American College of Neuropsychopharmacology

www.nature.com/npp

ARTICLE

Check for updates

Chemogenetic activation of corticotropin-releasing factor-expressing neurons in the anterior bed nucleus of the stria terminalis reduces effortful motivation behaviors

Isabella Maita[1,2], Allyson Bazer[1,3], Kiyeon Chae[1], Amlaan Parida[1], Mikyle Mirza[1], Jillian Sucher[1,3], Mimi Phan[1], Tonia Liu[1], Pu Hu[1], Ria Soni[1], Troy A. Roepke[4] and Benjamin Adam Samuels[1]✉

© The Author(s), under exclusive licence to American College of Neuropsychopharmacology 2023

Corticotropin-releasing factor (CRF) in the anterior bed nucleus of the stria terminalis (aBNST) is associated with chronic stress and avoidance behavior. However, CRF + BNST neurons project to reward- and motivation-related brain regions, suggesting a potential role in motivated behavior. We used chemogenetics to selectively activate CRF+ aBNST neurons in male and female CRF-ires-Cre mice during an effort-related choice task and a concurrent choice task. In both tasks, mice were given the option either to exert effort for high value rewards or to choose freely available low value rewards. Acute chemogenetic activation of CRF+ aBNST neurons reduced barrier climbing for a high value reward in the effort-related choice task in both males and females. Furthermore, acute chemogenetic activation of CRF+ aBNST neurons also reduced effortful lever pressing in high-performing males in the concurrent choice task. These data suggest a novel role for CRF+ aBNST neurons in effort-based decision and motivation behaviors.

Neuropsychopharmacology (2024) 49:377–385; https://doi.org/10.1038/s41386-023-01646-9

INTRODUCTION

Chronic exposure to stressful experiences can precipitate mood disorders. In rodents, decades of research demonstrate that chronic stress results in long-lasting changes to limbic circuitry and increased avoidance in behaviors historically associated with anxiety and/or depression. However, reward and motivation behavior deficits are observed in several psychiatric disorders [1, 2]. Importantly, similar versions of reward and motivation behavioral tasks can be performed in rodents and humans, with analogous interpretations [1]. Therefore, these cross-species behavioral measures may provide enhanced translational validity compared to traditional avoidance behaviors. Importantly, humans diagnosed with mood disorders [2] and rodents exposed to chronic stress [3–5] exhibit reduced motivation to exert effort for rewards.

Bed nucleus of the stria terminalis (BNST) is a limbic region associated with chronic stress and avoidance behaviors. Exposure to unpredictable fearful stimuli activates BNST in rodents [6] and humans [7–11]. BNST threat responsivity is increased in subjects diagnosed with post-traumatic stress disorder (PTSD) and generalized anxiety disorder [12–14]. Anterior BNST (aBNST) contains a subpopulation of GABAergic neurons that express corticotropin-releasing factor (CRF, also known as corticotropin-releasing hormone or CRH). Optogenetic activation of CRF-expressing (CRF+) aBNST neurons in rodents increases avoidance behavior [15–17]. Stress exposure produces long-lasting changes in CRF+ aBNST neurons [16, 18, 19], including hyperexcitability [16, 18, 20–22], which are necessary and sufficient for increases in avoidance [23]. Suppressing CRF signaling in the aBNST [16, 24, 25], or inhibiting CRF+ aBNST neurons with optogenetics [26], ameliorates stress-induced avoidance.

Neural circuitry and behavioral evidence suggest CRF+ aBNST neurons are also involved in stress effects on motivation behaviors. CRF+ aBNST neurons project to [27–29] and inhibit [17, 30] ventral tegmental area (VTA) and nucleus accumbens (NAc) shell. Stress-induced neuroplasticity increases the inhibitory actions of CRF in VTA and impairs reward responsivity [31–33]. CRF + aBNST neurons also promote aversive effects of withdrawal [34], drug-seeking [35, 36], and cocaine-induced dopamine release [30]. In a two-choice progressive ratio task for sucrose or optogenetic photostimulation of CRF + BNST neurons paired with sucrose, rats lever press less for paired sucrose and photostimulation [37]. These results suggest activating CRF+ aBNST neurons is aversive [17, 26, 38], but do not indicate if these neurons play a role in effortful behaviors.

Therefore, it remains unclear whether CRF+ aBNST neurons are involved in motivation behaviors, such as willingness to exert effort for high value rewards. Since chronic stress in mice decreases effortful behaviors and results in long-lasting changes to BNST CRF+ neurons, including hyperexcitability [16, 18, 20, 21, 39], we used chemogenetics to dissect the role of CRF+ aBNST neurons in effortful behaviors.

[1]Department of Psychology, School of Arts and Sciences, Rutgers, The State University of New Jersey, Piscataway, NJ, USA. [2]Neuroscience Graduate Program, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA. [3]Behavioral and Systems Neuroscience Graduate Program, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA. [4]Department of Animal Sciences, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA.
✉email: ben.samuels@rutgers.edu

Received: 22 March 2023 Revised: 23 June 2023 Accepted: 27 June 2023
Published online: 14 July 2023

MATERIALS AND METHODS

Transgenic and wildtype mice

Mice were group housed with ad libitum access to standard chow (Lab Diet 5001 Rodent Diet, St. Louis, MO) and water, except when fasting during behavioral experiments. Throughout effort-related choice, concurrent choice, and free feeding training and testing, mice were maintained at 85–90% of their body weight via a daily supply of reinforcer pellets and/or standard chow. Mice were maintained on a 12:12 light/dark schedule, with lights on at 6 AM. Training and testing was completed between 7 AM and 4 PM.

A total of 26 (14 F, 12 M) C57BL/6 J and 39 (19 F, 20 M) CRF-ires-Cre mice were used. Heterozygous CRF-ires-Cre transgenic mice were produced by crossing C57BL/6 J wildtype (Jackson Labs #000664) with homozygous *Crf-ires-Cre* mice (Jackson Labs #012704), a strain that expresses Cre recombinase in neurons with endogenous CRF expression [40]. 18 (10 F, 8 M) heterozygous CRF-ires-Cre and 26 WT C57BL/6 J (14 F, 12 M) underwent effort-related choice testing and 21 (9 F, 12 M) heterozygous CRF-ires-Cre mice underwent concurrent choice testing.

All experiments were carried out in accordance with the National Institutes of Health guidelines for animal care and with Rutgers Institutional Animal Care and Use Committee approval.

Stereotaxic surgery and viral injections

All heterozygous CRF-ires-Cre mice (6–9 weeks) underwent stereotaxic surgery to induce virally-mediated expression of Gq-coupled Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) or control. Mice were anesthetized with 1–1.5% isoflurane and head-fixed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Lidocaine (Lidoject, Covetrus) was topically injected, and skin was prepared with betadine and 10% ethanol. Mice were bilaterally infused with 300 nL of virus into the anterodorsolateral BNST at: +0.14 mm anterior to Bregma; +/−1.00 lateral to the midline; −3.60 mm ventral from the surface of the brain, which resulted in viral expression throughout aBNST. DREADD mice were injected with AAV9-hSyn-DIO-hM3D(Gq)-mCherry (AddGene #44361, Watertown, MA), virus titer 1.8×10^{13}. Control mice were injected with one of two control viruses: AAV9-hSyn-DIO-mCherry (AddGene #50459), virus titer 2.17×10^{13} or AAV9-hSyn.HI.eGFP-Cre-WPRE-SV40, virus titer 2.7×10^{13} (AddGene #105540). The skull was sealed with bone wax (Med Vet International, Mettawa, IL), and the scalp was closed with VetBond (3 M, St. Paul, MN). Mice were given 3 weeks for recovery and viral expression before training for behavioral experiments.

Clozapine-n-oxide (CNO)

CNO (Sigma-Aldrich, St. Louis, MO) was dissolved in 1 mL dimethyl sulfoxide (DMSO, Fisher Scientific) and adjusted to a final concentration of 0.5 mg/ml CNO in 7.5% v/v DMSO in sterile saline, then filtered through a syringe filter. Vehicle solution consisted of 7.5% v/v DMSO in sterile saline. 30 min before behavioral testing, the abdomen was wiped with 10% ethanol, then either 2 mg/kg CNO solution or Vehicle solution were injected intraperitoneally (i.p.).

Supplementary Methods contain detailed behavioral and tissue processing protocols, as well as statistical analysis and experimental design methods.

RESULTS

Chemogenetic activation of CRF + aBNST neurons increases avoidance behavior in female and male mice

We bilaterally injected aBNST of CRF-ires-Cre mice with AAV encoding Cre-dependent Gq (activating)-DREADD ($n = 22$) or Cre-dependent control (GFP/mCherry) virus ($n = 17$) (Fig. 1A). Viral expression endured throughout testing and was observed throughout aBNST (Fig. 1B). Following effort-related choice testing, 18 mice (Gq-DREADD, $n = 10$; GFP, $n = 8$) underwent timed perfusions to confirm chemogenetic activation of aBNST neurons. Mice were injected i.p. with CNO and sacrificed 120 min later to measure CNO-induced cFOS activation [41, 42]. More cFOS + aBNST neurons were observed in Gq-DREADD-injected mice than controls ($t(16) = 2.679$, $p < 0.05$) (Fig. 1C, D).

Activating CRF + BNST neurons increases avoidance without affecting overall locomotion [15, 16, 26]. Similarly, Gq-DREADD mediated activation (Fig. 1E) of CRF+ aBNST neurons reduced time spent in the center of an open field (OF) relative to mCherry controls (unpaired *t*-test, $t(19) = 2.331$, $p < 0.05$) (Fig. 1F). There was no effect of sex ($F(1,17) = 4.092$, $p = 0.059$) on center time (S1A), and no effect of chemogenetic activation on locomotion, as OF distance traveled was similar between DREADD and mCherry control mice ($t(19) = 0.242$, $p = 0.811$) (S1B).

Chemogenetic activation of CRF + aBNST neurons in both female and male mice decreases willingness to exert effort for high value rewards in effort-related choice

Chronic stress leads to sustained hyperactivity of CRF+ aBNST neurons [16, 18, 20–22] and impairs effortful motivation behaviors [3, 5]. Therefore, we wondered whether acute chemogenetic activation of CRF+ aBNST neurons is sufficient to mimic the effects of stress on effort-related choice. A cohort of CRF-ires-Cre mice received bilateral injections of AAV encoding Cre-dependent Gq-DREADD ($n = 12$; 6 F, 6 M) or GFP control ($n = 8$; 4 F, 4 M). Three weeks later mice were fasted to 85–90% of their ad libitum body weight and then underwent training and testing in a Y-maze barrier task [3] to assess the effects of CRF+ aBNST neuron activation on effort-related choice (Fig. 2A). First, mice were trained to select a high reward (HR) arm containing 4 reinforcer pellets over a low reward (LR) arm containing 2 reinforcer pellets in a Y-maze. After 16 days of training, mice reached a criterion of 70% HR arm choice (S2A). Notably, there were no differences between groups on the final day of free choice training (S2A–C).

Next, a barrier (10, 15, or 20 cm) was placed in the HR arm (Fig. 2B) to increase effort requirements for the higher value reward while the lower value reward remained freely available [43, 44]. Female and male mice exposed to chronic stress shift to choosing the LR arm at shorter barrier heights than controls [3, 5]. All mice were administered acute i.p. injections of 2 mg/kg CNO 30 min before testing to activate CRF+ aBNST neurons in Gq-DREADD mice. Mice underwent 10 trials per day over 3 days each of 10, 15, and 20 cm barrier testing. A repeated measures two-way ANOVA with virus (DREADD or GFP) as a between-subjects factor and barrier height (10, 15, 20 cm) as a within-subjects factor demonstrated a significant interaction between virus and barrier height ($F(2,32) = 6.24$, $p < 0.01$). Control mice chose the HR arm significantly more often than Gq-DREADD mice at 10 cm ($p < 0.001$), 15 cm ($p < 0.001$), and 20 cm ($p < 0.005$) barrier heights. As expected, all mice chose the HR arm significantly less when presented with a 20 cm tall barrier, versus when presented with 10 ($p < 0.0001$) or 15 ($p < 0.01$) cm barriers (Fig. 2C). These results combine both female and male mice, as we saw no effect of sex at any barrier height (S2D). Therefore, highly specific acute Gq-DREADD-mediated activation of CRF+ aBNST neurons mimics stress effects on effort-related choice.

Following 20 cm barrier testing, we placed 10 cm barriers in both arms for 3 days of discrimination testing. Surprisingly, a two-way ANOVA with virus treatment (DREADD or Control GFP) as a between-subjects factor and testing day (days 1, 2, and 3) as a within-subjects factor indicated a significant interaction between testing day and virus treatment ($F(2,32) = 4.02$, $p < 0.05$) (S2E). Multiple comparisons indicate DREADD mice chose the HR arm significantly less than GFP Control mice on days 2 ($p < 0.001$) and 3 ($p < 0.0001$). One possible explanation for this result is Gq-DREADD mice chose the HR arm so infrequently over the previous 9 days of barrier testing that they became overtrained to the LR arm.

CNO can be converted to clozapine, which could lead to the behavioral effects seen in effort-related choice [45, 46]. To confirm CNO itself does not alter behavior in the Y-maze barrier task, female ($n = 14$) and male ($n = 12$) WT C57BL6/J control mice underwent effort-related choice training and testing. Mice were injected with either CNO ($n = 13$) or Vehicle ($n = 13$) before Y-maze barrier testing, and no effects of CNO ($F(1,24) = 1.05$, $p = 0.324$) were observed (S3).

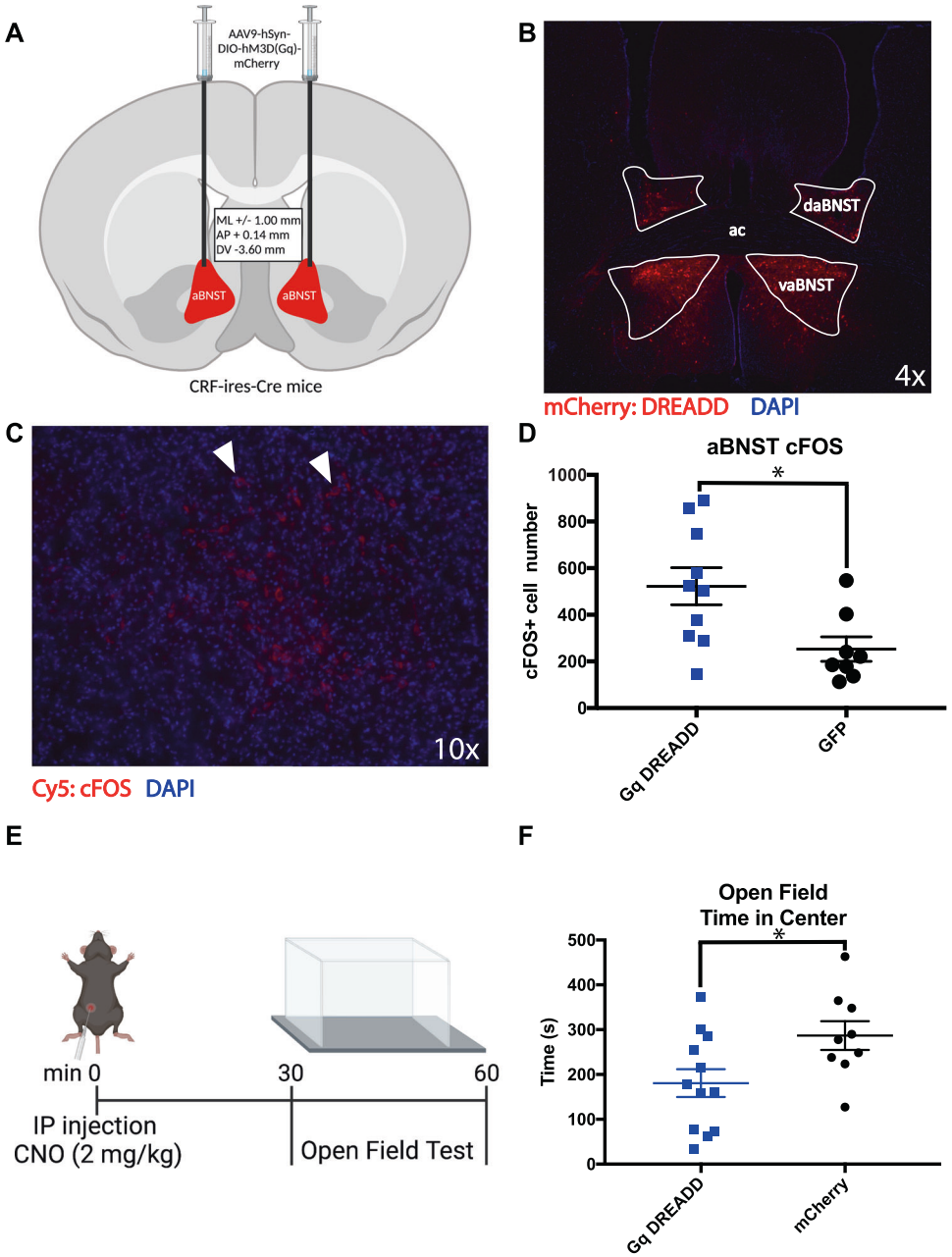


Fig. 1 Virally-mediated DREADD expression was confirmed to localize in the anterior bed nucleus of the stria terminalis (aBNST), a region associated with avoidance behaviors. Viral expression was induced through (**A**) bilateral infusion of Cre-dependent DREADD virus at the anterior dorsolateral BNST of transgenic CRF-ires-Cre mice (created with BioRender.com). **B** Viral expression, marked by red fluorescent marker mCherry, occurred throughout the aBNST. White lines delineate BNST borders of the dorsal aBNST, ventral aBNST (vaBNST) and the anterior commissure. Brain anatomy based on Allen Brain Atlas. **C** Gq-DREADD and GFP control mice were administered CNO, then sacrificed to image chemogenetically-induced cFOS expression in the aBNST. cFOS+ cells (Cy5, represented by arrowheads) in aBNST were (**D**) quantified and cFOS expression was higher in Gq-DREADD mice than GFP controls ($p < 0.05$). Gq-DREADD mice: $n = 10$, GFP mice: $n = 8$. **E** Gq-DREADD and control mCherry mice were administered clozapine-N-oxide (CNO) 30 min before behavioral testing (created with BioRender.com). **F** As expected, chemogenetic activation of the CRF-expressing neurons in the aBNST via CNO injection increases avoidance behavior in the open field, reducing time spent in the center for Gq-DREADD (M = 180.8 s, blue squares) but not mCherry control (M = 286.7 s, black points) mice ($t(19) = 2.331$, $p < 0.05$). Gq-DREADD mice: $n = 12$, mCherry mice: $n = 9$. Values are plotted as individual values and mean +/− SEM. *$p < 0.05$.

Male mice lever press more than females during progressive ratio and concurrent choice tasks

To evaluate the effect of chemogenetically activating CRF+ aBNST neurons on motivated behavior in a concurrent choice test, CRF-ires-Cre mice underwent stereotaxic surgery and received bilateral injection of either Gq-DREADD virus ($n = 12$; 5 F, 7 M) or mCherry control virus ($n = 9$; 7 F, 5 M) into the aBNST. After recovery, mice underwent training and testing in an instrumental progressive ratio with concurrent chow (PR/CHOW or concurrent choice) task (Fig. 3A). Mice first were trained to lever press in operant chambers, beginning with two days of magazine training, then two days of fixed ratio (FR)1 reinforcement schedule training, followed by single-sessions of variable ratio (VR)2, VR5, and VR20 training. Then, baseline progressive ratio (PR) measures were obtained via 10 daily 30-minute sessions, during which mice lever pressed on an FR(n + 1) x 15 schedule, requiring mice to press at

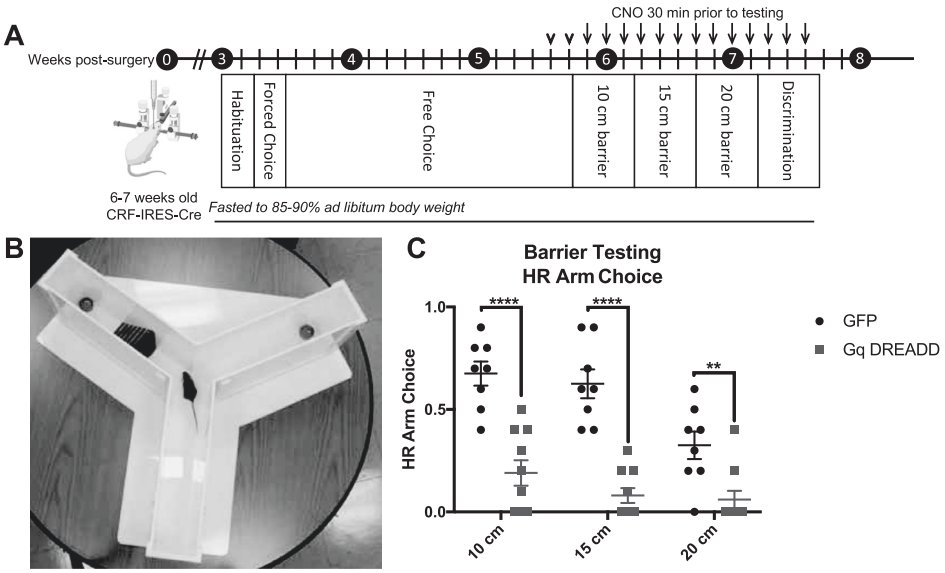


Fig. 2 Chemogenetic activation of CRF + BNST neurons reduced selection of the high reward (HR) arm in a Y-maze barrier task. **A** 6–7 week old CRF-ires-Cre mice were bilaterally injected with Cre-dependent Gq-DREADDs or a control Cre-dependent GFP virus. After recovery, mice were fasted and began training in the effort-related choice Y-maze barrier task. Training included 2 days of habituation, 2 days of forced chocie training, and 18 days of free choice training, wherein mice were trained in a Y-shaped maze to select an HR of of 4 sucrose pellets over a low reward (LR) of 2 pellets. On days 17 and 18 of free choice training, mice were injected with saline 30 min prior to training (arrowheads). Mice then underwent 3 days of 10 cm barrier testing, 3 days of 15 cm barrier testing, and 3 days of 20 cm barrier testing, prior to which mice were injected with CNO (arrows). During barrier testing, barriers were placed in the HR arm (**B**), so mice could choose to climb the barrier for an HR or traverse the other arm for an LR. **C** Gq-DREADD (blue squares) activation reduced HR arm choice, compared to GFP controls (black points), when tested in 10 ($p < 0.001$), 15 ($p < 0.001$) and 20 cm ($p < 0.005$) barrier conditions. HR arm choice is a proportion of 10 trials during which mice chose the HR arm. Gq-DREADD mice: $n = 10$, GFP mice: $n = 8$. Values are plotted as individual values and mean +/− SEM. $^{**}p < 0.01$; $^{****}p < 0.0001$.

an incrementally increasing frequency every 15 reinforcer pellets (FR1x15, FR2x15, etc) (Fig. 3B). A two-way ANOVA with sex as a between-subjects variable and training day (1–10) as a within-subjects variable found a main effect of sex ($F(1,19) = 11.02$, $p < 0.05$) on baseline PR lever pressing. Females lever pressed less than males throughout this progressive ratio task.

Baseline PR/CHOW lever pressing measures were then obtained over 5 days, during which mice could either exert effort by lever pressing on a PR schedule to consume palatable reinforcer pellets or consume standard lab chow freely available on the chamber floor (Fig. 3C). A two-way ANOVA revealed a main effect of sex ($F(1,19) = 19.93$, $p < 0.001$). Females lever pressed less than males throughout this concurrent choice task.

Prior to PR/CHOW testing, one trial of outcome devaluation assessed lever pressing when mice were in a refed state. All mice were given access in their home cages to standard lab chow 20 h before testing and reinforcers 4 h before testing. A two-way ANOVA with sex as a between-subjects variable and condition (PR, PR/CHOW, Refed) as a within-subjects variable revealed a significant interaction between sex and condition ($F(2,57) = 4.307$, $p < 0.05$), with main effects of both testing condition ($F(2,57) = 19.44$, $p < 0.0001$) and sex ($F(1,57)=$, $p < 0.0001$). Multiple comparisons indicate that male, but not female, mice lever pressed significantly less in the refed state, than during fasted PR ($p < 0.001$) and fasted PR/CHOW ($p < 0.0001$) (Fig. 3D). Therefore, the satiety-based outcome devaluation was effective in male mice.

Results from baseline training and outcome devaluation suggested individual differences in lever pressing may affect ability to detect reductions in motivation. Therefore, we separated mice into high- and low-performers, as described previously [47, 48]. Average lever pressing over the 5 days of PR/CHOW baseline training and median average lever pressing were calculated. Subjects with average lever presses above the median were designated high-performers, and those below the median were designated low performers (Fig. 3E). The divide between high-performing and low-performing mice was primarily driven by sex as no females were in the high-performing group. Since low-performing mice were not lever pressing enough to detect a possible Gq-DREADD mediated decrease in lever pressing, we focused our analyses on the high-performer male mice.

Chemogenetic activation of CRF + aBNST neurons decreases willingness to exert effort for high value rewards in a concurrent choice task in high-performing male mice

All high-performing male mice received CNO and Vehicle on alternating days of the 4 total days of PR/CHOW concurrent choice testing; thus, every mouse received two days of CNO injections and two days of Vehicle injections. Mice were injected i.p. 30 min before PR/CHOW testing and allowed to either lever press on a PR schedule or consume standard lab chow freely available on the chamber floor. A repeated measures two-way ANOVA of the high-performing male mice revealed a significant interaction between CNO treatment (CNO, VEH) and virus (Gq-DREADD, Control mCherry) ($F(1,9) = 0.022$, $p < 0.05$). Multiple comparisons established that Gq-DREADD mice lever pressed significantly less when injected with CNO than when injected with Vehicle ($p < 0.05$). CNO did not alter lever pressing in control mCherry mice ($p = 0.884$) (Fig. 3F). As expected, a two-way ANOVA of lever pressing suggests that Gq-DREADD mediated activation did not alter lever pressing behaviors in low-performing mice, and neither virus ($F(1,8) = 0.887$, $p = 0.374$) nor CNO ($F(1,8) = 2.314$, $p = 0.167$) significantly altered lever pressing in low performers (S4A). Time spent consuming the concurrently available standard lab chow was also quantified, and two-way ANOVAs revealed no effect of virus or sex ($F(3,16) = 0.744$, $p = 0.542$) or CNO treatment ($F(1,16) = 3.939$, $p = 0.065$) on time spent consuming chow (S4B). When only high performing males were analyzed, there was no effect of virus ($F(1,7) = 0.233$, $p = 0.644$) or CNO ($F(1,7) = 1.113$, $p = 0.326$) on time consuming chow (S4C). Therefore, Gq-DREADD mediated activation of CRF+ aBNST neurons in

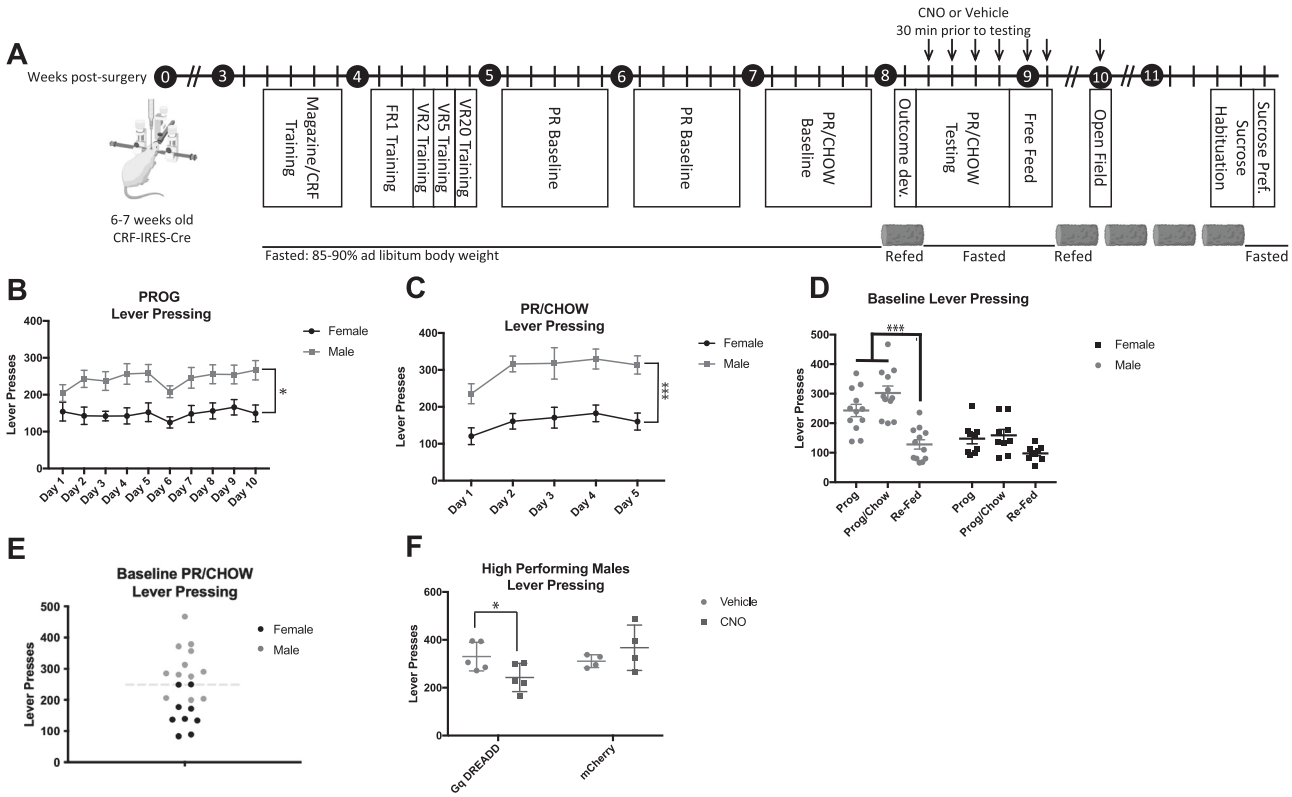


Fig. 3 Chemogenetic activation of CRF + BNST neurons decreased lever pressing for sucrose pellets in high-performing males. A 6–7-week-old CRF-ires-Cre transgenic mice were bilaterally injected with Cre-dependent Gq-DREADD or a control Cre-dependent mCherry virus. After recovery, mice were fasted and trained in operant chambers to lever press for sucrose pellets. Over 10 days, a progressive ratio (PR) baseline frequency of lever pressing was collected, wherein mice were trained to lever press at increasing frequencies on a (FR $(n + 1) \times 15$) PR reinforcement schedule. During PR/concurrent chow (CHOW) baseline training, concurrently available lab chow was freely available on the chamber floor. Mice were then re-fed and allowed ad libitum access to lab chow for 20 h, and sucrose pellets for 4 h, prior to an outcome devaluation trial. During 4 days of PR/CHOW testing, mice were injected with alternating treatments of CNO or Vehicle 30 min before PR/CHOW testing, to assess how chemogenetic activation of CRF + BNST neurons alters lever pressing in the PR/CHOW task. Appetite was assessed over two days of free feed testing, wherein mice were injected with alternating treatments of CNO or Vehicle. During open field and sucrose preference testing, all mice were injected with CNO solution. Mice were fasted to 85–90% of their ad libitum body weights throughout training and testing, except where marked with a chow icon, which represents free access to standard lab chow. Arrows represent daily CNO or Vehicle injections, 30 min before testing. **B** Males (grey) lever pressed significantly more frequently than females (black) during PR baseline training ($p < 0.05$) on days 2, 4, 5, 7, 8 ($p < 0.05$), and 10 ($p < 0.01$). **C** Males also pressed more than females during PR/CHOW ($p < 0.001$) baseline training, including days 1 ($p < 0.05$), 2 ($p < 0.001$), and 3–5 ($p < 0.01$). **D** Males lever pressed significantly less when re-fed during outcome devaluation, compared to PR ($p < 0.001$) or PR/CHOW ($p < 0.0001$) baseline. Female lever pressing did not significantly decline during outcome devaluation. Female: $n = 12$, male: $n = 12$. **E** Mice were designated high performers if their average PR/CHOW baseline lever pressing was greater than the median lever pressing (perforated line) for all mice. All high-performing mice were males (gray points), but all females (black points) were at or below the median baseline frequency. 3 low-performing males were excluded. **F** In the PR/Chow test, high-performing Gq-DREADD males, when injected with CNO, lever pressed less than when injected with Vehicle ($p < 0.05$). No effect of CNO was observed in mCherry control mice ($p = 0.884$). Gq-DREADD high-performers: $n = 5$, mCherry high-performers: $n = 4$. Values are plotted as individual values and mean +/− SEM. *$p < 0.05$; ***$p < 0.001$.

high-performing male mice decreased lever pressing for a high value reward when standard chow was freely available.

Chemogenetic activation of CRF + aBNST neurons does not decrease free feeding

To assess whether reductions in effortful behavior in effort-related choice or concurrent choice testing are due to decreased feeding behavior, CRF-ires-Cre mice underwent a two-day free feeding test 24 h following PR/CHOW testing. Mice remained fasted to 90% of their ad libitum body weight and, 30 min before testing, were injected with CNO or Vehicle on alternating days. Mice were then placed in their home cage with a pellet of standard lab chow for 1 h, and chow consumed (g) was measured. A two-way ANOVA revealed a significant interaction between virus treatment and CNO/VEH treatment ($F(1,19) = 15.45$, $p < 0.001$), but no main effect of CNO treatment alone ($F(1,19) = 1.302$, $p = 0.268$) or virus treatment alone ($F(1,19) = 0.992$, $p = 0.332$). Multiple comparisons found that DREADD mice injected with CNO consumed significantly more chow than when injected with Vehicle in this free feeding task ($p < 0.01$) (Fig. 4A). Analysis of high-performing males showed similar results. A two-way ANOVA revealed an interaction between virus treatment and CNO ($F(1,7) = 7.834$, $p < 0.05$) (S5A). Multiple comparisons, however, only revealed a non-significant increase in chow consumption in Gq-DREADD mice injected with CNO ($p = 0.071$). A two-way ANOVA with treatment and sex as between-subjects variables indicated a significant effect of sex on chow consumed ($F(1,34) = 24.32$, $p < 0.0001$), wherein DREADD females injected with vehicle ate less than their male counterparts ($p < 0.031$) (S5B), but high and low performers in PR/CHOW consumed similar amounts of chow in the free feeding task (S5C). Overall, these results indicate Gq-DREADD mediated activation of CRF+ aBNST neurons does not decrease free feeding.

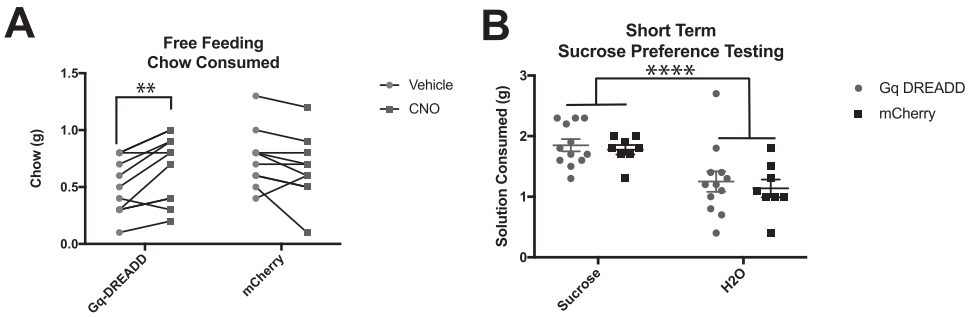


Fig. 4 Chemogenetic activation of CRF + BNST neurons increases appetite and does not affect sucrose preference. A Gq-DREADD and mCherry mice were injected with CNO or Vehicle over two alternating days, then chow consumption was measured in the home cage for 1 h. Chow consumption was significantly higher in Gq-DREADD mice injected with CNO (blue points), compared to those same mice injected with Vehicle (gray points) ($p < 0.01$). Gq-DREADD mice: $n = 12$, mCherry mice: $n = 9$ (**B**) Sucrose preference was measured in mice deprived of food and water for 18 h and injected with CNO 30 min before given the choice between 1% sucrose solution or water. All mice preferred sucrose solution over water ($p < 0.01$), and there was no effect of virus ($p = 0.567$) on sucrose consumption. Gq-DREADD mice: $n = 12$, mCherry mice: $n = 8$. Values are plotted as individual values and mean +/− SEM. **$p < 0.01$; ****$p < 0.0001$. Taken together, appetite and sucrose preference do not explain the reduction in effort observed in the Y-maze barrier task or PR/CHOW.

Chemogenetic activation of CRF + aBNST neurons does not alter sucrose preference

To exclude reward preference as a factor in reducing lever pressing and barrier-climbing, a short-term sucrose preference protocol was performed after the free feeding and open field testing. For 48 h, mice in their home cages habituated to a two-bottle choice between water and a 1% sucrose solution. Following habituation, mice were fasted for 18 h with no access to food or water. During short-term sucrose preference testing, all mice were administered CNO 30 min before testing, then mice were placed in their home cage with two-bottle choice for 1 h. A two-way ANOVA test with virus treatment as a between-subjects variable and solution type (water, sucrose) as a within-subjects variable established a main effect of solution type ($F(1,18) = 30.08$, $p < 0.0001$) on consumption, but no effect of virus treatment ($F(1,18) = 0.341$, $p = 0.567$). Multiple comparisons demonstrate that both DREADD ($p < 0.01$) and mCherry control ($p < 0.01$) mice preferred sucrose solution over water (Fig. 4B). Analysis of high-performing males suggested no effect of virus or CNO treatment on sucrose preference (S5D). There was no effect of sex (S5E) or PR/Chow performance (S5F) on sucrose preference. Therefore, Gq-DREADD mediated activation of CRF+ aBNST neurons does not alter reward preference.

DISCUSSION

Exposure to chronic stress increases avoidance and decreases motivation behaviors. aBNST CRF+ neurons are involved in stress-induced avoidance [16, 18, 20, 21, 39], but it was unknown whether they also play a role in effortful responding. A potential mechanism is that stress drives drug-seeking [31] and activates CRF + BNST projections that inhibit dopaminergic reward-related regions, including the VTA and NAc [30, 33], thereby reducing motivated behavior. Here, we tested whether highly specific chemogenetic activation of CRF+ aBNST neurons mimics stress effects on willingness to exert effort for a high value reward when a low value reward is freely available in both effort-related choice and concurrent choice.

The Y-maze barrier task used for effort-related choice [3] was adapted from Salamone et al. [49] to assess cost/benefit analysis in mice. Chemogenetically activating CRF+ aBNST neurons resulted in both female and male mice choosing the LR arm more often than control mice at all barrier heights. This reduction in effortful behavior is similar to what is observed in male and female mice exposed to chronic stress and in male mice exposed to chronic corticosterone [3–5]. Therefore, specific activation of CRF+ aBNST neurons is sufficient to mimic chronic stress effects on effort-related choice.

After effort-related choice testing, mice underwent three days of discrimination testing, with 10 cm barriers in both the HR and LR arms. Surprisingly, Gq-DREADD mice continued to choose the LR arm during discrimination. This could be a learning deficit, however, lesion studies suggest BNST is not necessary for spatial learning [50]. More likely Gq-DREADD mice became overtrained after repeatedly choosing the LR arm over the 90 total barrier testing trials. They failed to reverse their arm choice as a function of habitual behavior, wherein choosing the LR arm became automatic and resistant to change. Interestingly, stress can augment tendencies to repeat behaviors [4]. WT DREADD-free controls confirmed that CNO alone does not alter arm choice or barrier climbing abilities.

We also tested whether chemogenetically activating CRF+ aBNST neurons decreased willingness to exert effort for high value rewards in concurrent choice. Mice were presented with a progressively higher-effort/high-reward choice to lever press on a progressive ratio schedule for a palatable reinforcer pellet or a low-effort/low-reward choice to consume standard chow freely available on the chamber floor. Concurrent choice measures motivated behavior [51] and is associated with dopamine signaling and activity in mesolimbic reward-related regions [52]. Baseline PR showed a clear sex difference, where female mice lever pressed significantly less than males in both PR and PR/CHOW conditions. Therefore, a floor effect existed in the females where it was difficult to measure decreased lever pressing from a low baseline in both an outcome devaluation task and with Gq-DREADD mediated activation of CRF+ aBNST neurons in concurrent choice. Previous studies found sex differences in appetitive learning and lever pressing tasks, where females are less engaged than males [53–56]. Future experiments must determine the cause of these sex differences in lever pressing.

Previous studies [47, 48] have segregated rodents into high- and low-performers in PR/CHOW [47, 48, 57], so we similarly grouped mice using a median-split of baseline lever pressing. All high-performers were males and most low-performers were females. During concurrent choice testing, high-performing male mice received injections of either CNO or Vehicle on alternating days. These high-performers lever pressed significantly less during DREADD-mediated activation of CRF+ aBNST neurons relative to when they received vehicle solution. This reduction in lever pressing is reminiscent of chronic stress exposure or corticosterone administration [3–5]. To confirm that reduced lever pressing was not caused by chemogenetically-induced appetite suppression, we performed a free feeding task. Chemogenetic activation of CRF+ aBNST neurons increased chow consumption, so appetite suppression is unlikely to drive reductions in lever pressing. These

results are consistent with studies demonstrating BNST stimulation increases appetite, possibly via GABAergic BNST projections to ventrolateral PAG [58] and LH [59]. Taken together, these results suggest activating CRF+ aBNST neurons is sufficient to mimic stress effects on concurrent choice in high-performing male mice. However, the lack of female engagement limits the interpretation of the concurrent choice assay. Another interpretation is that activating CRF+ aBNST neurons may have a greater effect on motivation in assays involving a physical barrier than in time-limited operant assays.

Role of CRF+ aBNST neurons in motivated behavior

We used chemogenetics to mimic the activating effects of stress on CRF+ aBNST neurons [16, 18, 20, 21, 39]. Results from both sexes in effort-related choice and from high-performing males in concurrent choice demonstrate that activating CRF+ aBNST neurons reduces effortful motivation without affecting appetite, sucrose preference, or locomotion. These results demonstrate the critical role of CRF+ aBNST neurons in mediating effortful motivation behaviors.

Experiments over the past decade support a "valence surveillance" [60] role of BNST, using self-stimulation and real-time place preference (RTPP) tests to show that stimulating specific BNST circuits [38] and cell types [15, 38] can be rewarding and promote responding in a Pavlovian reward task [61]. However, RTPP, self-stimulation, and reward learning tests do not assess effortful behavior. Baumgartner et al. [37] found that rats lever pressed less when sucrose rewards were paired with optogenetic activation of CRF + BNST neurons, concluding that these neurons promote aversive motivation (the drive to avoid negatively valenced states). This concurs with findings that CRF + BNST neurons drive avoidance in aversive environments [15–17]. An aversive motivation role could also explain why CRF + BNST neurons promote drug-seeking behaviors [35, 36], possibly to avoid withdrawal [62]. Our experiments demonstrate a role for CRF + BNST neurons in stress-induced reductions in incentive motivation (the drive to approach and work for natural rewards, such as food). Motivation behavior is associated with dopaminergic mesolimbic pathways. Optogenetically activating VTA-projecting GABAergic BNST neurons is anxiolytic and rewarding [38]. Here, we used cell-specific chemogenetic activation of CRF+ aBNST neurons, which release GABA [63, 64] in the VTA [17, 27, 29, 30] and NAc [17]. We hypothesize that chemogenetics activated the inhibitory $BNST^{CRF}$-VTA circuit, reducing effortful motivation behaviors.

CRF+ aBNST neurons also co-release CRF [63, 64], which has complex effects on mesolimbic reward areas [65]. Bryce and Floresco [66] described an effort discounting task that allowed rats to either lever press once for a low reward of one pellet or press 4 times for more food. After stress, or intracerebroventricular or intra-VTA infusion of CRF, rats chose the low effort/low reward behavior. Intra-VTA infusions of CRF may increase [67] or decrease [33] dopamine signaling, and may drive [32, 67] or block [33] reward-seeking behaviors. A potential "valence flip" may explain these opposing results, wherein the effects of CRF depend on environmental conditions, such as substance abuse or stress [65, 68]. Further experiments should investigate whether chemogenetically activating CRF+ aBNST neurons results in co-release of CRF, and the nature of CRF modulation in mesolimbic reward areas.

REFERENCES

1. Der-Avakian A, Barnes SA, Markou A, Pizzagalli DA. Translational assessment of reward and motivational deficits in psychiatric disorders. Curr Top Behav Neurosci. 2016;28:231–62.
2. Cooper JA, Arulpragasam AR, Treadway MT. Anhedonia in depression: biological mechanisms and computational models. Curr Opin Behav Sci. 2018;22:128–35.
3. Dieterich A, Yohn CN, Samuels BA. Chronic stress shifts effort-related choice behavior in a Y-maze barrier task in mice. J Vis Exp. 2020;162:e61548.
4. Dieterich A, Srivastava P, Sharif A, Stech K, Floeder J, Yohn SE, et al. Chronic corticosterone administration induces negative valence and impairs positive valence behaviors in mice. Transl Psychiatry. 2019;9:337.
5. Dieterich A, Liu T, Samuels BA. Chronic non-discriminatory social defeat stress reduces effort-related motivated behaviors in male and female mice. Transl Psychiatry. 2021;11:125.
6. Goode TD, Ressler RL, Acca GM, Miles OW, Maren S. Bed nucleus of the stria terminalis regulates fear to unpredictable threat signals. Elife. 2019;8:e46525.
7. Alvarez RP, Chen G, Bodurka J, Kaplan R, Grillon C. Phasic and sustained fear in humans elicits distinct patterns of brain activity. Neuroimage. 2011;55:389–400.
8. Choi JM, Padmala S, Pessoa L. Impact of state anxiety on the interaction between threat monitoring and cognition. Neuroimage. 2012;59:1912–23.
9. Klumpers F, Kroes MC, Heitland I, Everaerd D, Akkermans SE, Oosting RS, et al. Dorsomedial prefrontal cortex mediates the impact of serotonin transporter linked polymorphic region genotype on anticipatory threat reactions. Biol Psychiatry. 2015;78:582–9.
10. McMenamin BW, Langeslag SJ, Sirbu M, Padmala S, Pessoa L. Network organization unfolds over time during periods of anxious anticipation. J Neurosci. 2014;34:11261–73.
11. McMenamin BW, Pessoa L. Discovering networks altered by potential threat ("anxiety") using quadratic discriminant analysis. Neuroimage. 2015;116:1–9.
12. Brinkmann L, Buff C, Neumeister P, Tupak SV, Becker MP, Herrmann MJ, et al. Dissociation between amygdala and bed nucleus of the stria terminalis during threat anticipation in female post-traumatic stress disorder patients. Hum Brain Mapp. 2017;38:2190–205.
13. Buff C, Brinkmann L, Bruchmann M, Becker MPI, Tupak S, Herrmann MJ, et al. Activity alterations in the bed nucleus of the stria terminalis and amygdala during threat anticipation in generalized anxiety disorder. Soc Cogn Affect Neurosci. 2017;12:1766–74.
14. Buff C, Brinkmann L, Neumeister P, Feldker K, Heitmann C, Gathmann B, et al. Specifically altered brain responses to threat in generalized anxiety disorder relative to social anxiety disorder and panic disorder. Neuroimage Clin. 2016;12:698–706.
15. Giardino WJ, Eban-Rothschild A, Christoffel DJ, Li SB, Malenka RC, de Lecea L. Parallel circuits from the bed nuclei of stria terminalis to the lateral hypothalamus drive opposing emotional states. Nat Neurosci. 2018;21:1084–95.
16. Hu P, Liu J, Maita I, Kwok C, Gu E, Gergues MM, et al. Chronic stress induces maladaptive behaviors by activating corticotropin-releasing hormone signaling in the mouse oval bed nucleus of the stria terminalis. J Neurosci. 2020;40:2519–37.
17. Marcinkiewcz CA, Mazzone CM, D'Agostino G, Halladay LR, Hardaway JA, DiBerto JF, et al. Serotonin engages an anxiety and fear-promoting circuit in the extended amygdala. Nature. 2016;537:97–101.
18. Dabrowska J, Hazra R, Guo JD, Li C, Dewitt S, Xu J, et al. Striatal-enriched protein tyrosine phosphatase-STEPs toward understanding chronic stress-induced activation of corticotrophin releasing factor neurons in the rat bed nucleus of the stria terminalis. Biol Psychiatry. 2013;74:817–26.
19. Hammack SE, Cheung J, Rhodes KM, Schutz KC, Falls WA, Braas KM, et al. Chronic stress increases pituitary adenylate cyclase-activating peptide (PACAP) and brain-derived neurotrophic factor (BDNF) mRNA expression in the bed nucleus of the stria terminalis (BNST): roles for PACAP in anxiety-like behavior. Psychoneuroendocrinology. 2009;34:833–43.
20. Partridge JG, Forcelli PA, Luo R, Cashdan JM, Schulkin J, Valentino RJ, et al. Stress increases GABAergic neurotransmission in CRF neurons of the central amygdala and bed nucleus stria terminalis. Neuropharmacology. 2016;107:239–50.
21. Snyder AE, Salimando GJ, Winder DG, Silberman Y. Chronic intermittent ethanol and acute stress similarly modulate BNST CRF neuron activity via noradrenergic signaling. Alcohol Clin Exp Res. 2019;43:1695–701.
22. Ventura-Silva AP, Pego JM, Sousa JC, Marques AR, Rodrigues AJ, Marques F, et al. Stress shifts the response of the bed nucleus of the stria terminalis to an anxiogenic mode. Eur J Neurosci. 2012;36:3396–406.
23. Maita I, Roepke TA, Samuels BA. Chronic stress-induced synaptic changes to corticotropin-releasing factor-signaling in the bed nucleus of the stria terminalis. Front Behav Neurosci. 2022;16:903782.
24. Valdez GR, Zorrilla EP, Roberts AJ, Koob GF. Antagonism of corticotropin-releasing factor attenuates the enhanced responsiveness to stress observed during protracted ethanol abstinence. Alcohol. 2003;29:55–60.
25. Sahuque LL, Kullberg EF, McGeehan AJ, Kinder JR, Hicks MP, Blanton MG, et al. Anxiogenic and aversive effects of corticotropin-releasing factor (CRF) in the bed nucleus of the stria terminalis in the rat: role of CRF receptor subtypes. Psychopharmacol (Berl). 2006;186:122–32.
26. Kim SY, Adhikari A, Lee SY, Marshel JH, Kim CK, Mallory CS, et al. Diverging neural pathways assemble a behavioural state from separable features in anxiety. Nature. 2013;496:219–23.
27. Dabrowska J, Martinon D, Moaddab M, Rainnie DG. Targeting corticotropin-releasing factor projections from the oval nucleus of the bed nucleus of the stria

terminalis using cell-type specific neuronal tracing studies in mouse and rat brain. J Neuroendocrinol. 2016;28:12.
28. Dong HW, Swanson LW. Organization of axonal projections from the anterolateral area of the bed nuclei of the stria terminalis. J Comp Neurol. 2004;468:277–98.
29. Rodaros D, Caruana DA, Amir S, Stewart J. Corticotropin-releasing factor projections from limbic forebrain and paraventricular nucleus of the hypothalamus to the region of the ventral tegmental area. Neuroscience. 2007;150:8–13.
30. Silberman Y, Matthews RT, Winder DG. A corticotropin releasing factor pathway for ethanol regulation of the ventral tegmental area in the bed nucleus of the stria terminalis. J Neurosci. 2013;33:950–60.
31. Wang B, Shaham Y, Zitzman D, Azari S, Wise RA, You ZB. Cocaine experience establishes control of midbrain glutamate and dopamine by corticotropin-releasing factor: a role in stress-induced relapse to drug seeking. J Neurosci. 2005;25:5389–96.
32. Lodge DJ, Grace AA. Acute and chronic corticotropin-releasing factor 1 receptor blockade inhibits cocaine-induced dopamine release: correlation with dopamine neuron activity. J Pharm Exp Ther. 2005;314:201–6.
33. Wanat MJ, Bonci A, Phillips PE. CRF acts in the midbrain to attenuate accumbens dopamine release to rewards but not their predictors. Nat Neurosci. 2013;16:383–5.
34. Wenzel JM, Cotten SW, Dominguez HM, Lane JE, Shelton K, Su ZI, et al. Noradrenergic beta-receptor antagonism within the central nucleus of the amygdala or bed nucleus of the stria terminalis attenuates the negative/anxiogenic effects of cocaine. J Neurosci. 2014;34:3467–74.
35. Vranjkovic O, Gasser PJ, Gerndt CH, Baker DA, Mantsch JR. Stress-induced cocaine seeking requires a beta-2 adrenergic receptor-regulated pathway from the ventral bed nucleus of the stria terminalis that regulates CRF actions in the ventral tegmental area. J Neurosci. 2014;34:12504–14.
36. Erb S, Stewart J. A role for the bed nucleus of the stria terminalis, but not the amygdala, in the effects of corticotropin-releasing factor on stress-induced reinstatement of cocaine seeking. J Neurosci. 1999;19:RC35.
37. Baumgartner HM, Schulkin J, Berridge KC. Activating corticotropin-releasing factor systems in the nucleus accumbens, amygdala, and bed nucleus of stria terminalis: incentive motivation or aversive motivation? Biol Psychiatry. 2021;89:1162–75.
38. Jennings JH, Sparta DR, Stamatakis AM, Ung RL, Pleil KE, Kash TL, et al. Distinct extended amygdala circuits for divergent motivational states. Nature. 2013;496:224–8.
39. Hu P, Maita I, Phan ML, Gu E, Kwok C, Dieterich A, et al. Early-life stress alters affective behaviors in adult mice through persistent activation of CRH-BDNF signaling in the oval bed nucleus of the stria terminalis. Transl Psychiatry. 2020;10:396.
40. Taniguchi H, He M, Wu P, Kim S, Paik R, Sugino K, et al. A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. Neuron. 2011;71:995–1013.
41. Hoffman GE, Smith MS, Verbalis JG. c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. Front Neuroendocrinol. 1993;14:173–213.
42. Jendryka M, Palchaudhuri M, Ursu D, van der Veen B, Liss B, Katzel D, et al. Pharmacokinetic and pharmacodynamic actions of clozapine-N-oxide, clozapine, and compound 21 in DREADD-based chemogenetics in mice. Sci Rep. 2019;9:4522.
43. Salamone JD, Yohn SE, Lopez-Cruz L, San Miguel N, Correa M. Activational and effort-related aspects of motivation: neural mechanisms and implications for psychopathology. Brain. 2016;139:1325–47.
44. Salamone JD, Correa M, Ferrigno S, Yang JH, Rotolo RA, Presby RE. The psychopharmacology of effort-related decision making: dopamine, adenosine, and insights into the neurochemistry of motivation. Pharm Rev. 2018;70:747–62.
45. MacLaren DA, Browne RW, Shaw JK, Krishnan Radhakrishnan S, Khare P, Espana RA, et al. Clozapine n-oxide administration produces behavioral effects in long-evans rats: implications for designing DREADD experiments. eNeuro. 2016;3:5.
46. Manvich DF, Webster KA, Foster SL, Farrell MS, Ritchie JC, Porter JH, et al. The DREADD agonist clozapine N-oxide (CNO) is reverse-metabolized to clozapine and produces clozapine-like interoceptive stimulus effects in rats and mice. Sci Rep. 2018;8:3840.
47. Randall PA, Lee CA, Podurgiel SJ, Hart E, Yohn SE, Jones M, et al. Bupropion increases selection of high effort activity in rats tested on a progressive ratio/chow feeding choice procedure: implications for treatment of effort-related motivational symptoms. Int J Neuropsychopharmacol. 2014;18.2:pyu017.
48. Randall PA, Lee CA, Nunes EJ, Yohn SE, Nowak V, Khan B, et al. The VMAT-2 inhibitor tetrabenazine affects effort-related decision making in a progressive ratio/chow feeding choice task: reversal with antidepressant drugs. PLoS One. 2014;9:e99320.
49. Salamone JD, Cousins MS, Bucher S. Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure. Behav Brain Res. 1994;65:221–9.
50. Pezuk P, Goz D, Aksoy A, Canbeyli R. BNST lesions aggravate behavioral despair but do not impair navigational learning in rats. Brain Res Bull. 2006;69:416–21.
51. Salamone JD, Steinpreis RE, McCullough LD, Smith P, Grebel D, Mahan K. Haloperidol and nucleus accumbens dopamine depletion suppress lever pressing for food but increase free food consumption in a novel food choice procedure. Psychopharmacol (Berl). 1991;104:515–21.
52. Salamone JD, Correa M, Yohn S, Lopez Cruz L, San Miguel N, Alatorre L. The pharmacology of effort-related choice behavior: dopamine, depression, and individual differences. Behav Process. 2016;127:3–17.
53. Becker-Krail DD, Ketchesin KD, Burns JN, Zong W, Hildebrand MA, DePoy LM, et al. Astrocyte molecular clock function in the nucleus accumbens is important for reward-related behavior. Biol Psychiatry. 2022;92:68–80.
54. Bryce CA, Floresco SB. Central CRF and acute stress differentially modulate probabilistic reversal learning in male and female rats. Behav Brain Res. 2021;397:112929.
55. Mishima N, Higashitani F, Teraoka K, Yoshioka R. Sex differences in appetitive learning of mice. Physiol Behav. 1986;37:263–8.
56. Orsini CA, Willis ML, Gilbert RJ, Bizon JL, Setlow B. Sex differences in a rat model of risky decision making. Behav Neurosci. 2016;130:50–61.
57. Randall PA, Pardo M, Nunes EJ, Lopez Cruz L, Vemuri VK, Makriyannis A, et al. Dopaminergic modulation of effort-related choice behavior as assessed by a progressive ratio chow feeding choice task: pharmacological studies and the role of individual differences. PLoS One. 2012;7:e47934.
58. Hao S, Yang H, Wang X, He Y, Xu H, Wu X, et al. The lateral hypothalamic and BNST GABAergic projections to the anterior ventrolateral periaqueductal gray regulate feeding. Cell Rep. 2019;28:616–24.
59. Jennings JH, Rizzi G, Stamatakis AM, Ung RL, Stuber GD. The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding. Science. 2013;341:1517–21.
60. Lebow MA, Chen A. Overshadowed by the amygdala: the bed nucleus of the stria terminalis emerges as key to psychiatric disorders. Mol Psychiatry. 2016;21:450–63.
61. Kaouane N, Ada S, Hausleitner M, Haubensak W. Dorsal bed nucleus of the stria terminalis-subcortical output circuits encode positive bias in pavlovian fear and reward. Front Neural Circuits. 2021;15:772512.
62. Silberman Y, Winder DG. Corticotropin releasing factor and catecholamines enhance glutamatergic neurotransmission in the lateral subdivision of the central amygdala. Neuropharmacology. 2013;70:316–23.
63. Dabrowska J, Hazra R, Guo JD, Dewitt S, Rainnie DG. Central CRF neurons are not created equal: phenotypic differences in CRF-containing neurons of the rat paraventricular hypothalamus and the bed nucleus of the stria terminalis. Front Neurosci. 2013;7:156.
64. Pomrenze MB, Millan EZ, Hopf FW, Keiflin R, Maiya R, Blasio A, et al. A transgenic rat for investigating the anatomy and function of corticotrophin releasing factor circuits. Front Neurosci. 2015;9:487.
65. Hollon NG, Burgeno LM, Phillips PE. Stress effects on the neural substrates of motivated behavior. Nat Neurosci. 2015;18:1405–12.
66. Bryce CA, Floresco SB. Perturbations in effort-related decision-making driven by acute stress and corticotropin-releasing factor. Neuropsychopharmacology. 2016;41:2147–59.
67. Wanat MJ, Hopf FW, Stuber GD, Phillips PE, Bonci A. Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih. J Physiol. 2008;586:2157–70.
68. Lemos JC, Wanat MJ, Smith JS, Reyes BA, Hollon NG, Van Bockstaele EJ, et al. Severe stress switches CRF action in the nucleus accumbens from appetitive to aversive. Nature. 2012;490:402–6.

AUTHOR CONTRIBUTIONS

IM, AB, BAS, and TAR conceived of the experiments. IM, AB, KC, AP, MM, JS, TL, RS, MP, PH conducted or assisted with experiments. IM wrote the first draft of the paper, and BAS and TAR revised.

FUNDING

This work was supported by National Institutes of Mental Health (NIMH) Grant R01 MH123544 (BAS, TAR).

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41386-023-01646-9.

Correspondence and requests for materials should be addressed to Benjamin Adam Samuels.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.