouts of burying (W=-7, p= 0.656, **Fig. 2H**), pile height (W=12, p= 0.281, **Fig. 2I**), and latency to begin burying (W=2, p=0.966**, Fig. 2J**) were unaffected, as were probe investigations (W=8, p=0.765, **Fig. 2K**), freezing (W=-1, p>0.9999) and time attempting escape (W=2, p=0.966). Specificity of CNO effects to DREADD-expressing animals were largely confirmed with Drug (VEH/CNO) x DREADD Group (GAD1:Cre/Control) ANOVAs on log-transformed data, with a significant interaction of Drug x DREADD for burying duration ($F_{1,28}$=4.196, p=0.050) and bouts ($F_{1,29}$=6.687, p=0.015), and trends toward an increased pile height ($F_{1,34}$=2.068, p=0.160) and decreased latency ($F_{1,33}$=2.585, p=0.117).

During initial drug-free learning of the shock probe threat, GAD1:Cre and Control rats acquired the task similarly, indicating that observed VP^{GABA} inhibition effects did not result from pre-existing group differences in training history or efficacy. All rats contacted the probe at least once during initial shock training, and with similar latency in GAD1:Cre and Control groups (Control: m±SEM=13.13±3.317sec; GAD1:Cre: m±SEM= 6.730±0.991sec; Mann-Whitney U=24, p=0.173). During acquisition, the duration of defensive burying did not differ between GAD1:Cre and Control rats (U=41, p=0.5516), nor did the height of bedding piles (Control: m±SEM=4.125in±0.625in; GAD1:Cre: m±SEM = 2.913in±0.476in; U=135.500, p=0.168).

3.2.2 Similar Effects of VP^{GABA} Inhibition in Both Sexes

Minor behavioral sex differences were found in this probe fear expression test. Males spent slightly more time burying than females (Main effect of Sex, log-normalized data: $F_{1,31}$=5.128, p=0.031), and built taller piles of bedding ($F_{1,31}$=8.620, p=0.006), while females spent more time attempting to escape than males (Main effect of Sex: $F_{1,37}$=32.630, p<0.0001). There were no other sex differences in probe-directed behaviors (burying bouts, latency, pile height, probe investigations) or freezing (Fs<3.636, ps>0.05; **Fig. S1A-D)**.

No sex differences were seen in effects of VP^{GABA} inhibition on defensive responses to the probe, with no significant Sex x Drug interactions observed for any behavior in GAD1:Cre rats (all Fs<2.573, ps>0.120), including burying, pile height, latency to bury, freezing, escape attempts, or probe investigations (**Fig. S1A-D**). No sex-specific effects of CNO were observed in

Control animal behaviors either (no Sex x Drug interactions, log-normalized data; Fs<5.020, ps>0.05).

3.3 VP^{GABA} Inhibition Does Not Alter Learning About the Shock Probe

3.3.1 Effects of VP^{GABA} Inhibition on Active Fear Acquisition

Next we asked whether inhibiting VP^{GABA} impacts learning about the shock-delivering probe, by training a cohort of GAD1:Cre (n=15;6F) and Control rats (n=15;6F) to acquire a fear memory in the presence of CNO or VEH (**Fig. 3A**). VP^{GABA} neuron inhibition did not affect defensive burying directed at the probe during this acquisition session (Mann-Whitney test; duration: U=85, p=0.563, **Fig. 3B**; bouts: U=65.5, p=0.140, **Fig. 3C**; pile height: U=89, p=0.689, **Fig. 3D**; latency: U=65, p=0.137, **Fig. 3E**). Probe investigations (U=95, p=0.834, **Fig. 3F**) and freezing (U=88, p=0.667) were similarly unaffected. Rats were then tested 48hrs later with the non-electrified probe, drug-free, thus querying retention of the threat learning that occurred in the presence or absence of VP^{GABA}. Again, no significant differences were observed in defensive responses (duration: U=87.5, p=0.643, **Fig. 3G**; bouts: U=84.5, p=0.765, **Fig. 3H**; pile height: U=88.5, 0.673, **Fig. 3I**; latency: U=95.5, p=0.918, **Fig. 3J**) or probe investigations (U=62, p=0.104, **Fig. 3K**), and freezing was not observed. These data suggest that VP^{GABA} inhibition does not alter unconditioned responses to an electrified shock probe itself, nor learning or consolidation of memories about this threat.

3.3.2 Minor Sex Differences in Active Fear Acquisition Tests

Female rats displayed slightly less burying behavior than males during shock probe acquisition (Main Effect of Sex; burying duration: $F_{1,22}$=3.378, p=0.080; bouts: $F_{1,22}$=5.864, p=0.024; pile height: $F_{1,24}$=7.225, p=0.013; and increased latency to bury: $F_{1,24}$=6.907, p=0.015). Accordingly, during the subsequent retention test females were also slower to initiate defensive responses ($F_{1,24}$=8.519, p=0.008), and they created shorter piles than males ($F_{1,24}$=5.226, p=0.032).

VP^{GABA} inhibition did not significantly influence defensive behaviors during the acquisition phase of the shock probe task in a sex-dependent manner, as no Sex × DREADD interactions were observed (all Fs<0.600, ps>0.446). However, during the drug-free expression test conducted 24hrs later, there was a significant Sex × DREADD interaction, such that prior VP^{GABA} inhibition appeared to enhance defensive behaviors in females while reducing them in males (duration: $F_{1,21}$=11.76, p=0.003; bouts: $F_{1,21}$=9.446, p=0.006; pile height: $F_{1,24}$=7.832, p=0.010). Given the lack of significant post hoc effects between DREADD and control groups within each sex, additional studies are needed to explore the potential for sex-dependent effects of VP^{GABA} inhibition on threat memory.

3.4 VP^{GABA} Inhibition Enhances Freezing to a Shock-Predictive Auditory Cue

Next we asked whether VP^{GABA} neurons are also involved in responses to cues that are temporally- but not spatially-predictive of an aversive shock, and which elicit passive (freezing) rather than active (probe burying) defensive responses.

Rats first learned to associate a 20sec auditory cue that terminates coincident with a foot shock, delivered 3 times during an 8.5min fear acquisition session (low intensity 0.3mA/2sec shock group: n=16 GAD1:Cre, n=17 Control. High intensity 0.75mA/2sec shock group: n=16 GAD1:Cre, n=19 Control). 48h later they underwent a fear expression test in a novel context, starting 30min after injection of CNO or VEH. The shock-paired cue (without shock) was played 24 times in this 47min session, allowing examination of VP^{GABA} effects on fear cue extinction (**Fig. 4A)**.

3.4.1 Effects of VP^{GABA} Inhibition on Defensive Responses to Auditory Threat Cues

In the low-shock intensity (0.3mA) cohort, VP^{GABA} inhibition did not alter behaviors during a 3min baseline period (Mann-Whitney test, GAD1:Cre versus Control; Low-shock cohort: U=110.5, p=0.103), suggesting no change in general arousal or exploration of the novel chamber. When the shock-paired cue was played for the first time on the expression test, when rats maximally expected to be imminently shocked, VP^{GABA} inhibited rats froze more than Controls (U=56.5, p=0.005. **Fig. 4B** bar graph). Cues were played 23 more times in the session without shocks, and VP^{GABA}-inhibited rats froze more throughout this training than Controls (Main effect of DREADD Group (GAD1:Cre/Control): $F_{1,30}$=6.398, p=0.017; including during the 1st and 23rd cue presentations (Holm-Šídák, t_{720}=3.152, p=0.038; t_{720}=3.229, p=0.031). Both groups showed similar extinction rates (Main effect of Cue#: $F_{23,690}$=20.430, p<0.0001; No DREADD x Cue# interaction: $F_{23,690}$=0.944, p=0.539).

In addition to measuring behavior during 20sec cues, we also examined the 90sec periods immediately after each cue, finding that VP^{GABA} inhibited rats froze more than controls across all cues, especially late in the session (Main effect of DREADD group: $F_{1,30}$=5.341, p=0.028; DREADD x Cue# interaction: $F_{24,720}$=1.532, p=0.050, **Fig. 4C**).

For the cohort receiving 0.75mA shock during training, VP^{GABA} inhibition did not notably alter behavior during baseline (U=122, p=0.293), during the first cue presentation (U=196.5, p=0.930), or across the 24 cues (no main effect of DREADD group: $F_{1, 33}$= 1.701, p=0.201, nor DREADD x Cue# interaction: $F_{24,792}$=0.629, p=0.916), potentially due to a ceiling effect caused by high rates of freezing in both groups. In post-cue periods, increased freezing in VP^{GABA} rats was seen in early, but not late extinction trials (DREADD x Cue# interaction: $F_{23,759}$=2.316, p=0.001, **Fig. 4E**), potentially suggestive of VP^{GABA} inhibition causing “spillover” of fear into these otherwise relatively “safe” post-cue periods.

Group differences were again not attributable to differential Pavlovian training prior to VP^{GABA} inhibition. GAD1:Cre and Control rats behaved similarly during acquisition, with freezing during baseline (Us>119, ps>0.05), during each of the three 20sec cue presentations (Us>82.5, ps>0.05), and during 90sec periods following each shock (Us>97.5, ps>0.05).

VP^{GABA} inhibition thus enhanced passive defensive freezing responses to auditory threat cues, and this effect was specific to freezing. Other measured behaviors were either unaffected by VP^{GABA} inhibition (rearing, head movements, escape attempts), or decreased both initially (sniffing during the 1st cue in the low-shock group: U=64, p=0.009) and throughout the session (grooming, low-shock group, Main Effect of DREADD; during cue: $F_{1,30}$=10.06, p=0.004; post-cue: $F_{1,30}$=7.648, p=0.010). We note that defensive burying was never seen in this experiment, since a grid floor was placed above bedding in test chambers.

3.4.2 Sex-Specific Effects of Passive Fear Expression and VP^{GABA} Inhibition

Females froze more than males in general, with increased freezing during cues in low and high shock cohorts (**Fig S2,** Main Effect of Sex; low-shock cohort: $F_{1,28}$=7.945, p=0.009; High-shock cohort: $F_{1,31}$=40.820, p<0.0001), and in post-cue periods also in the low-shock cohort ($F_{1, 28}$=9.943, p=0.004; high-shock cohort: $F_{1,31}$=0.597, p=0.446).

There was some evidence that VP^{GABA} inhibition had greater ability to augment conditioned freezing in females than in males (**Fig. S2**; Sex x DREADD interaction for freezing during cues in the low shock cohort: $F_{1,28}$=4.479, p=0.043; high shock cohort: $F_{1,31}$=0.754, p=0.392). Similar

effects were observed in post-cue periods (Low-shock cohort: $F_{1,29}$=5.883, p=0.022; high-shock cohort: $F_{1,31}$=0.962, p=0.334). This intriguing finding is a rare example of a potentially sex-dependent effect of VP^{GABA} inhibition which should be replicated.

3.5 VP^{GABA} Inhibition Effects on Neural Activity in LHb, and in Neighboring VP Neurons

We next sought to interrogate the neural circuits that mediate augmented threat responses when VP^{GABA} neurons are inhibited. To do so, we examined activity that was elicited by a threatening shock probe in VP subpopulations and in LHb, and determined how inhibiting VP^{GABA} altered this activity. Male rats expressing hM4Di DREADDs in VP^{GABA} (n=12; n=4 per test condition) were trained as described above with a metal-wrapped probe that delivered shocks when touched (n=8), or that did not deliver shocks (n=4). 24hr later they encountered the probe again, now unelectrified, starting 30min after injection of VEH (n=4 shock-experienced rats, n=4 never shocked rats) or CNO (n=4 shock-experienced rats; **Fig. 5A**).

We first examined Fos in the LHb, an aversion-related brain region to which VP^{GABA} (and VP glutamatergic) neurons strongly project (Faget et al., 2018; Groenewegen et al., 1993; Haber et al., 1993; Liu et al., 2020; Root et al., 2014; Tooley et al., 2018). Test condition strongly impacted LHb Fos ($F_{2,9}$=16.120, p=0.001, **Fig. 5B,C**), with the threatening probe increasing LHb Fos over a non-threating one (Holm-Šídák, noShock+VEH versus Shock+VEH: t_9=2.481, p=0.035), and VP^{GABA} inhibition further augmenting this threat-induced LHb activity (Shock+VEH vs. Shock+CNO: t_9=3.183, p=0.022).

We also examined how threat and VP^{GABA} neuron inhibition impacted Fos expression in populations of cells within VP itself. We used our ability to Cre-dependently label VP^{GABA} neurons with mCherry to identify GAD1+ cells within VP **(Fig. 5D)**, and to distinguish them from unlabeled cells in the immediate vicinity, which are presumably largely non-GABAergic, and are therefore mostly glutamatergic (Faget et al., 2018; Tooley et al., 2018).

We did not find significant group differences in Fos within mCherry+ VP^{GABA} neurons themselves ($F_{2,\ 8}$=3.113, p=0.100, **Fig. 5F**), including in the pre-hoc planned comparison between shock-VEH and shock-CNO rats, which showed only a non-significant trend toward Gi DREADD inhibition of GABA neuron Fos (t-test: t_6=1.961, p= 0.098). We note that this trend is consistent with our prior functional validation of VP^{GABA} neuron inhibition by DREADDs in these transgenic rats (Farrell et. al. 2021).

In contrast to VP^{GABA} cells, activity of non-labeled, putatively non-GABA VP cells did significantly differ by test condition ($F_{2,\ 8}$=9.243, p=0.008, **Fig. 5E**). Threat alone did not seem to activate VP non-GABA cells (noShock+VEH vs. Shock+VEH, t_8=1.338, p=0.218), but VP^{GABA} inhibition potentiated threat-induced activity in VP non-GABA neurons, relative to threat alone (Shock+VEH vs Shock+CNO, t_8=3.003, p=0.034). We expect these non mCherry-labeled VP cells are largely glutamatergic, since only about 10% of VP cells contain neither GABA nor glutamate mRNA, but instead acetylcholine (Faget et al., 2018; Tooley et al., 2018). We tested this prediction by examining Fos in VP ChAT+ acetylcholine cells in adjacent sections from the same rats. As expected, no group differences were seen ($F_{2,9}$=0.390, p=0.688, **Fig. 5G**), suggesting that non-GABA VP cells that potentiated by VP^{GABA} inhibition during shock are likely glutamatergic.

Finally, we also sought to examine how Fos in VP populations relates to Fos in LHb of the same animals, toward understanding how VP^{GABA} inhibition affects both cell populations. We found that activity in LHb approaches significant correlation with Fos in non-GABA-labeled (putatively glutamatergic) VP neurons (r=0.561, p=0.073, **Fig. 5H**). A similar relationship between LHb and

VP^{GABA} Fos (r=0.077, p=0.823, **Fig. 5I**), or LHb and VP ChAT Fos (r=0.327, p=0.326, **Fig. 5J**) is not apparent.

These findings are consistent with shock probe threat inducing activity directly in LHb, but not VP non-GABA neurons, and with VP^{GABA} inhibition disinhibiting LHb both directly, and potentially also by disinhibiting local VP glutamate neurons as well (Liu et al., 2020; Soares-Cunha & Heinsbroek, 2023; Tooley et al., 2018), which would also lead to excitation of LHb via direct excitatory projections from VP.

4. Discussion

Here we show that in addition to promoting reward seeking, VP^{GABA} neurons can also regulate aversively motivated behaviors. Our findings suggest that chemogenetically inhibiting VP^{GABA} neurons "releases the brakes" on context-appropriate defensive responses to learned threats, likely via direct or indirect disinhibition of LHb. This suggests that VP^{GABA} cells may promote reward pursuit both by imbuing reward-associated stimuli with incentive salience (Berridge & Robinson, 2003; Smith et al., 2009), but also by concurrently suppressing conditioned fear and aversion that would otherwise limit reward seeking. We found that VP^{GABA} inhibition consistently enhanced either active or passive defensive responses to shock threats, depending on which response was most appropriate for the perceived threat encountered. In contrast, inhibiting VP^{GABA} did not affect acquisition of defensive responses to threat, and non-defensive behaviors were unaffected, suggesting a specific role for VP^{GABA} in suppressing fear and aversion elicited by threat cues that were spatially or temporally associated with a noxious shock. These findings lend insight into VP modulation of motivation, and may also reflect broader principles by which intermeshed subcortical circuits of valanced motivation govern affective decision making (Carlezon & Thomas, 2009; Gordon-Fennell & Stuber, 2021; Waraczynski, 2016), and thus inform future strategies for intervening in disorders like addiction and depression.

4.1 VP^{GABA} Inhibition Augments Defensive Responses to Learned Threats

VP^{GABA} neurons have been described as primarily helping drive appetitive behavior like highly-motivated reward seeking (Faget et al., 2018, 2024; Prasad et al., 2020; Scott et al., 2023; Soares-Cunha & Heinsbroek, 2023; Stephenson-Jones et al., 2020; Wulff et al., 2019), while intermingled VP glutamate neurons instead seem to mediate aversive and avoidance processes that suppress reward pursuit (Levi et al., 2020; Liu et al., 2020; Tooley et al., 2018; Wang et al., 2020). Other reports have also suggested a role for VP in fear or aversion (Hernández-Jaramillo et al., 2024; Moaddab et al., 2021; Russell & McDannald, 2024), and one showed VP^{GABA} cells respond to noxious stimuli (Faget et al., 2024), but their roles in aversive motivation in particular was unknown.

We previously showed that chemogenetically inhibiting VP^{GABA} in rats failed to alter either primary unconditioned responses to shocks, or to markedly alter instrumental avoidance or escape from foot shocks (Farrell et al., 2021), and here we recapitulated the finding that primary behavioral reactions to shock do not require VP^{GABA}. In contrast, we found an unappreciated role for VP^{GABA} in conditioned threat responses, with inactivation causing markedly *enhanced* defensive responses to shock-associated cues. This implies that VP^{GABA} neurons, in addition to directly promoting appetitive responses, may engage safety signaling mechanisms that dampen aversive motivation through the relief from anticipated threat like other valence-processing circuits (Duvarci, 2024; Luo et al., 2018; Sangha et al., 2020).

In the natural world of rats, where both opportunities and threats often co-occur, this type of push-pull mechanism would be adaptive in situations in which reward pursuit must be prioritized even in the face of danger. Consistent with this idea, when we previously asked how inhibiting VP^{GABA} influences risky decision making, we found a shift to a more conservative strategy—promoting choice of small, safe reward (one food pellet) over a larger, but potentially dangerous option (two pellets, plus a chance of foot shock)(Farrell et al., 2021) We interpreted this as reflecting primarily a suppression of motivation to receive reward when VP^{GABA} was suppressed, but the present data suggest that such decision making may also have been influenced by an augmented perception of threat. We therefore propose that VP^{GABA}, and potentially other pallidal, hypothalamic, and extended amygdala GABA circuits with a seemingly similar specific role in promoting reward seeking and consumption (Gordon-Fennell & Stuber, 2021; Jennings et al., 2013; Nieh et al., 2015; Nieh, Vander Weele, et al., 2016; Waraczynski, 2016; Zhang et al., 2021), may play a more nuanced role than previously appreciated when balancing conflicting motivational influences to guide high-stakes decision making.

Given the VP's long-known role in motor activation (Austin & Kalivas, 1990; Churchill et al., 1998; Heimer et al., 1982), it is particularly striking that inhibiting VP^{GABA} led to either active, threat-directed defensive treading, or instead to passive immobility in response to threat—depending on the nature of the shock cue presented. The shock probe used here was a localized stimulus that was spatially paired with shock—analogous to naturalistic threats like a predator chasing a rat down an underground tunnel (Berridge et al., 1988; Craft et al., 1988; Pinel & Treit, 1978). Such proximate, localized threats must either be escaped, or if this is impossible, opposed—for example by pushing and throwing any available material toward the threat, as occurs here when rats attempt to bury the probe in bedding material. VP^{GABA} inhibition selectively augmented this defensive behavior in response to the learned probe threat. In contrast, when a diffuse auditory cue is played just prior to delivery of a foot shock, most rats exhibit freezing behavior when the cue is re-encountered. When VP^{GABA} was inhibited under these circumstances, passive freezing was significantly augmented. Non-defensive behaviors were not altered by VP^{GABA} inhibition in either threat task, nor were defensive behaviors inappropriate to the threat at hand altered (freezing to the probe, treading to the auditory cue). Therefore, VP^{GABA} inhibition seems to elicit a fear-like state that is upstream of action selection and generation circuits, which determine the best behavioral manifestation of fear under the circumstances. A similar position in threat-processing circuits has been proposed for hypothalamic corticotropin releasing factor cells (Bains et al., 2015; Füzesi et al., 2016), but how exactly these interact with VP, or how either population interacts with downstream mechanisms generating specific defensive actions remains to be determined.

Notably, VP^{GABA} inhibition amplified freezing in response to the auditory shock-predictive cue, but did not increase freezing in a novel context before the cue was presented, suggesting no unusual generalization of fear to this environment. This indicates a specific enhancement of conditioned fear responses, rather than, for example, perception of novelty as threatening. However, we did observe that VP^{GABA} inhibition caused rats to continue to freeze even after termination of a cue that was played without delivery of the predicted shock. This suggests that VP^{GABA} inhibition may prolong or intensify cue-elicited fear, thereby interfering with the normal resolution or extinction of the fear response. These data imply that VP^{GABA} neurons are involved in shaping not only the selection of appropriate defensive motor strategies, but also the ability of rats to appropriately terminate defensive states once immediate danger has passed, and to extinguish conditioned fear responses.

4.2 VP^{GABA} Inhibition Does not Alter Responses to Shock, or Learning About the Shock Probe

VP^{GABA} inhibition enhanced conditioned fear elicited by previously-learned threat cues, so we next asked whether this manipulation also altered either unconditioned responses to shock itself, or affected the ability of rats to learn about a threat. Replicating and extending a prior result (Farrell et al., 2021), unconditioned responses to footshock (0.3 or 0.75mA) or probe-delivered shock (1.5mA) were unaltered by VP^{GABA} inhibition, suggesting these cells do not mediate affective or sensory aspects of the unconditioned experience of a noxious stimulus. Likewise, the acquisition of initial defensive burying responses to the probe after the first time being shocked by it was also unaltered by ongoing VP^{GABA} inhibition, as was subsequent retrieval of aversive memories formed during inhibition. This suggests that neither the ability to form aversive Pavlovian memories or the strength of the memories formed, nor the consolidation of those memories was altered by disrupting VP^{GABA}. There is some evidence for a role for VP in other types of learning (Dusa et al., 2022; Floresco et al., 1999; Kaplan et al., 2020; Leung & Balleine, 2013; Macpherson et al., 2019; Péczely et al., 2014; Roman-Ortiz et al., 2021; Stephenson-Jones, 2019; Stephenson-Jones et al., 2020), though many of these findings were not designed to disambiguate motivational from learning processes, or VP cell types. One report found that stimulating predominantly GABAergic VP neurons that co-express enkephalin disrupts formation of an inhibitory avoidance memory (Macpherson et al., 2019), and another showed that optogenetically stimulating VP^{GABA} suppressed expression of auditory cue-induced freezing, without affecting acquisition of new fear learning—both findings are fundamentally consistent with the present results. This said, further work is required to fully understand VP^{GABA} roles in formation, storage, and retrieval of different types of memory and stages of learning, consolidation, and recall (McGaugh, 1989; Packard & Cahill, 2001).

4.3 VP^{GABA} Inhibition "Releases Brakes" on LHb, and on Nearby "Non-GABA" Neurons

The LHb, a major efferent target of VP^{GABA} as well as VP glutamate neurons (Faget et al., 2018; Liu et al., 2020; Stephenson-Jones et al., 2020; Tooley et al., 2018), encodes negative affect and learning, and plays a role in aversion, avoidance, and anhedonia (Bromberg-Martin & Hikosaka, 2011; Li et al., 2013; Liu et al., 2020; Matsumoto & Hikosaka, 2009; Tooley et al., 2018; Y. Yang et al., 2018). We therefore determined how neural activity in LHb and in VP cell populations is affected by exposure to a threatening shock prod, and whether inhibition of VP^{GABA} cells affects this threat-induced Fos.

As expected, encountering a threatening probe increased activity in LHb relative to an inert probe, and we found that this activation was further potentiated by inhibiting VP^{GABA}. This is consistent with a disinhibition mechanism, whereby silencing VP^{GABA} removes direct inhibitory input to LHb, amplifying its response to threat. Activation of GABAergic inputs to the LHb from other deep brain regions has even been shown to induce place preference (Barker et al., 2017; Stamatakis et al., 2013). While global inhibition of VP^{GABA}—but not VP glutamate neurons—induces place avoidance (Faget et al., 2018), the specific role of the VP^{GABA} to LHb projection in active defensive responses remains less understood. Optogenetic activation of VP^{GABA} terminals in LHb, unlike in the VTA, failed to promote appetitive behavior in mice (Faget et al., 2018). Another group has shown optogenetic inhibition of this projection strongly suppresses conditioned reward-seeking, and modestly reduces conditioned avoidance of a noxious stimulus (Stephenson-Jones et al., 2020). Together with our finding of enhanced threat-induced LHb Fos following VP^{GABA} inhibition, suggests that VP^{GABA} neurons likely regulate motivation in part via their direct inhibition of LHb.

However, it is also possible that VP^{GABA} inhibition also indirectly impacts LHb activity via an intermediary, such as nearby populations of VP glutamate neurons. VP glutamate cells also project robustly to LHb, and this pathway mediates aversion related processes, especially avoidance behaviors (Faget et al., 2018; Heinsbroek et al., 2020; Stephenson-Jones et al.,

2020; Tooley et al., 2018; Wulff et al., 2019). Moreover, VP^{GABA} cells connect directly with their glutamatergic neighbors (Faget et al., 2018; Levi et al., 2020; Tripathi et al., 2013), so it is possible that inhibiting VP^{GABA} here disinhibited VP glutamate, thus yielding net excitation in those which project to LHb. We therefore sought to examine Fos in VP glutamate neurons, but since IHC methods cannot reliably be used to identity VP glutamate cells at this time, we did so indirectly by labeling VP^{GABA} cells using the aforementioned Cre-dependent system, and examining Fos expression in cells that were 1) within VP borders, and 2) within the zone around virus injection sites in which GABA cells were robustly labeled, and 3) not labeled with mCherry. VP contains only about 10% cells that are neither GABA nor glutamate (Faget et al., 2018; Tooley et al., 2018), and these are cholinergic (Bengtson & Osborne, 2000; Gritti et al., 1993; Záborsky & Cullinan, 1992). Since we found no effects of VP^{GABA} inhibition on ChAT+ VP cells in the vicinity, we inferred that cells meeting these 3 criteria were likely mostly glutamatergic. This interpretation should be tested directly by examining Fos in verified VP glutamate cells (which cannot be reliably identified using the IHC methods available to us).

These "putative VP glutamate cells" expressed statistically comparable levels of Fos in non-shock and shock-experienced rats, suggesting that unlike LHb, putative VP non-GABA cells are not robustly activated by probe threat alone. However, threat-induced activity in these cells was observed when we acutely inhibited VP^{GABA} cells—an effect we also did not see in identified VP cholinergic cells. This supports the possibility that VP glutamate neurons, but not acetylcholine neurons were disinhibited by suppressed activity in their GABA neighbors, and thus that VP glutamate may also contribute to fear potentiation by helping excite LHb. Supporting this idea, we found promising trend toward correlation between Fos expression in putative VP non-GABA neurons and in LHb of the same rats. No similar correlation was seen in VP mCherry-labeled GABA cells, or in VP acetylcholine cells.

Therefore, our finding that VP^{GABA} inhibition promotes aversive motivation may involve both suppression of VP^{GABA} inputs directly to LHb, as well as disinhibition of VP glutamate projections to LHb—in other words VP^{GABA} neuron inhibition may both remove inhibition from LHb via direct projections and simultaneously excite LHb indirectly via disinhibition of neighboring VP glutamate neurons that also project to LHb. The relative contributions of each mechanism should be verified in future studies.

4.4 Sex Differences in VP^{GABA}

VP (DiBenedictis et al., 2020; Lee et al., 2024; Scott et al., 2025; L. Yang et al., 2024) and the circuits in which it is embedded (Becker & Chartoff, 2019; Lim et al., 2004; Trainor, 2011; Zachry et al., 2021), appear to differ based on sex; as does prevalence and symptoms of the psychiatric disorders these circuits contribute to (Bangasser & Cuarenta, 2021; Cosgrove et al., 2007; Green et al., 2018; Kessler, 2003; Trainor, 2011; Young & Pfaff, 2014). In this context it is surprising that our lab's prior work in VP has rarely observed sex differences in the effects of VP manipulations on drug and natural reward seeking (Farrell et al., 2019, 2021, 2022; Mahler et al., 2014). Here, we again found only subtle sex differences in the effects of VP^{GABA} inhibition on threat responses, specifically an apparently stronger enhancement of certain aspects of auditory cue-elicited freezing in females, relative to males. We also noticed VP-independent sex differences in threat responding, in that females passively froze more than males to auditory fear cues, while males exhibited more active burying of the probe cue. Further work should examine how the conditions under which sex importantly modulates VP roles in aversively motivated behavior (Blanchard et al., 1991).

4.5 Caveats and Limitations

These studies have certain limitations that should be noted, and potentially resolved with further research. VP^{GABA} inhibition enhanced freezing only to a low-intensity shock, likely reflecting a ceiling effect at higher shock intensities. We also limit the aversive stimulus here to shock delivered two different ways—it would be valuable to learn whether VP^{GABA} inhibition also augments other responses to learned (e.g. context fear conditioning) or innate threats (e.g predator odors or looming stimuli). Further, naturalistic threat responding also depends critically on factors like threat imminence or proximity which could be manipulated in follow-up studies. Further, other potential responses to threats like escape or defensive aggression were not possible in these experiments, and how VP^{GABA} helps prioritize such strategies remains to be seen. With regard to neural activity data, we acknowledge that adding a group that was VP^{GABA} inhibited in the absence of threat would have been an interesting comparison, though its absence does not impact our main conclusions. No VP^{GABA}-mediated sex-differences were observed in the shock-probe expression task, however conclusions from the Fos analysis performed in male rats could be distinct from females and should be followed up.

4.6 Conclusions

Here we report a novel role for VP^{GABA} neurons in suppressing responses to threat cues, complementing their established role in promoting reward seeking. These cells seem to facilitate commitment to reward pursuit even in the face of danger, thus prevent vacillation between behavioral strategies once a decision to pursue reward is made. In addition to clarifying roles for VP circuits in motivation, these findings might also be relevant to the organization of wider subcortical circuits involving seemingly analogous intermixed populations of GABA and glutamate cells that mediate reward and aversion, respectively (Gordon-Fennell & Stuber, 2021; Hu, 2016; Root et al., 2015, 2020; Soares-Cunha & Heinsbroek, 2023; Wulff et al., 2019). These data also open new avenues of investigation for understanding how VP^{GABA} interacts with other VP cell populations involved in stress (Chang & Grace, 2014; Eckenwiler et al., 2025; Ji et al., 2022), and wider brain motivation circuits. Given that imbalance in motivational drives is likely involved in numerous psychiatric disorders including addiction, depression, schizophrenia and PTSD, understanding the functions of these brain networks will be essential for developing next-generation strategies for treating these devastating conditions.

5. Authorship Contribution Statement

EMR and MXM equally contributed to conceptualization and implementation of the project, and with SVM analyzed data and prepared the manuscript. EMR, MXM, CMR, RKR, MRF, VAV, SAW, and GJK contributed data, and to manuscript preparation.

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References

Austin, M. C., & Kalivas, P. W. (1990). Enkephalinergic and GABAergic modulation of motor activity in the ventral pallidum. *The Journal of Pharmacology and Experimental Therapeutics*, *252*(3), 1370–1377. https://doi.org/10.1016/S0022-3565(25)20204-X

Bains, J. S., Cusulin, J. I. W., & Inoue, W. (2015). Stress-related synaptic plasticity in the hypothalamus. *Nature Reviews Neuroscience 2015 16:7*, *16*(7), 377–388. https://doi.org/10.1038/nrn3881

Bangasser, D. A., & Cuarenta, A. (2021). Sex differences in anxiety and depression: circuits and mechanisms. *Nature Reviews Neuroscience 2021 22:11*, *22*(11), 674–684. https://doi.org/10.1038/s41583-021-00513-0

Barker, D. J., Miranda-Barrientos, J., Zhang, S., Root, D. H., Wang, H.-L., Liu, B., Calipari, E. S., & Morales, M. (2017). Lateral Preoptic Control of the Lateral Habenula through Convergent Glutamate and GABA Transmission. *Cell Reports*, *21*(7), 1757–1769. https://doi.org/10.1016/j.celrep.2017.10.066

Becker, J. B., & Chartoff, E. (2019). Sex differences in neural mechanisms mediating reward and addiction. *Neuropsychopharmacology*, *44*(1), 166–183. https://doi.org/10.1038/S41386-018-0125-6;SUBJMETA=1662,1689,378,5,631;KWRD=ADDICTION,MOTIVATION

Bengtson, C. P., & Osborne, P. B. (2000). Electrophysiological properties of cholinergic and noncholinergic neurons in the ventral pallidal region of the nucleus basalis in rat brain slices. *Journal of Neurophysiology*, *83*(5), 2649–2660. https://doi.org/10.1152/JN.2000.83.5.2649/ASSET/IMAGES/LARGE/9K0500958009.JPEG

Berridge, K. C., Fentress, J. C., & Treit, D. (1988). A triggered hyperkinesia induced in rats by lesions of the corpus striatum. *Experimental Neurology*, *99*(2), 259–268. https://doi.org/10.1016/0014-4886(88)90146-X

Berridge, K. C., & Robinson, T. E. (2003). Parsing reward. *Trends in Neurosciences*, *26*(9), 507–513. https://doi.org/10.1016/S0166-2236(03)00233-9

Blanchard, D. C., Shepherd, J. K., Carobrez, A. D. P., & Blanchard, R. J. (1991). Sex effects in defensive behavior: Baseline differences and drug interactions. *Neuroscience & Biobehavioral Reviews*, *15*(4), 461–468. https://doi.org/10.1016/S0149-7634(05)80132-0

Bonaventura, J., Eldridge, M. A. G., Hu, F., Gomez, J. L., Sanchez-Soto, M., Abramyan, A. M., Lam, S., Boehm, M. A., Ruiz, C., Farrell, M. R., Moreno, A., Galal Faress, I. M., Andersen, N., Lin, J. Y., Moaddel, R., Morris, P. J., Shi, L., Sibley, D. R., Mahler, S. V, … Michaelides, M. (2019). High-potency ligands for DREADD imaging and activation in rodents and monkeys. *Nature Communications*, *10*(1), 4627. https://doi.org/10.1038/s41467-019-12236-z

Bromberg-Martin, E. S., & Hikosaka, O. (2011). Lateral habenula neurons signal errors in the prediction of reward information. *Nature Neuroscience*, *14*(9), 1209–1218. https://doi.org/10.1038/NN.2902

Carlezon, W. A., & Thomas, M. J. (2009). Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis. *Neuropharmacology*, *56 Suppl 1*(Suppl 1), 122–132. https://doi.org/10.1016/J.NEUROPHARM.2008.06.075

Chang, C. H., & Grace, A. A. (2014). Amygdala-Ventral Pallidum Pathway Decreases Dopamine Activity After Chronic Mild Stress in Rats. *Biological Psychiatry*, *76*(3), 223–230. https://doi.org/10.1016/J.BIOPSYCH.2013.09.020

Churchill, L., Klitenick, M. A., & Kalivas, P. W. (1998). Dopamine depletion reorganizes projections from the nucleus accumbens and ventral pallidum that mediate opioid-induced

motor activity. *Journal of Neuroscience*, *18*(19), 8074–8085. https://doi.org/10.1523/JNEUROSCI.18-19-08074.1998

Cosgrove, K. P., Mazure, C. M., & Staley, J. K. (2007). Evolving Knowledge of Sex Differences in Brain Structure, Function, and Chemistry. *Biological Psychiatry*, *62*(8), 847–855. https://doi.org/10.1016/J.BIOPSYCH.2007.03.001

Craft, R. M., Howard, J. L., & Pollard, G. T. (1988). Conditioned defensive burying as a model for identifying anxiolytics. *Pharmacology Biochemistry and Behavior*, *30*(3), 775–780. https://doi.org/10.1016/0091-3057(88)90098-6

DiBenedictis, B. T., Cheung, H. K., Nussbaum, E. R., & Veenema, A. H. (2020). Involvement of ventral pallidal vasopressin in the sex-specific regulation of sociosexual motivation in rats. *Psychoneuroendocrinology*, *111*, 104462. https://doi.org/10.1016/J.PSYNEUEN.2019.104462

Dusa, D., Ollmann, T., Kállai, V., Lénárd, L., Kertes, E., Berta, B., Szabó, Á., László, K., Gálosi, R., Zagoracz, O., Karádi, Z., & Péczely, L. (2022). The antipsychotic drug sulpiride in the ventral pallidum paradoxically impairs learning and induces place preference. *Scientific Reports*, *12*(1), 19247. https://doi.org/10.1038/s41598-022-23450-z

Duvarci, S. (2024). Dopaminergic circuits controlling threat and safety learning. *Trends in Neurosciences*, *47*(12), 1014–1027. https://doi.org/10.1016/J.TINS.2024.10.001/ASSET/54F2EAC0-FB9C-4738-8BA3-E5CA2FB60E71/MAIN.ASSETS/GR2.JPG

Eckenwiler, E. A., Ingebretson, A. E., Stolley, J. J., Fusaro, M. A., Romportl, A. M., Ross, J. M., Petersen, C. L., Kale, E. M., Clark, M. S., Schattauer, S. S., Zweifel, L. S., & Lemos, J. C. (2025). Corticotropin-Releasing Factor Release From a Unique Subpopulation of Accumbal Neurons Constrains Action-Outcome Acquisition in Reward Learning. *Biological Psychiatry*, *97*(6), 637–650. https://doi.org/10.1016/J.BIOPSYCH.2024.08.006

Faget, L., Oriol, L., Lee, W.-C., Zell, V., Sargent, C., Flores, A., Hollon, N. G., Ramanathan, D., & Hnasko, T. S. (2024). Ventral pallidum GABA and glutamate neurons drive approach and avoidance through distinct modulation of VTA cell types. *Nature Communications*, *15*(1), 4233. https://doi.org/10.1038/S41467-024-48340-Y

Faget, L., Zell, V., Souter, E., McPherson, A., Ressler, R., Gutierrez-Reed, N., Yoo, J. H., Dulcis, D., & Hnasko, T. S. (2018). Opponent control of behavioral reinforcement by inhibitory and excitatory projections from the ventral pallidum. *Nat Commun*, *9*(1), 849. https://doi.org/10.1038/s41467-018-03125-y

Fanselow, M. S. (1979). Naloxone attenuates rat's preference for signaled shock. *Physiological Psychology*, *7*(1), 70–74.

Fanselow, M. S. (1980). Conditional and unconditional components of post-shock freezing. *The Pavlovian Journal of Biological Science: Official Journal of the Pavlovian*, *15*(4), 177–182.

Farrell, M. R., Esteban, J. S. D., Faget, L., Floresco, S. B., Hnasko, T. S., & Mahler, S. V. (2021). Ventral Pallidum GABA Neurons Mediate Motivation Underlying Risky Choice. *Journal of Neuroscience*, *41*(20), 4500–4513. https://doi.org/10.1523/JNEUROSCI.2039-20.2021

Farrell, M. R., Ruiz, C. M., Castillo, E., Faget, L., Khanbijian, C., Liu, S., Schoch, H., Rojas, G., Huerta, M. Y., Hnasko, T. S., & Mahler, S. V. (2019). Ventral pallidum is essential for

cocaine relapse after voluntary abstinence in rats. *Neuropsychopharmacology*, *44*(13), 2174–2113. https://doi.org/10.1038/s41386-019-0507-4

Farrell, M. R., Ye, Q., Xie, Y., Esteban, J. S. D., & Mahler, S. V. (2022). Ventral pallidum GABA neurons bidirectionally control opioid relapse across rat behavioral models. *Addiction Neuroscience*, *3*. https://doi.org/10.1016/J.ADDICN.2022.100026

Floresco, S. B., Braaksma, D. N., & Phillips, A. G. (1999). Involvement of the Ventral Pallidum in Working Memory Tasks With or Without a Delay. *Annals of the New York Academy of Sciences*, *877*(1), 711–716. https://doi.org/10.1111/J.1749-6632.1999.TB09308.X

Fucich, E. A., & Morilak, D. A. (2018). Shock-probe Defensive Burying Test to Measure Active versus Passive Coping Style in Response to an Aversive Stimulus in Rats. *Bio-Protocol*, *8*(17). https://doi.org/10.21769/BIOPROTOC.2998

Füzesi, T., Daviu, N., Wamsteeker Cusulin, J. I., Bonin, R. P., & Bains, J. S. (2016). Hypothalamic CRH neurons orchestrate complex behaviours after stress. *Nature Communications 2016 7:1*, *7*(1), 1–14. https://doi.org/10.1038/ncomms11937

Gomez, J. L., Bonaventura, J., Lesniak, W., Mathews, W. B., Sysa-Shah, P., Rodriguez, L. A., Ellis, R. J., Richie, C. T., Harvey, B. K., Dannals, R. F., Pomper, M. G., Bonci, A., & Michaelides, M. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science*, *357*(6350), 503–507. https://doi.org/10.1126/science.aan2475

Gordon-Fennell, A., & Stuber, G. D. (2021). Illuminating Subcortical GABAergic and Glutamatergic Circuits for Reward and Aversion. *Neuropharmacology*, *198*, 108725. https://doi.org/10.1016/J.NEUROPHARM.2021.108725

Green, T., Flash, S., & Reiss, A. L. (2018). Sex differences in psychiatric disorders: what we can learn from sex chromosome aneuploidies. *Neuropsychopharmacology 2018 44:1*, *44*(1), 9–21. https://doi.org/10.1038/s41386-018-0153-2

Gritti, I., Mainville, L., & Jones, B. E. (1993). Codistribution of GABA- with acetylcholine-synthesizing neurons in the basal forebrain of the rat. *Journal of Comparative Neurology*, *329*(4), 438–457. https://doi.org/10.1002/CNE.903290403

Groenewegen, H. J., & Berendse, H. W. (1990). Connections of the subthalamic nucleus with ventral striatopallidal parts of the basal ganglia in the rat. *Journal of Comparative Neurology*, *294*(4), 607–622. https://doi.org/10.1002/CNE.902940408

Groenewegen, H. J., Berendse, H. W., & Haber, S. N. (1993). Organization of the output of the ventral striatopallidal system in the rat: ventral pallidal efferents. *Neuroscience*, *57*(1), 113–142.

Haber, S. N., Groenewegen, H. J., Grove, E. A., & Nauta, W. J. H. (1985). Efferent connections of the ventral pallidum: Evidence of a dual striato pallidofugal pathway. *Journal of Comparative Neurology*, *235*(3), 322–335. https://doi.org/https://doi.org/10.1002/cne.902350304

Haber, S. N., Lynd-Balta, E., & Mitchell, S. J. (1993). The organization of the descending ventral pallidal projections in the monkey. *Journal of Comparative Neurology*, *329*(1), 111–128. https://doi.org/https://doi.org/10.1002/cne.903290108

Haber, S. N., & Nauta, W. J. H. (1983). Ramifications of the globus pallidus in the rat as indicated by patterns of immunohistochemistry. *Neuroscience*, *9*(2), 245–260. https://doi.org/https://doi.org/10.1016/0306-4522(83)90291-9

Heimer, L., Switzer, R. D., & Van Hoesen, G. W. (1982). Ventral striatum and ventral pallidum: Components of the motor system? *Trends in Neurosciences*, *5*, 83–87.

Heimer, L., Zahm, D. S., Churchill, L., Kalivas, P. W., & Wohltmann, C. (1991). Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience*, *41*(1), 89–125. https://doi.org/10.1016/0306-4522(91)90202-Y

Heinsbroek, J. A., Bobadilla, A.-C., Dereschewitz, E., Assali, A., Chalhoub, R. M., Cowan, C. W., & Kalivas, P. W. (2020). Opposing Regulation of Cocaine Seeking by Glutamate and GABA Neurons in the Ventral Pallidum. *Cell Reports*, *30*(6), 2018-2027.e3. https://doi.org/https://doi.org/10.1016/j.celrep.2020.01.023

Hernández-Jaramillo, A., Illescas-Huerta, E., & Sotres-Bayon, F. (2024). Ventral Pallidum and Amygdala Cooperate to Restrain Reward Approach under Threat. *Journal of Neuroscience*, *44*(23). https://doi.org/10.1523/JNEUROSCI.2327-23.2024

Hu, H. (2016). Reward and Aversion. *Annual Review of Neuroscience*, *39*(Volume 39, 2016), 297–324. https://doi.org/10.1146/ANNUREV-NEURO-070815-014106/CITE/REFWORKS

Jann, M. W., Lam, Y. W., & Chang, W. H. (1994). Rapid formation of clozapine in guinea-pigs and man following clozapine-N-oxide administration. *Archives Internationales de Pharmacodynamie et de Therapie*, *328*(2), 243–250. http://europepmc.org/abstract/MED/7710309

Jennings, J. H., Sparta, D. R., Stamatakis, A. M., Ung, R. L., Pleil, K. E., Kash, T. L., & Stuber, G. D. (2013). Distinct extended amygdala circuits for divergent motivational states. *Nature*, *496*(7444), 224–228. https://doi.org/10.1038/nature12041

Ji, M. J., Gao, Z. Q., Yang, J., Cai, J. H., Li, K. X., Wang, J., Zhang, H., Zhou, C. H., Cao, J. L., & Liu, C. (2022). Dynorphin promotes stress-induced depressive behaviors by inhibiting ventral pallidal neurons in rats. *Acta Physiologica*, *236*(3), e13882. https://doi.org/10.1111/APHA.13882;JOURNAL:JOURNAL:17481716A;REQUESTEDJOURNAL:JOURNAL:17481716;PAGE:STRING:ARTICLE/CHAPTER

Kalivas, P. W., Churchill, L., & Klitenick, M. A. (1993). GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. *Neuroscience*, *57*(4), 1047–1060.

Kaplan, A., Mizrahi-Kliger, A. D., Israel, Z., Adler, A., & Bergman, H. (2020). Dissociable roles of ventral pallidum neurons in the basal ganglia reinforcement learning network. *Nature Neuroscience*, *23*(4), 556–564.

Kessler, R. C. (2003). Epidemiology of women and depression. *Journal of Affective Disorders*, *74*(1), 5–13. https://doi.org/10.1016/S0165-0327(02)00426-3

Lammel, S., Lim, B. K., Ran, C., Huang, K. W., Betley, M. J., Tye, K. M., Deisseroth, K., & Malenka, R. C. (2012). Input-specific control of reward and aversion in the ventral tegmental area. *Nature*, *491*(7423), 212–217. https://doi.org/10.1038/nature11527

Lawson, K. A., Flores, A. Y., Hokenson, R. E., Ruiz, C. M., & Mahler, S. V. (2021). Nucleus Accumbens Chemogenetic Inhibition Suppresses Amphetamine-Induced Ultrasonic Vocalizations in Male and Female Rats. *Brain Sciences 2021, Vol. 11, Page 1255*, *11*(10), 1255. https://doi.org/10.3390/BRAINSCI11101255

Lawson, K. A., Ruiz, C. M., & Mahler, S. V. (2023). A head-to-head comparison of two DREADD agonists for suppressing operant behavior in rats via VTA dopamine neuron inhibition.

Psychopharmacology, *240*(10), 2101–2110. https://doi.org/10.1007/S00213-023-06429-0/FIGURES/3

Lee, J. D. A., Reppucci, C. J., Huez, E. D. M., Bredewold, R., & Veenema, A. H. (2024). Sex differences in the structure and function of the vasopressin system in the ventral pallidum are associated with the sex-specific regulation of social play behavior in juvenile rats. *Hormones and Behavior*, *163*, 105563. https://doi.org/10.1016/J.YHBEH.2024.105563

Leung, B. K., & Balleine, B. W. (2013). The Ventral Striato-Pallidal Pathway Mediates the Effect of Predictive Learning on Choice between Goal-Directed Actions. *The Journal of Neuroscience*, *33*(34), 13848. https://doi.org/10.1523/JNEUROSCI.1697-13.2013

Levi, L. A., Inbar, K., Nachshon, N., Bernat, N., Gatterer, A., Inbar, D., & Kupchik, Y. M. (2020). Projection-specific potentiation of ventral pallidal glutamatergic outputs after abstinence from cocaine. *Journal of Neuroscience*, *40*(20), 1276–1285. https://doi.org/10.1523/JNEUROSCI.0612-20.2020

Li, K., Zhou, T., Liao, L., Yang, Z., Wong, C., Henn, F., Malinow, R., Yates, J. R., & Hu, H. (2013). βCaMKII in lateral habenula mediates core symptoms of depression. *Science*, *341*(6149), 1016–1020. https://doi.org/10.1126/SCIENCE.1240729/SUPPL_FILE/1240729.LI.SM.PDF

Lim, M. M., Murphy, A. Z., & Young, L. J. (2004). Ventral striatopallidal oxytocin and vasopressin V1a receptors in the monogamous prairie vole (Microtus ochrogaster). *Journal of Comparative Neurology*, *468*(4), 555–570. https://doi.org/10.1002/CNE.10973

Liu, B., Cao, Y., Wang, J., & Dong, J. (2020). Excitatory transmission from ventral pallidum to lateral habenula mediates depression. *The World Journal of Biological Psychiatry*, *21*(8), 627–633. https://doi.org/10.1080/15622975.2020.1725117

Luo, R., Uematsu, A., Weitemier, A., Aquili, L., Koivumaa, J., McHugh, T. J., & Johansen, J. P. (2018). A dopaminergic switch for fear to safety transitions. *Nature Communications 2018 9:1*, *9*(1), 1–11. https://doi.org/10.1038/s41467-018-04784-7

Macpherson, T., Mizoguchi, H., Yamanaka, A., & Hikida, T. (2019). Preproenkephalin-expressing ventral pallidal neurons control inhibitory avoidance learning. *Neurochemistry International*, *126*, 11–18.

Mahler, S. V, & Aston-Jones, G. (2018). CNO Evil? Considerations for the Use of DREADDs in Behavioral Neuroscience. *Neuropsychopharmacology*, *43*(5), 934–936. https://doi.org/10.1038/npp.2017.299

Mahler, S. V, Vazey, E. M., Beckley, J. T., Keistler, C. R., McGlinchey, E. M., Kaufling, J., Wilson, S. P., Deisseroth, K., Woodward, J. J., & Aston-Jones, G. (2014). Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. *Nature Neuroscience*, *17*(4), 577–585.

Martinez, M. X., Alizo Vera, V., Ruiz, C. M., Floresco, S. B., & Mahler, S. V. (2024). Adolescent THC impacts on mPFC dopamine-mediated cognitive processes in male and female rats. *Psychopharmacology 2024 242:2*, *242*(2), 309–326. https://doi.org/10.1007/S00213-024-06676-9

Martinez, M. X., Farrell, M. R., & Mahler, S. V. (2023). Pathway-specific chemogenetic manipulation by applying ligand to axonally expressed DREADDs. In *Vectorology for Optogenetics and Chemogenetics* (pp. 207–220). Springer.

Matsumoto, M., & Hikosaka, O. (2009). Representation of negative motivational value in the primate lateral habenula. *Nature Neuroscience*, *12*(1), 77–84. https://doi.org/10.1038/NN.2233

McGaugh, J. L. (1989). Modulation of Memory Storage Processes. *Memory: Interdisciplinary Approaches*, 33–64. https://doi.org/10.1007/978-1-4612-3500-2_3

Moaddab, M., Ray, M. H., & McDannald, M. A. (2021). Ventral pallidum neurons dynamically signal relative threat. *Communications Biology 2021 4:1*, *4*(1), 1–14. https://doi.org/10.1038/s42003-020-01554-4

Nieh, E. H., Matthews, G. A., Allsop, S. A., Presbrey, K. N., Leppla, C. A., Wichmann, R., Neve, R., Wildes, C. P., & Tye, K. M. (2015). Decoding neural circuits that control compulsive sucrose seeking. *Cell*, *160*(3), 528–541. https://doi.org/10.1016/J.CELL.2015.01.003/ATTACHMENT/A7A8641E-9760-4CF4-8113-D209EC66DF99/MMC5.PDF

Nieh, E. H., Vander Weele, C. M., Matthews, G. A., Presbrey, K. N., Wichmann, R., Leppla, C. A., Izadmehr, E. M., & Tye, K. M. (2016). Inhibitory Input from the Lateral Hypothalamus to the Ventral Tegmental Area Disinhibits Dopamine Neurons and Promotes Behavioral Activation. *Neuron*, *90*(6), 1286–1298. https://doi.org/10.1016/J.NEURON.2016.04.035

Nieh, E. H., Vander Weele, C. M., Matthews, G. A., Presbrey, K. N., Wichmann, R., Leppla, C. A., Izadmehr, E. M., & Tye, K. M. (2016). Inhibitory Input from the Lateral Hypothalamus to the Ventral Tegmental Area Disinhibits Dopamine Neurons and Promotes Behavioral Activation. *Neuron*, *90*(6), 1286–1298. https://doi.org/10.1016/j.neuron.2016.04.035

Ottenheimer, D., Richard, J. M., & Janak, P. H. (2018). Ventral pallidum encodes relative reward value earlier and more robustly than nucleus accumbens. *Nat Commun*, *9*(1), 4350. https://doi.org/10.1038/s41467-018-06849-z

Packard, M. G., & Cahill, L. (2001). Affective modulation of multiple memory systems. *Current Opinion in Neurobiology*, *11*(6), 752–756. https://doi.org/10.1016/S0959-4388(01)00280-X

Pardo-Garcia, T. R., Garcia-Keller, C., Penaloza, T., Richie, C. T., Pickel, J., Hope, B. T., Harvey, B. K., Kalivas, P. W., & Heinsbroek, J. A. (2019). Ventral Pallidum Is the Primary Target for Accumbens D1 Projections Driving Cocaine Seeking. *The Journal of Neuroscience*, *39*(11), 2041. https://doi.org/10.1523/JNEUROSCI.2822-18.2018

Péczely, L., Ollmann, T., László, K., Kovács, A., Gálosi, R., Szabó, Á., Karádi, Z., & Lénárd, L. (2014). Role of D1 dopamine receptors of the ventral pallidum in inhibitory avoidance learning. *Behavioural Brain Research*, *270*, 131–136. https://doi.org/https://doi.org/10.1016/j.bbr.2014.04.054

Pinel, J. P., & Treit, D. (1978). Burying as a defensive response in rats. *Journal of Comparative and Physiological Psychology*, *92*(4), 708–712. https://doi.org/10.1037/H0077494

Prasad, A. A., Xie, C., Chaichim, C., Nguyen, J. H., McClusky, H. E., Killcross, S., Power, J. M., & McNally, G. P. (2020). Complementary Roles for Ventral Pallidum Cell Types and Their Projections in Relapse. *Journal of Neuroscience*, *40*(4), 880–893. https://doi.org/10.1523/JNEUROSCI.0262-19.2019

Roman-Ortiz, C., Guevara, J. A., & Clem, R. L. (2021). GABAergic basal forebrain projections to the periaqueductal gray promote food consumption, reward and predation. *Scientific Reports*, *11*(1), 22638. https://doi.org/10.1038/s41598-021-02157-7

Root, D. H., Barker, D. J., Estrin, D. J., Miranda-Barrientos, J. A., Liu, B., Zhang, S., Wang, H. L., Vautier, F., Ramakrishnan, C., Kim, Y. S., Fenno, L., Deisseroth, K., & Morales, M. (2020). Distinct Signaling by Ventral Tegmental Area Glutamate, GABA, and Combinatorial Glutamate-GABA Neurons in Motivated Behavior. *Cell Reports*, *32*(9), 108094. https://doi.org/10.1016/j.celrep.2020.108094

Root, D. H., Ma, S., Barker, D. J., Megehee, L., Striano, B. M., Ralston, C. M., Fabbricatore, A. T., & West, M. O. (2013). Differential roles of ventral pallidum subregions during cocaine self-administration behaviors. *Journal of Comparative Neurology*, *521*(3), 558–588. https://doi.org/10.1002/CNE.23191

Root, D. H., Mejias-Aponte, C. A., Qi, J., & Morales, M. (2014). Role of Glutamatergic Projections from Ventral Tegmental Area to Lateral Habenula in Aversive Conditioning. *The Journal of Neuroscience*, *34*(42), 13906. https://doi.org/10.1523/JNEUROSCI.2029-14.2014

Root, D. H., Melendez, R. I., Zaborszky, L., & Napier, T. C. (2015). The ventral pallidum: Subregion-specific functional anatomy and roles in motivated behaviors. *Progress in Neurobiology*, *130*, 29–70. https://doi.org/10.1016/J.PNEUROBIO.2015.03.005

Russell, E. L., & McDannald, M. A. (2024). Ventral Pallidum Neurons Are Necessary to Generalize and Express Fear-Related Responding in a Minimal Threat Setting. *ENeuro*, *11*(11). https://doi.org/10.1523/ENEURO.0124-24.2024

Sangha, S., Diehl, M. M., Bergstrom, H. C., & Drew, M. R. (2020). Know safety, no fear. *Neuroscience & Biobehavioral Reviews*, *108*, 218–230. https://doi.org/10.1016/J.NEUBIOREV.2019.11.006

Scott, A., Palmer, D., Newell, B., Lin, I., Cayton, C. A., Paulson, A., Remde, P., & Richard, J. M. (2023). Ventral Pallidal GABAergic Neuron Calcium Activity Encodes Cue-Driven Reward Seeking and Persists in the Absence of Reward Delivery. *Journal of Neuroscience*, *43*(28), 51915203. https://doi.org/10.1523/JNEUROSCI.0013-23.2023

Scott, A., Paulson, A., Prill, C., Kermoade, K., Newell, B., Eckenwiler, E. A., Lemos, J. C., & Richard, J. M. (2025). Ventral Pallidal GABAergic Neurons Drive Consumption in Male, But Not Female, Rats. *ENeuro*, *12*(2). https://doi.org/10.1523/ENEURO.0245-24.2025,

Smith, K. S., & Berridge, K. C. (2007). Opioid Limbic Circuit for Reward: Interaction between Hedonic Hotspots of Nucleus Accumbens and Ventral Pallidum. *Journal of Neuroscience*, *27*(7), 1594–1605. https://doi.org/10.1523/jneurosci.4205-06.2007

Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2009). Ventral Pallidum Roles in Reward and Motivation. *Behavioural Brain Research*, *196*(2), 155. https://doi.org/10.1016/J.BBR.2008.09.038

Soares-Cunha, C., & Heinsbroek, J. A. (2023). Ventral pallidal regulation of motivated behaviors and reinforcement. *Frontiers in Neural Circuits*, *17*. https://doi.org/10.3389/FNCIR.2023.1086053/FULL

Stamatakis, A. M., Jennings, J. H., Ung, R. L., Blair, G. A., Weinberg, R. J., Neve, R. L., Boyce, F., Mattis, J., Ramakrishnan, C., & Deisseroth, K. (2013). A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward. *Neuron*, *80*(4), 1039–1053.

Stephenson-Jones, M. (2019). Pallidal circuits for aversive motivation and learning. *Current Opinion in Behavioral Sciences*, *26*, 82–89. https://doi.org/https://doi.org/10.1016/j.cobeha.2018.09.015

Stephenson-Jones, M., Bravo-Rivera, C., Ahrens, S., Furlan, A., Xiao, X., Fernandes-Henriques, C., & Li, B. (2020). Opposing contributions of GABAergic and glutamatergic ventral pallidal neurons to motivational behaviors. *Neuron*, *105*(5), 921–933. https://doi.org/10.1016/j.neuron.2019.12.006

Tooley, J., Marconi, L., Alipio, J. B., Matikainen-Ankney, B., Georgiou, P., Kravitz, A. V., & Creed, M. C. (2018). Glutamatergic Ventral Pallidal Neurons Modulate Activity of the Habenula–Tegmental Circuitry and Constrain Reward Seeking. *Biological Psychiatry*, *83*(12), 1012–1023. https://doi.org/10.1016/J.BIOPSYCH.2018.01.003

Trainor, B. C. (2011). Stress responses and the mesolimbic dopamine system: Social contexts and sex differences. *Hormones and Behavior*, *60*(5), 457–469. https://doi.org/10.1016/J.YHBEH.2011.08.013

Tripathi, A., Prensa, L., & Mengual, E. (2013). Axonal branching patterns of ventral pallidal neurons in the rat. *Brain Structure and Function*, *218*, 1133–1157.

Wang, F., Zhang, J., Yuan, Y., Chen, M., Gao, Z., Zhan, S., Fan, C., Sun, W., & Hu, J. (2020). Salience processing by glutamatergic neurons in the ventral pallidum. *Science Bulletin*, *65*(5), 389–401. https://doi.org/10.1016/J.SCIB.2019.11.029

Waraczynski, M. (2016). Toward a systems-oriented approach to the role of the extended amygdala in adaptive responding. *Neuroscience & Biobehavioral Reviews*, *68*, 177–194. https://doi.org/https://doi.org/10.1016/j.neubiorev.2016.05.015

Wulff, A. B., Tooley, J., Marconi, L. J., & Creed, M. C. (2019). Ventral pallidal modulation of aversion processing. *Brain Research*, *1713*, 62–69. https://doi.org/10.1016/J.BRAINRES.2018.10.010

Yang, L., Fang, L. Z., Lynch, M. R., Xu, C. S., Hahm, H. J., Zhang, Y., Heitmeier, M. R., Costa, V. D., Samineni, V. K., & Creed, M. C. (2024). Transcriptomic landscape of mammalian ventral pallidum at single-cell resolution. *Science Advances*, *10*(50), 6017. https://doi.org/10.1126/SCIADV.ADQ6017

Yang, Y., Cui, Y., Sang, K., Dong, Y., Ni, Z., Ma, S., & Hu, H. (2018). Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nature 2018 554:7692*, *554*(7692), 317–322. https://doi.org/10.1038/nature25509

Young, L. J., & Pfaff, D. W. (2014). Sex differences in neurological and psychiatric disorders. *Frontiers in Neuroendocrinology*, *35*(3), 253–254. https://doi.org/10.1016/j.yfrne.2014.05.005

Záborsky, L., & Cullinan, W. E. (1992). Projections from the nucleus accumbens to cholinergic neurons of the ventral pallidum: a correlated light and electron microscopic double-immunolabeling study in rat. *Brain Research*, *570*(1–2), 92–101. https://doi.org/10.1016/0006-8993(92)90568-T

Zachry, J. E., Nolan, S. O., Brady, L. J., Kelly, S. J., Siciliano, C. A., & Calipari, E. S. (2021). Sex differences in dopamine release regulation in the striatum. *Neuropsychopharmacology*, *46*(3), 491–499. https://doi.org/10.1038/S41386-020-00915-1;SUBJMETA=1788,1964,378,548,631;KWRD=NEUROTRANSMITTERS,REWARD

Zahm, D. S. (1989). The ventral striatopallidal parts of the basal ganglia in the rat—II. Compartmentation of ventral pallidal efferents. *Neuroscience*, *30*(1), 33–50. https://doi.org/10.1016/0306-4522(89)90351-5

Zahm, D. S., & Heimer, L. (1988). Ventral striatopallidal parts of the basal ganglia in the rat: I. Neurochemical compartmentation as reflected by the distributions of neurotensin and substance P immunoreactivity. *Journal of Comparative Neurology*, *272*(4), 516–535. https://doi.org/https://doi.org/10.1002/cne.902720406

Zahm, D. S., & Heimer, L. (1990). Two transpallidal pathways originating in the rat nucleus accumbens. *Journal of Comparative Neurology*, *302*(3), 437–446.

Zahm, D. S., Williams, E., & Wohltmann, C. (1996). Ventral striatopallidothalamic projection: IV. Relative involvements of neurochemically distinct subterritories in the ventral pallidum and adjacent parts of the rostroventral forebrain. *Journal of Comparative Neurology*, *364*(2), 340–362.

Zahm, D. S., Zaborszky, L., Alheid, G. F., & Heimer, L. (1987). The ventral striatopallidothalamic projection: II. The ventral pallidothalamic link. *Journal of Comparative Neurology*, *255*(4), 592–605. https://doi.org/https://doi.org/10.1002/cne.902550410

Zhang, G. W., Shen, L., Tao, C., Jung, A. H., Peng, B., Li, Z., Zhang, L. I., & Tao, H. W. (2021). Medial preoptic area antagonistically mediates stress-induced anxiety and parental behavior. *Nature Neuroscience 2021 24:4*, *24*(4), 516–528. https://doi.org/10.1038/s41593-020-00784-3

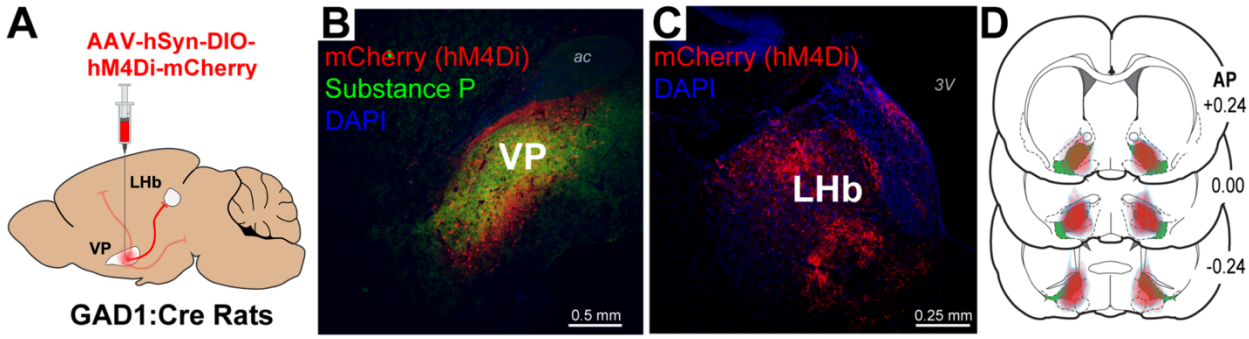

Figure 1: DREADD Methods to Assess Active and Passive Defensive Responses to Stress
(A) Diagram of hM4Di-vector injection to the VP. **(B)** Representative image of mCherry (red) expression in the Substance P (green)-defined VP from an included GAD1:Cre rat. DAPI is blue. **(C)** mCherry-expressing VP^{GABA} axons (red) in LHb are shown, DAPI is blue. **(D)** Location of virus expression in each GAD1:Cre rat is shown in red, with Substance P-defined VP borders represented in green.

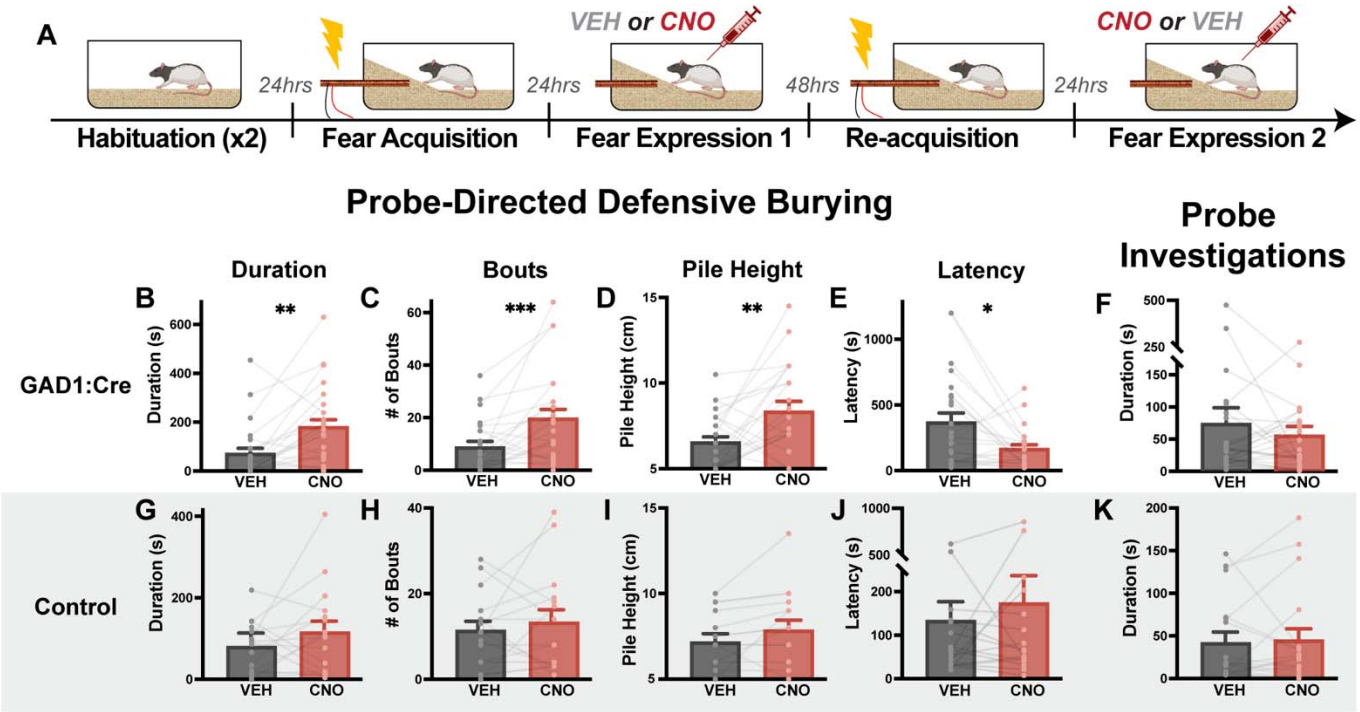

Figure 2: Enhanced Active Defensive Burying Responses to a Localized Threat Under VP^{GABA} Inhibition
(A) Behavioral protocol for probe fear expression tests. **(B-E)** Enhanced defensive burying behaviors from GAD1:Cre positive animals in counterbalanced VEH/CNO test days. **(F)** No change GAD1:Cre probe investigations. Control rats displayed no change in defensive burying **(G-J)** or investigations **(K).** *p<0.05 in

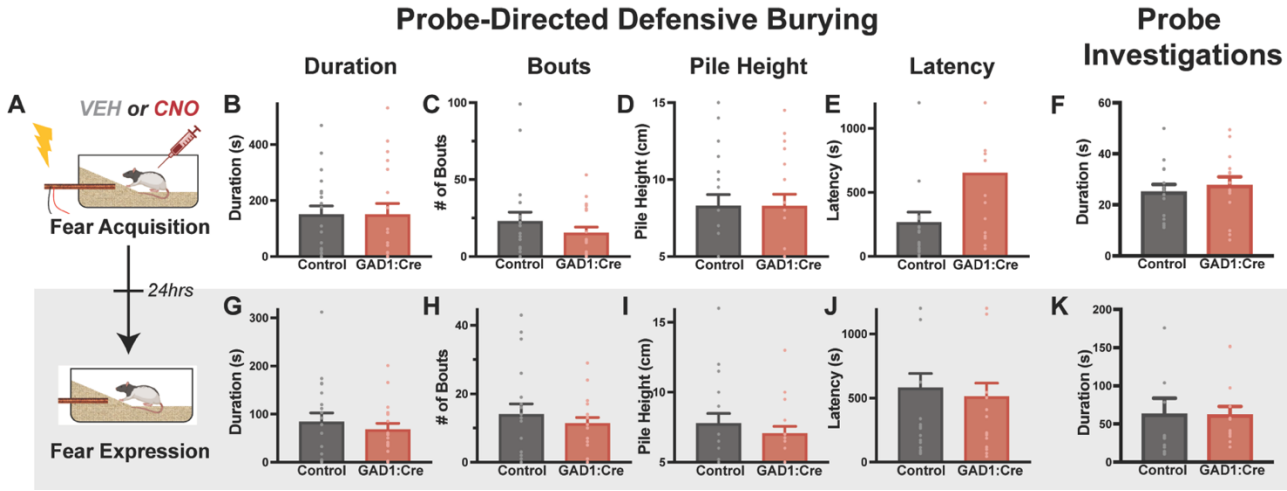

Figure 3: VP^{GABA} Inhibition During Training Does Not Affect Acquisition of Defensive Burying
(A) Diagram displaying behavior protocol with CNO or vehicle injections given during training day and a subsequent memory test with a dormant probe. Training day results are shown for defensive burying measures including duration **(B)**, bouts **(C)**, pile height **(D)**, and latency **(E)**, and also probe investigation duration **(F)**.The same measures on the expression test are respectively shown in **(G-K).** No statistically significant results were

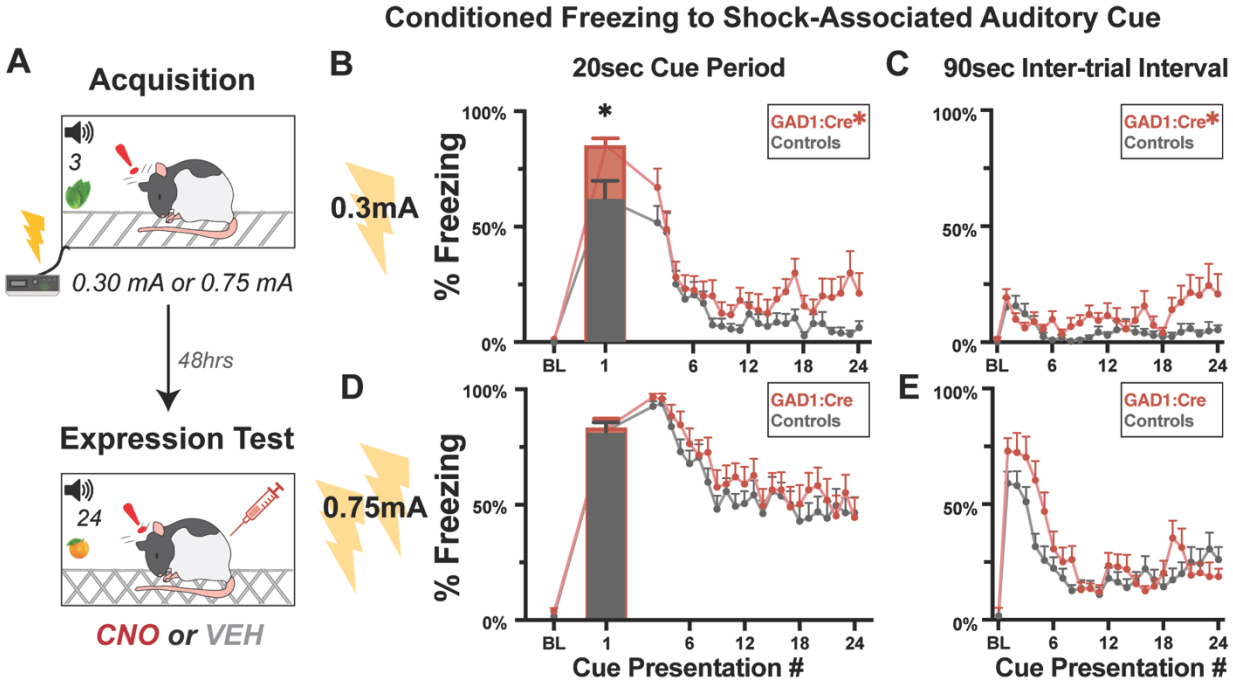

Figure 4: Enhanced Passive Defensive Freezing Responses to A Learned Cue Under VP^{GABA} Inhibition
(A) Diagram of the passive auditory fear expression protocol. Extinction of the conditioned stimulus occurred in a different context. **(B)** The low-shock experiment yielded enhanced freezing in VP^{GABA} -inhibited animals during the first 20s cue presentation and throughout the extinction session. **(C)** VP^{GABA}-inhibited animals have enhanced freezing during 90sec post-cue period. **(D)** In the high-shock group, no significant differences in freezing were observed between groups during cue presentations, however, **(E)** higher freezing was observed in the post-cue period. BL-Baseline. Numbers correspond to cue-period. *p<0.05 in an unpaired t-test for the first cue. In box: Red * main effect of Inhibition p<0.05 in a 2-way

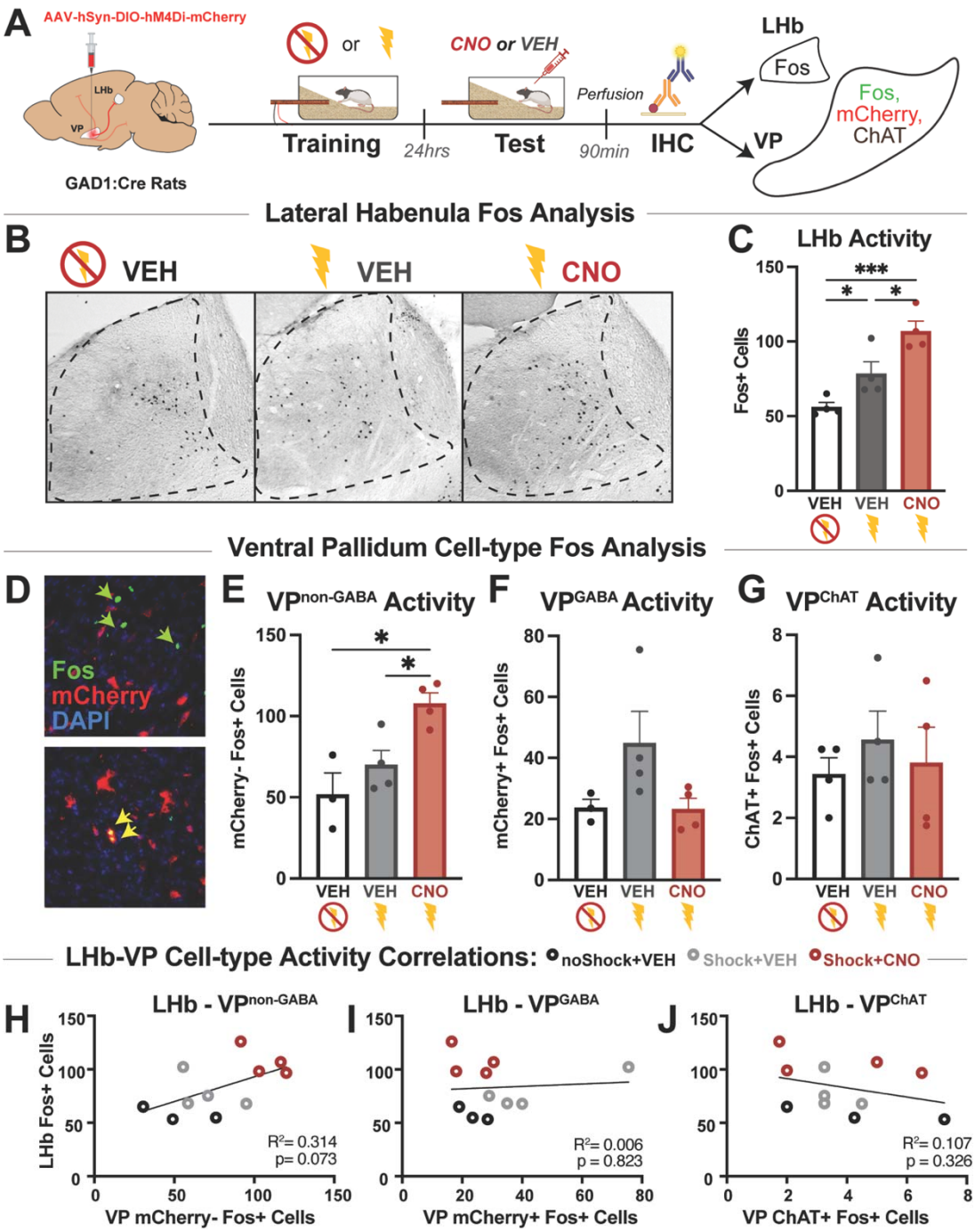

Figure 5: VP^{GABA} Inhibition Increases Threat-induced Fos in the Lhb and in Local Putative VP^{GLUT} Cells: (A) Schematic of Fos immunohistochemistry (IHC) experiment protocol. GABA neurons are visualized with mCherry expressed Cre-dependently in GAD1:Cre rats. **(B)** Representative images of LHb Fos expression in no shock+VEH, shock+VEH, and shock+CNO rats. **(C)** Fos was increased by threatening probe exposure in shocked animals compared to no-shock Controls, and this increase is further augmented when VP^{GABA} is inhibited during threat. **(D)** Representative images identifying active cell-types in VP region near the virus injection site co-stained for mCherry, Fos, and DAPI. Yellow markers show active GABA cells; green markers show active non-GABA cells. **(E)** Quantification of Fos activation in VP mCherry- cells is shown. Shock alone did not increase non-GABA VP cell Fos, but inhibiting VP^{GABA} potentiated shock-induced activation in these cells. **(F)** No significant differences between test conditions were observed in VP^{GABA} cells labeled with mCherry or **(G)** in VP ChAT+ acetylcholine cells. **(H-J)** Correlations between Fos in LHb and VP populations are shown, including VP **(H)** non-GABA cells, **(I)** VP^{GABA} cells, and **(J)** VP acetylcholine cells. *One-Way ANOVA and Holm-Sidak, $p<0.05$ two-tailed.