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Activating Corticotropin-Releasing Factor Systems in the Nucleus Accumbens, Amygdala, and Bed Nucleus of Stria Terminalis: Incentive Motivation or Aversive Motivation?

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ABSTRACT

BACKGROUND: Corticotropin-releasing factor (CRF) neural systems are important stress mechanisms in the central amygdala (CeA), bed nucleus of stria terminalis (BNST), nucleus accumbens (NAc), and related structures. CRF-containing neural systems are traditionally posited to generate aversive distress states that motivate overconsumption of rewards and relapse in addiction. However, CRF-containing systems may alternatively promote incentive motivation to increase reward pursuit and consumption without requiring aversive states.

METHODS: We optogenetically stimulated CRF-expressing neurons in the CeA, BNST, or NAc using *Crh*-Cre+ rats (n = 37 female, n = 34 male) to investigate roles in incentive motivation versus aversive motivation. We paired CRF-expressing neuronal stimulations with earning sucrose rewards in two-choice and progressive ratio tasks and investigated recruitment of distributed limbic circuitry. We further assessed valence with CRF-containing neuron laser self-stimulation tasks.

RESULTS: Channelrhodopsin excitation of CRF-containing neurons in the CeA and NAc amplified and focused incentive motivation and recruited activation of mesocorticolimbic reward circuitry. CRF systems in both the CeA and NAc supported laser self-stimulation, amplified incentive motivation for sucrose in a breakpoint test, and focused "wanting" on laser-paired sucrose over a sucrose alternative in a two-choice test. Conversely, stimulation of CRF-containing neurons in the BNST produced negative valence or aversive effects and recruited distress-related circuitry, as stimulation was avoided and suppressed motivation for sucrose.

CONCLUSIONS: CRF-containing systems in the NAc and CeA can promote reward consumption by increasing incentive motivation without involving aversion. In contrast, stimulation of CRF-containing systems in the BNST is aversive but suppresses sucrose reward pursuit and consumption rather than increase, as predicted by traditional hedonic self-medication hypotheses.

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Corticotropin-releasing factor (CRF) is triggered by diverse aversive stressors to initiate behavioral and physiological stress responses (1–11). CRF-expressing neurons are concentrated in the hypothalamic paraventricular nucleus (PVN) but also occur in the nucleus accumbens (NAc) and in extended amygdala components such as the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST) (12–24).

Stress can trigger relapse in addiction or eating disorders (25–27). Traditional views suggest that CRF-containing systems increase reward consumption primarily by mediating the negative valence of stress, creating unpleasant states that promote drug relapse or eating for hedonic self-medication (27–29). In the opponent-process theory of addiction (30–32), taking addictive drugs activates a pleasant A-process, which is posited to trigger underlying longer-lasting aversive B-processes to create an unpleasant opponent B-state of withdrawal. In particular, opponent-process neuroscience models of addiction have posited that activation of CeA and BNST CRF-containing systems generates unpleasant withdrawal symptoms, again leading to relapse via hedonic self-medication (27–29,32–35).

However, CRF systems may also activate to changing events that mobilize biobehavioral responses, whether stressful or not (9–11). For example, CRF-containing neurons can be activated by positive reward stimuli (10–15,36). Some CRF systems may have positively valenced roles in promoting appetitive incentive motivation without inducing negative distress or withdrawal. For instance, NAc CRF microinjections in rats increase cue-triggered "wanting" for sucrose during Pavlovian instrumental transfer testing comparable with dopamine-stimulating amphetamine microinjections (13). NAc CRF microinjections can also establish positive conditioned place preference and increase NAc dopamine release in mice, only becoming aversive following severe stress (14).

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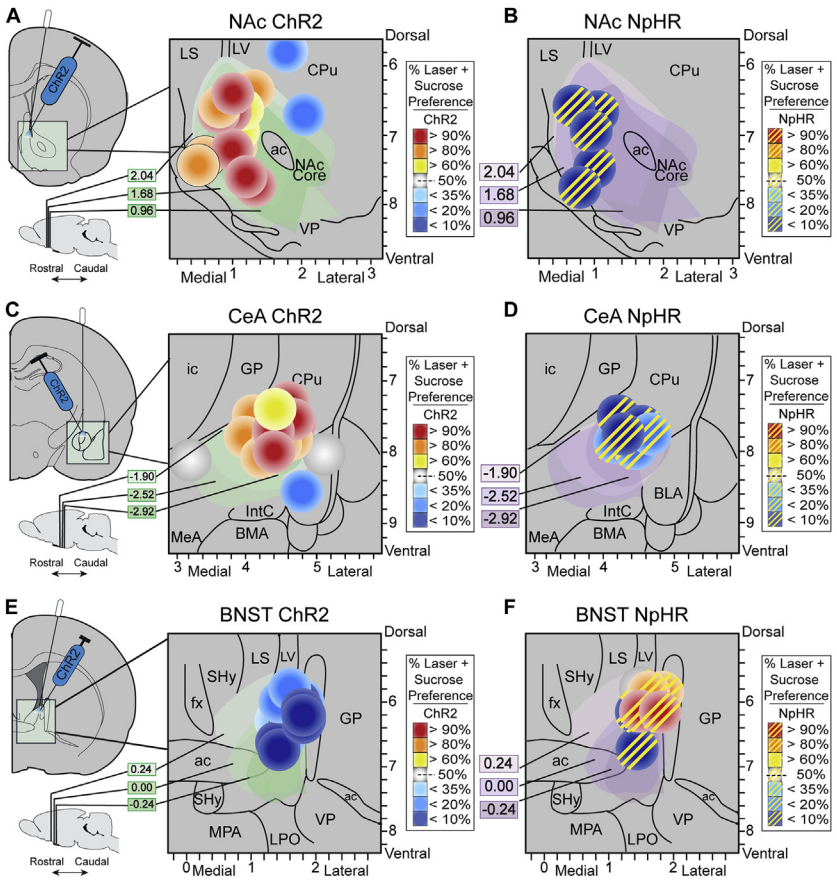

Figure 1. Localization of function maps. Function maps of effects on sucrose preference in two-choice task of ChR2 stimulation of CRF-expressing neurons in the **(A)** NAc, **(C)** CeA, and **(E)** BNST. Maps for inhibitory halorhodopsin effects of laser illumination on CRF-expressing neurons are shown in the **(B)** NAc, **(D)** CeA, and **(F)** BNST (striped symbols). Symbol sizes reflect size of optogenetic Fos plumes (Figure 2 and Supplemental Results). Yellow, orange, or red symbol colors show intensity of enhancement of laser-induced preference for the laser+sucrose option over the sucrose-alone option produced at that site (effects shown for days 6–8 of two-choice task). Conversely, blue colors show intensity of avoidance of laser+sucrose (i.e., preference instead for sucrose alone) (Table 1). ac, anterior commissure; BLA, basolateral amygdala; BMA, basomedial amygdala; BNST, bed nucleus of stria terminalis; CeA, central nucleus of amygdala; ChR2, channelrhodopsin-2; CPu, caudate putamen; CRF, corticotropin-releasing factor; fx, fornix; GP, globus pallidus; ic, internal capsule; IntC, intercalated amygdala; LPO, lateral preoptic area; LS, lateral septum; LV, lateral ventricle; MeA, medial amygdala; MPA, medial preoptic area; NAc, nucleus accumbens; NpHR, halorhodopsin; SHy, septohypothalamic nucleus; VP, ventral pallidum.

Additionally, mice self-stimulate for optogenetic excitation of CeA CRF-expressing neurons, suggesting incentive motivation (12). CRF in rats does mediate stress-induced reinstatement for addictive drugs but does not require either withdrawal or corticosterone (37–39). Overall, CRF seems not simply tied to an aversive affective dimension but instead has a larger regulatory role in affective valence and organization of behaviors (40–42).

Here, we examined potential positively valenced versus negatively valenced motivational roles of CRF-expressing neurons in the NAc, CeA, or BNST, using BAC transgenic *Crh*-Cre+ rats (21) to optogenetically stimulate CRF-containing neurons in each structure. During two-choice incentive motivation tests, rats could choose between 1) earning sucrose paired with laser stimulations and 2) equivalent sucrose option without laser (43). In progressive ratio breakpoint tests, laser stimulation effects on incentive motivation magnitude for sucrose was assessed. Finally, laser self-stimulation tests assessed whether CRF-containing neuronal stimulation was rewarding on its own. We found that NAc and CeA CRF-containing neuron stimulation enhanced sucrose incentive motivation, was reinforcing, and recruited activation of mesolimbic circuitry. Conversely, BNST CRF-containing neuronal stimulation was avoided, suppressed sucrose pursuit, and recruited pain-related circuitry.

METHODS AND MATERIALS

Animals

Female (n = 37) and male (n = 34) *Crh*-Cre+ Wistar rats (>250 g at surgery) (21) were bred and phenotyped in-house. Same-sex groups were housed on a 12-hour reverse light/dark cycle (~21°C) with ad libitum food (Purina, St. Louis, MO) and water. All experimental procedures were approved by the University of Michigan Institutional Animal Care & Use Committee in accordance with NIH animal care and use guidelines.

Surgery

Surgeries followed previous methods (Supplemental Methods) (43–45). Bilateral 1.0-μL infusions in the NAc, CeA, or BNST contained either active AAV-DIO-ChR2-eYFP virus (n = 33) or optically inactive control virus AAV-DIO-eYFP (n = 19) to infect only neurons containing Cre-recombinase. We note that the *Crh*-Cre BAC rats used here express Cre primarily in CRF neurons that are also GABAergic (gamma-aminobutyric acidergic) (21). This makes them suitable for our study, given that CeA, BNST, and NAc CRF-expressing neurons predominantly coexpress GABA. A separate group received halorhodopsin AAV-DIO-NpHR-eYFP (n = 19) virus for CRF-containing neuronal inhibition. NAc shell, lateral CeA, or dorsolateral BNST sites were staggered across individuals (Figure 1 and

Table 1), and optic fibers were secured with surgical screws and acrylic.

Stimulation Parameters

Channelrhodopsin-2 (ChR2) laser illumination (2–3 mW; 473 nm) was tested at 10 Hz and 40 Hz (46–48). Inhibitory halorhodopsin (NpHR) testing used constant illumination (8–10 mW; yellow 592 nm) (49).

Two-Choice Sucrose

An instrumental two-choice task evaluated whether pairing CRF-expressing neuronal stimulation in the NAc, CeA, or BNST with one sucrose reward made it more or less desirable than an identical sucrose reward delivered without laser (Supplemental Methods) (43). Briefly, rats learned that presses on one lever earned sucrose pellets plus 8-second laser illuminations and an 8-second tone or white noise (laser+sucrose). Presses on a different lever earned sucrose and noise/tone but no laser (sucrose alone). Lever and tone/noise assignments were balanced across rats but remained permanent for each rat.

Reinforcement schedules increased across 8 test days: fixed ratio (FR) 1 (days 1–3), FR4 (day 4), random ratio (RR) 4 (day 5), RR6 (days 6–8). Each day, rats were required to earn rewards twice from each lever presented alone, before free choice. The alternate laser frequency (10 Hz/40 Hz) was tested on 3 subsequent RR6 days. Separate halorhodopsin rats underwent identical procedures with yellow laser.

Progressive Ratio

Progressive ratio tests assessed whether ChR2 stimulation of CRF-containing neurons affects magnitude of sucrose incentive motivation (Supplemental Methods) (43). Briefly, rats were tested one day with only the laser+sucrose (10 Hz/40 Hz) lever available, another day with sucrose alone, and a third day with laser+sucrose using the alternate frequency. Within each session, the responses required to earn the next reward increased after each reward, and breakpoint or ratio reached during 30-minute sessions was assessed. Separate halorhodopsin rats underwent testing with inhibition.

Spout-Touch Laser Self-stimulation

Incentive properties of laser alone without sucrose were tested in instrumental spout-touch self-stimulation tests. With 2 empty waterspouts available, each touch on a designated laser spout provided stimulation (3 s; 10 Hz/40 Hz; 30 min). Touches on the other inactive spout earned nothing as a baseline exploration measure. Rats were classified on day 1 as robust, low, or non–self-stimulators, and days 2–3 evaluated consistency of self-stimulation (Supplemental Methods) (45).

Place-Based Self-stimulation

In a different place-based self-stimulation test, rats could earn laser self-stimulations by remaining in a designated laser-delivering chamber within a 3-chamber apparatus (2 major, 1 smaller center; Supplemental Methods) after an initial session without laser evaluated baseline preference. For 3 test days, laser-delivering chamber entries triggered laser (3 s on/4 s off), which continued cycling as long as rats remained, terminating on exit. Time in laser-delivering minus time in alternative no-laser chamber difference scores were assessed.

Histology

Briefly, laser stimulations preceded lethal doses of sodium pentobarbital and transcardial perfusions for Fos assessment (Supplemental Methods) (44). Brains were extracted, postfixed, sectioned into 40-μm slices via cryostat (Leica, Buffalo Grove, IL), processed for GFP (green fluorescent protein) and cFos immunohistochemistry (Figure 2), and imaged using a digital camera (Qimaging, Teledyne Photometrics, Tucson, AZ) and fluorescence microscope (Leica).

Coronal sections were imaged (10× magnification) to quantify distributed Fos using Paxinos & Watson atlas (50). Laser-recruited changes in Fos expression in the NAc/CeA/BNST groups were compared with eYFP (enhanced yellow fluorescent protein) control levels in several mesocorticolimbic structures (Box 1).

Table 1. Histological Placements of Experimental Animals

	Confirmed Placement Ranges, mm From Bregma			ChR2, *n*		eYFP, *n*		NpHR, *n*		
Target	A/P	M/L	D/V	Uni	Bil	Uni	Bil	Uni	Bil	Contralateral Misses, Locations
CeA	−2.16 to −3.00	±4.2 to 4.7	−7.0 to −7.6	3	7	2	5	3	4	BLA, MeA, optic tract
NAc	+1.44 to 0.96	±0.8 to 1.6	−6.3 to −7.6	7	5	3	3	3	3	DS, MS, NAc core
BNST	+0.24 to −0.24	±1.6 to 2.0	−5.8 to −6.4	4	7	3	3	2	4	ac, GP

Table shows anatomical confirmed placement ranges for experimental animals targeting either the lateral CeA, NAc shell, or dorsolateral BNST. Confirmed placement ranges are determined from Paxinos and Watson brain atlas (50) and display A/P, M/L, and D/V coordinates in mm from bregma. *n* values for excitatory ChR2, inactive control eYFP virus, and inhibitory NpHR groups include those with bilateral fiber and virus placements or unilateral virus/fiber placements in one hemisphere, with a contralateral miss in the other hemisphere. For CeA rats (top), contralateral miss sites were located in either the BLA, MeA, or optic tract, with no substantial virus expression found in these missed hemispheres. For NAc rats (middle), placements for unilateral misses were located in the DS, MS, or NAc core, and no substantial viral expression in these structures was observed. For BNST rats (bottom), sites of unilateral misses were in either the ac or GP without substantial virus expression. See Figure 1.

ac, anterior commissure; A/P, anterior/posterior; Bil, bilateral; BLA, basolateral amygdala; BNST, bed nucleus of stria terminalis; CeA, central nucleus of amygdala; ChR2, channelrhodopsin-2; DS, dorsal striatum; D/V, dorsal/ventral; eYFP, enhanced yellow fluorescent protein; GP, globus pallidus; MeA, medial amygdala; M/L, medial/lateral; MS, medial septum; NAc, nucleus accumbens; NpHR, halorhodopsin; Uni, unilateral.

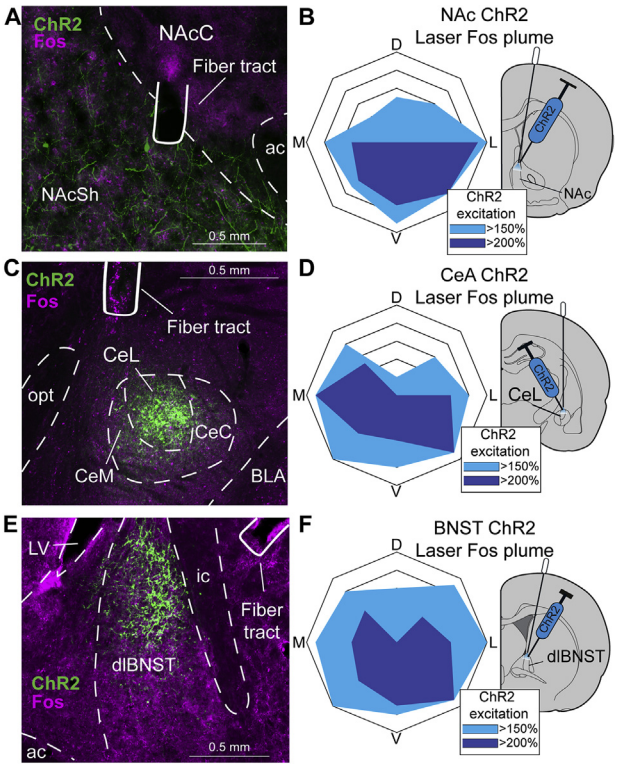

Figure 2. Virus expression and local Fos plumes. Photomicrograph (×10 magnification) shows ChR2 virus expression (green), and neuronal Fos protein expression (magenta) immediately surrounding optic fiber tips for *Crh*-Cre+ rats in the **(A)** NAc shell, **(C)** lateral division of CeA, and **(E)** dorsolateral division of BNST. Blue diagrams at right show maps displaying size and intensity of local Fos plumes produced in each structure by laser stimulation of CRF-containing neurons expressing ChR2 (i.e., zones of >150% Fos elevation and >200% Fos elevation over baselines [100%]) measured in laser-illuminated enhanced yellow fluorescent protein control rats. Average Fos plume diameters are shown for *Crh*-Cre+ ChR2 rats after laser illumination in **(B)** NAc, **(D)** CeA, and **(F)** BNST CRF-containing neurons. ac, anterior commissure; BLA, basolateral amygdala; BNST, bed nucleus of stria terminalis; CeA, central nucleus of amygdala; CeC, capsular central amygdala; CeL, lateral central amygdala; CeM, medial central amygdala; ChR2, channelrhodopsin-2; CRF, corticotropin-releasing factor; D, dorsal to fiber tip; dlBNST, dorsolateral BNST; ic, internal capsule; L, lateral to fiber tip; LV, lateral ventricle; M, medial to fiber tip; NAc, nucleus accumbens; NAcC, NAc core; NAcSh, NAc shell; V, ventral to fiber tip.

CRF and Cre Expression Assessed by RNAScope Fluorescence In Situ Hybridization

Colocalization of Cre and CRF in infected neurons was verified with fluorescence in situ hybridization (Supplement) (16,51). Cells containing *Cre* and *Crh* messenger RNA were manually counted in 100 × 100 × 17 μm volumes from core samples in the NAc, CeA, and BNST (n = 6).

Statistical Analyses

Mixed-model analyses of variance evaluated within-group (e.g., laser-pairings) and between-group effects (e.g., ChR2/eYFP) followed by post hoc comparisons with Bonferroni corrections. Distant Fos was evaluated by unpaired t tests. Effect sizes are Cohen's d. For all analyses, significance level was p = .05, two-tailed.

RESULTS

Cre and CRF Colocalization

Crh and *Cre* messenger RNAs were visualized using fluorescence in situ hybridization in slices from *Crh*-Cre+ rats (n = 6) and found to typically occur together in the same neurons. CRF+ and Cre+ coexpressing neurons were densely concentrated within the lateral CeA (10.1 ± 0.9 colabeled neurons per 100 × 100 × 17 μm volume) and dorsolateral BNST (10.0 ± 0.7). In the NAc, CRF+ neurons were sparsely distributed throughout the medial shell (6.0 ± 0.7 colabeled neurons, or nearly one-half CeA/BNST density) (Figure 3 and Supplemental Results).

NAc and CeA CRF-Expressing Neurons Recruit Similar Structures, BNST Shows Distinct Activation

Recruitment of Fos elevation in distant brain circuitry was assessed following CRF-expressing neuron excitation in the NAc, CeA, or BNST (Box 1 and Table S1).

Laser ChR2 excitation of CRF-containing neurons in the NAc shell recruited 150%–200% increases in distant Fos expression over eYFP control levels in reward-related mesocorticolimbic structures, including the NAc core, CeA, ventral tegmentum (VTA), ventral pallidum (VP), and lateral hypothalamus (LH) (Figure 4A). Similarly, CeA stimulation of CRF-expressing neurons excitation increased Fos expression 150%–250% in the NAc shell, VTA, VP, and LH (Figure 4B).

Conversely, in BNST ChR2 rats, CRF-containing neuron excitation recruited distant Fos 150%–200% elevation in several structures related to pain, aversion, fear, or satiety, the midbrain periaqueductal gray, PVN, and basolateral amygdala, in addition to 150% elevation in some mesocorticolimbic structures (Figure 4C).

NAc and CeA CRF-Expressing Neuronal Stimulation Enhances Paired-Sucrose Value

NAc CRF-Containing Neuron Incentive Enhancement. Pairing ChR2 stimulation of CRF-containing neurons in the NAc (n = 8) with earning sucrose rewards in the two-choice task caused rats to pursue the paired laser+sucrose option nearly exclusively over the other identical sucrose-alone option without laser ($F_{1,6}$ = 46.700; p < .001) (Figure 5A). Rats reached a 7:1 ratio preference by final day 8 (t_7 = 5.846; p = .001; 95% CI, 208–491; d = 2.66). Both female and male ChR2 *Crh*-Cre+ rats showed strong preferences for the NAc laser+sucrose lever over the sucrose-alone lever (females, 5:1 ± 1 ratio; males, 7:1 ± 1) (Figure S1A). Both 10 Hz (n = 5; $F_{1,4}$ = 24.540; p = .008) and 40 Hz frequencies of NAc laser excitation (n = 7; $F_{1,6}$ = 39.209; p = .001) supported similar laser+sucrose preference, with no difference between frequencies ($F_{1,10}$ = 1.186; p = .302) (Figure S2C). In contrast, NAc eYFP control rats with inactive virus chose randomly between

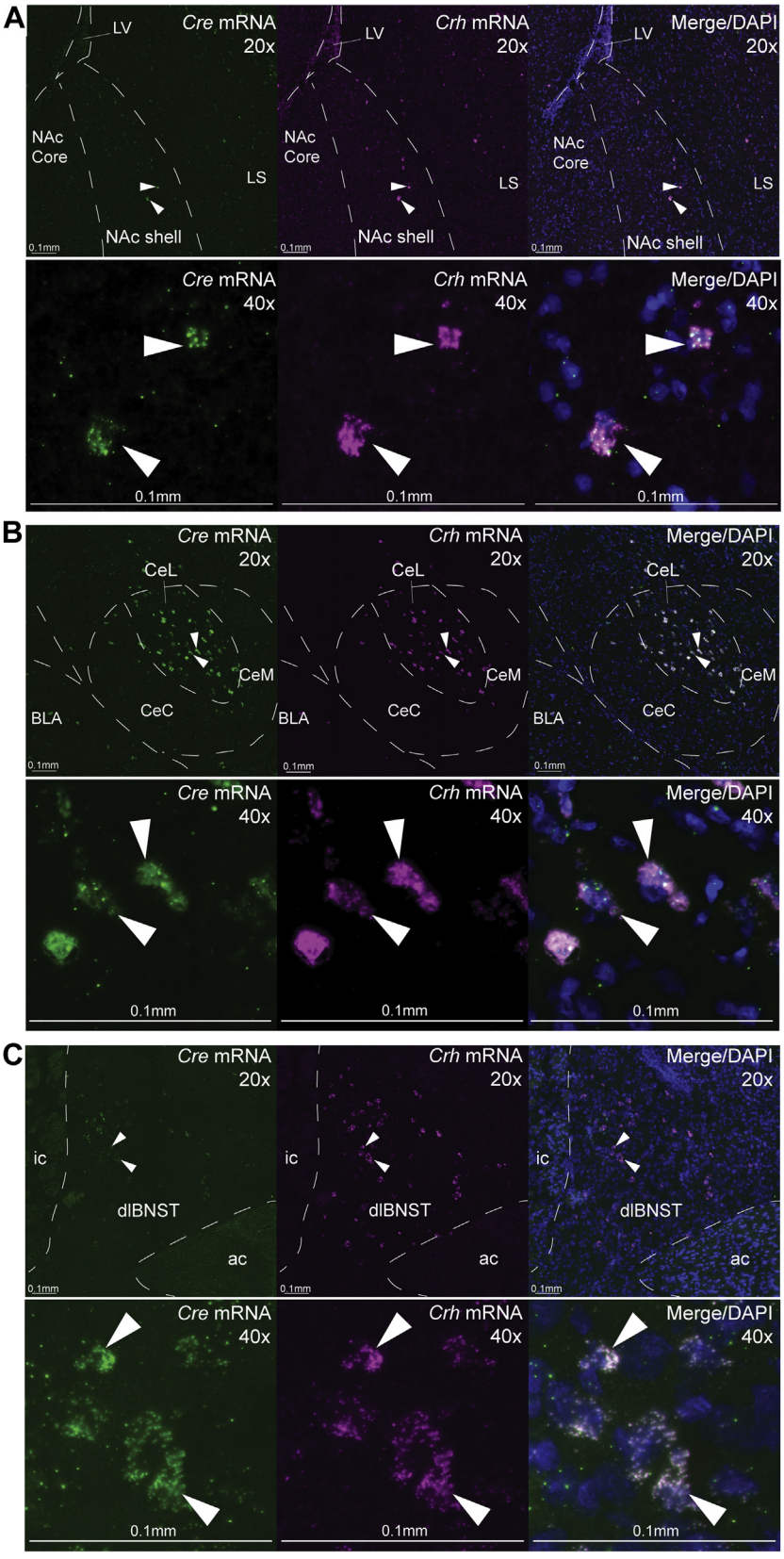

Figure 3. CRF and Cre colocalization verification through fluorescence in situ hybridization. Representative images for *Cre* mRNA expression and *Crh* mRNA expression in the **(A)** NAc shell, **(B)** lateral division of CeL, and **(C)** dlBNST in *Crh*-Cre+ rats (n = 6). Low-power (20×) and high-power (40×) images show localization of neurons expressing *Cre* mRNA (green) or *Crh* mRNA (magenta) and Cre/CRF colocalization with cell bodies stained with DAPI (blue). Arrows point to examples of cells coexpressing *Cre* and *Crh* mRNAs. Scale bars = 0.1 mm. See Supplemental Methods and Supplemental Results. ac, anterior commissure; BLA, basolateral amygdala; CeC, capsular central amygdala; CeL, central amygdala; CeM, medial central amygdala; CRF, corticotropin-releasing factor; dlBNST, dorsolateral bed nucleus of stria terminalis; ic, internal capsule; LS, lateral septum; LV, lateral ventricle; mRNA, messenger RNA; NAc, nucleus accumbens.

Box 1. Brain Regions Assessed for Laser-Recruited Changes in Fos Expression

Orbitofrontal cortex (OFC)
Infralimbic cortex (IF)
Nucleus accumbens core (NAcC)
Anterior nucleus accumbens shell (aNAcSh)
Posterior nucleus accumbens shell (pNAcSh)
Anterior bed nucleus of stria terminalis (aBNST)
Posterior bed nucleus of stria terminalis (pBNST)
Anterior ventral pallidum (aVP)
Posterior ventral pallidum (pVP)
Anterior lateral hypothalamus (aLH)
Posterior lateral hypothalamus (pLH)
Paraventricular nucleus hypothalamus (PVN)
Medial amygdala (MeA)
Central amygdala (CeA)
Basolateral amygdala (BLA)
Ventral tegmentum (VTA)
Substantia nigra (SN)
Midbrain periaqueductal gray (PAG)

Laser-induced enhancements in Fos expression were assessed in the listed mesocorticolimbic brain regions, following CRF-expressing neuronal excitation in the NAc, CeA, or BNST. Distant Fos levels in ChR2 animals were compared with levels assessed in inactive eYFP control rats that underwent identical Fos induction procedures. See Figure 4 and Supplemental Methods.

ChR2, channelrhodopsin-2; CRF, corticotropin-releasing factor; eYFP, enhanced yellow fluorescent protein.

laser+sucrose and sucrose-alone options (n = 6; $F_{1,5}$ = 0.014; p = .911) (Figure 5B).

NAc CRF-Containing Neuron Inhibition Paired-Avoidance. Separate inhibition rats, with NpHR in the NAc, developed strong avoidance of the paired laser+sucrose option and instead preferred sucrose alone by a 20:1 ratio (n = 6; $F_{1,5}$ = 25.741; p = .004) (Figure 5C).

CeA CRF-Containing Neuron Incentive Enhancement. In the CeA, ChR2 stimulation of CRF-containing neurons induced similar near-exclusive pursuit of the paired laser+sucrose option (n = 9; $F_{1,7}$ = 19.227; p = .003) (Figure 6A), growing to a >10:1 ratio over sucrose alone by day 8 (t_8 = 5.110; p = .001; 95% CI, 241–638; d = 3.09). Female and male ChR2 *Crh*-Cre+ rats had similar preference ratios for CeA laser+sucrose over sucrose alone (females, 13:1 ± 2; males, 10:1 ± 2) (Figure S1A). Both 10 Hz (n = 9; $F_{1,8}$ = 59.101; p < .001) (Figure S2D) and 40 Hz frequencies of CeA laser excitation (n = 5; $F_{1,4}$ = 90.572; p = .001) supported comparable levels of preference ($F_{1,12}$ = 0.534; p = .479). By contrast, control CeA eYFP rats chose equally between sucrose options (n = 7; $F_{1,6}$ = 0.003; p = .959) and so differed significantly from CeA ChR2 rats ($F_{1,14}$ = 4.853; p = .045) (Figure 6B).

CeA CRF-Containing Neuron Inhibition Paired-Avoidance. NpHR CeA inhibition of CRF-containing neurons (n = 7) produced avoidance of the laser-paired sucrose option, instead causing a 10:1 ratio preference for sucrose alone ($F_{1,6}$ = 72.960, p < .001) (Figure 6C).

NAc and CeA CRF-Expressing Neuronal Excitation Increases Breakpoint

Progressive ratio breakpoint tests assessed whether CRF-containing neuron stimulation changed the intensity of incentive motivation to obtain sucrose reward. NAc ChR2 rats (n = 6) worked twice as hard in the progressive ratio task on their laser+sucrose day and achieved 200% higher effort breakpoints than on the sucrose-alone day (t_5 = 6.010; p = .002; 95% CI, 23–58; d = 2.6) (Figure 5D). Both female (210 ± 16%) and male rats doubled their breakpoints in the laser+sucrose condition (170 ± 24%) (Figure S1B). Similarly, 10 Hz (t_3 = 4.841; p = .017; n = 4) and 40 Hz (t_5 = 6.010; p = .002; n = 6) (Figure S3D) laser frequencies supported similar doubling of breakpoint. NAc eYFP control rats showed no breakpoint differences between laser+sucrose and sucrose-alone days (n = 5; t_4 = 0.533; p = .62) (Figure 5D) and so differed significantly from ChR2 rats ($F_{1,9}$ = 6.689; p = .029).

In the CeA, excitation of CRF-containing neurons also increased laser+sucrose breakpoint by >200% over sucrose alone (n = 7; t_6 = 6.712; p = .001; 95% CI, 34–73; d = 3.58) (Figure 6D). CeA stimulation doubled breakpoint in both females (250 ± 56%) and males (250 ± 25%) (Figure S1B) and at both 10 Hz (n = 7; t_6 = 4.992; p = .002) and 40 Hz frequencies (n = 5; t_4 = 4.3981; p = .012) (Figure S3D). Control CeA eYFP rats (n = 5) showed no laser effect on breakpoint (t_4 = 0.314; p = .769) (Figure 6D) and significantly differed from ChR2 rats ($F_{1,10}$ = 9.590; p = .011).

BNST CRF-Containing Neuron Excitation Induces Laser-Paired Sucrose Avoidance

In the two-choice task, BNST ChR2 rats avoided the laser+sucrose option and instead preferred sucrose alone (n = 8; $F_{1,6}$ = 13.927; p = .010) (Figure 7A), reaching an 8:1 sucrose-alone preference by day 8 (n = 8; t_7 = 6.059; p = .001; 95% CI, 214–488; d = 4.72). ChR2 males showed numerically stronger avoidance of BNST laser+sucrose (10:1 ± 3 preference for sucrose alone) than females (5:1 ± 1), but the small group sizes were not adequately powered to statistically evaluate sex differences here (Figure S1). Both 10 Hz (n = 7; $F_{1,6}$ = 30.241; p = .002) and 40 Hz frequencies supported similar laser+sucrose avoidance (n = 5; $F_{1,4}$ = 9.474; p = .037) with no difference in magnitude ($F_{1,10}$ = 0.996; p = .342). In contrast, BNST eYFP control rats chose equally between the two sucrose options (n = 6; $F_{1,5}$ = 0.054; p = .826) (Figure 7B).

BNST NpHR Two-Choice. BNST NpHR rats (n = 6) showed no statistical difference in choice between sucrose options ($F_{1,5}$ = 0.167; p = .700) (Figure 7C), although there was a nonsignificant trend toward preferring the laser+sucrose option paired with halorhodopsin inhibition.

BNST CRF-Containing Neuron Excitation Suppresses Sucrose Incentive Motivation. Excitation of BNST CRF-

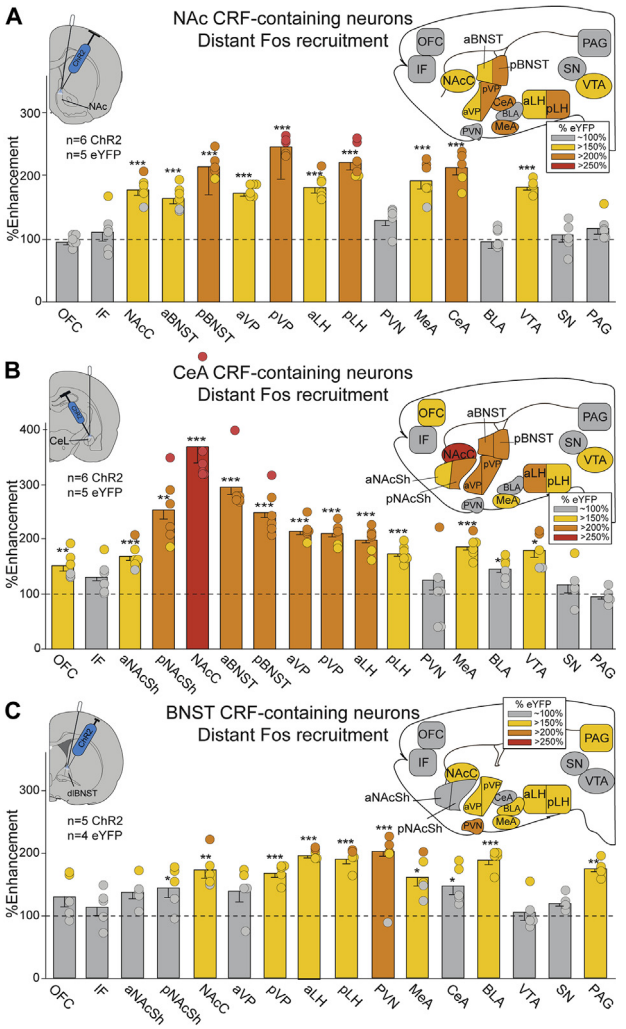

Figure 4. Laser-enhancements in distant Fos expression. Brain maps show recruitment of distant Fos elevation in mesocorticolimbic structures following CRF-containing neuron ChR2 stimulation in the NAc, CeA, or BNST (colors denote percent Fos elevation vs. eYFP control rats, all two-way unpaired *t* tests). **(A)** NAc ChR2 stimulation (n = 3 female, n = 3 male): NAcC, VTA, aVP, pVP, aLH, pLH, MeA, CeA, aBNST, and pBNST. **(B)** CeA ChR2 stimulation (n = 3 female, n = 3 male): OFC, aNAcSh, pNAcSh, NAcC, aVP, pVP, aLH, pLH, MeA, VTA, aBNST, pBNST, and minor increases in BLA (<150%). **(C)** BNST ChR2 stimulation (n = 2 female, n = 3 male): BLA, PAG, hypothalamic PVN, NAcC, pVP, aLH, pLH, MeA, and minor increases in pNAcSh (<150%) and CeA (<150%). See Table S1. Mean and SEM reported; $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. aBNST, anterior BNST; aLH, anterior LH; aNAcSh, anterior NAc shell; aVP, anterior VP; BLA, basolateral amygdala; BNST, bed nucleus of stria terminalis; CeA, central nucleus of amygdala; CeL, lateral central amygdala; ChR2, channelrhodopsin-2; CRF, corticotropin-releasing factor; dlBNST, dorsolateral BNST; eYFP, enhanced yellow fluorescent protein; IF, infralimbic cortex; LH, lateral hypothalamus; MeA, medial amygdala; NAc, nucleus accumbens; NAcC, NAc core; OFC, orbitofrontal cortex; PAG, periaqueductal gray; pBNST, posterior BNST; pLH, posterior LH; pNAcSh, posterior NAc shell; PVN, paraventricular nucleus; pVP, posterior VP; SN, substantia nigra; VP, ventral pallidum; VTA, ventral tegmentum.

containing neurons suppressed incentive motivation to earn sucrose, reducing the laser+sucrose breakpoint effort to half that of sucrose alone (t_7 = 5.492; p = .001; 95% CI, 20–49; d = 2.25) (Figure 7D). Female rats (49 ± 27%) and male rats (54 ± 14%) showed similar breakpoint reductions, and 10 Hz (t_7 = 6.178; $p < .001$; n = 8) and 40 Hz frequencies were comparably effective (t_3 = 5.333; p = .013; n = 4). By contrast, BNST eYFP control rats showed no breakpoint laser effects (n = 5; t_4 = 0.441; p = .682) (Figure 7D) and so differed from BNST ChR2 rats ($F_{1,11}$ = 5.874, p = .034).

Opposite Breakpoint Effects for CRF-Expressing Neuronal Inhibition

Halorhodopsin inhibition of CRF-containing neurons in the NAc (n = 6; Figure 5D) or CeA (n = 7; Figure 6D) suppressed laser+sucrose breakpoint to ~50% that of sucrose alone (NAc: t_5 = 5.308; p = .003; 95% CI, 19–53; d = 2.58; CeA: t_6 = 4.032; p = .007; 95% CI, 13–55; d = 2.33). BNST CRF-containing neuronal inhibition did not significantly alter breakpoint effort, although there was a nonsignificant trend toward a higher breakpoint for laser + sucrose (n = 6; t_5 = 0.717; p = .506) (Figure 7D).

Spout-Touch Self-stimulation: NAc and CeA Stimulation of CRF-Expressing Neurons by Itself Is a Moderate Reward

In the instrumental self-stimulation task, each touch on the designated laser spout earned 3 seconds of laser excitation, whereas inactive spout touches delivered nothing. No NAc ChR2 rats met the criterion for robust self-stimulation of >50 touches on the laser spout on day 1 (45). However, 7 of 8 NAc ChR2 rats demonstrated low-level self-stimulation, meeting a lesser criterion of only >10 laser spout touches and >2 times more touches on the laser spout as on the inactive spout. On days 2–3, those 7 NAc rats achieved 25–35 self-stimulations per 30-minute session, roughly 4 times more than inactive spout touches (n = 7; $F_{1,5}$ = 7.823; p = .038) (Figure 8A) and ~1.5 times more laser spout touches than eYFP control rats ($F_{1,9}$ = 9.949; p = .012). Female and male NAc ChR2 rats showed similar levels of self-stimulation (males: 29 ± 16 illuminations; females: 29 ± 8), and 10 Hz and 40 Hz frequencies both supported self-stimulation (10 Hz: 25 ± 10, n = 3; 40 Hz: 32 ± 10, n = 4).

CeA Self-stimulation. Overall, 2 of 8 CeA ChR2 rats met the >50 illuminations criterion for robust self-stimulation, whereas 7 met the lower >10 moderate self-stimulation criterion. These 7 CeA ChR2 rats self-stimulated ~25–35 times on days 2–3, which was >300% more than the inactive spout ($F_{1,5}$ = 12.009; p = .018) (Figure 8B), and earned >300% more illuminations than eYFP control rats ($F_{1,9}$ = 17.576; p = .002). The two most robust self-stimulators were both females and reached 40 ± 3 self-stimulations per day (vs. males: n = 5, 23 ± 7 self-stimulations per day). Both 10 Hz (n = 4) and 40 Hz (n = 3) frequencies supported similar levels of CeA self-stimulation (10 Hz: 27 ± 10 self-stimulations; 40 Hz: 29 ± 8).

BNST Fails to Support Self-stimulation. No BNST ChR2 rats met any criteria for self-stimulation of CRF-containing neurons, responding equally at low rates on both spouts (n = 8; $F_{1,6}$ = 0.006; p = .939) (Figure 8C).

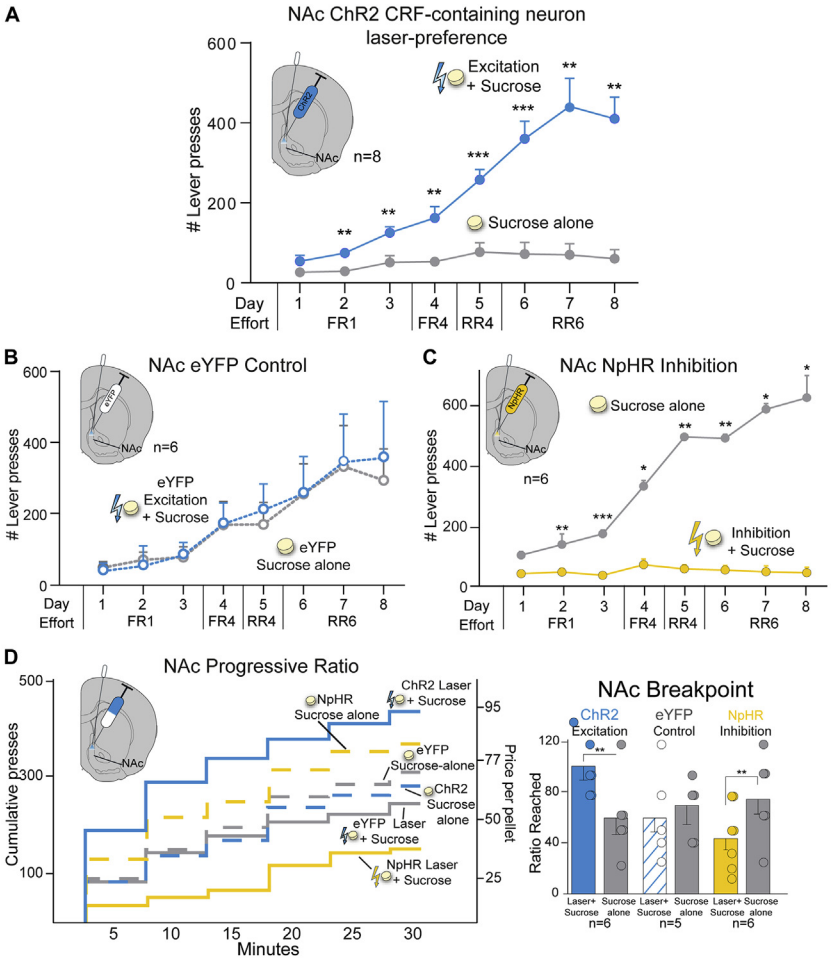

Figure 5. CRF-containing neuron stimulation in the NAc biases and amplifies sucrose motivation. **(A)** ChR2 excitation of CRF-containing neurons in the NAc shell caused preference for paired laser+sucrose over sucrose alone in two-choice test (n = 3 female, n = 5 male), reaching a 7:1 ratio by day 8. In contrast, **(B)** control NAc eYFP rats chose equally between options. **(C)** NpHR inhibition of CRF-containing neurons in the NAc shell (n = 3 female, n = 3 male) caused avoidance of laser+sucrose and sucrose-alone preference. **(D)** In a progressive ratio test, NAc ChR2 CRF-containing neuron excitation enhanced incentive motivation breakpoint of laser+sucrose over sucrose alone (n = 3 female, n = 3 male). ChR2 rats had higher laser+sucrose breakpoints than eYFP control rats (n = 5). Laser did not affect NAc eYFP control breakpoint between progressive ratio test days. NAc NpHR inhibition of CRF-containing neurons reduced laser+sucrose breakpoint motivation (n = 3 female, n = 3 male). Mean and SEM reported; $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. ChR2, channelrhodopsin-2; CRF, corticotropin-releasing factor; eYFP, enhanced yellow fluorescent protein; FR, fixed ratio; NAc, nucleus accumbens; NpHR, halorhodopsin; RR, random ratio.

CeA and NAc Self-stimulation Does Not Account for Laser Effects on Sucrose Motivation. Did laser self-stimulation in the CeA and NAc substantially drive laser's ability to control sucrose pursuit in two-choice or progressive ratio tasks? The answer appears to be no; there was no correlation between self-stimulation values, which were generally low, and control of sucrose pursuit in the two-choice test (NAc: n = 6, r = .624, p = .098; CeA: n = 7, r = −.024, p = .926), nor was there a correlation between self-stimulation and enhancement of progressive ratio breakpoint, which was relatively strong in most NAc or CeA ChR2 rats (Pearson's correlation; NAc: n = 6, r = −.349, p = .498; CeA: n = 7, r = .605, p = .280). Finally, even CeA (n = 1) and NAc (n = 1) rats that failed to self-stimulate showed ∼200% laser-induced enhancements of breakpoint and control of laser+sucrose preference (11:1 ratio) as strong as in self-stimulators (∼200%, 9:1).

NAc and CeA Place-Based Self-stimulation, BNST Place-Avoidance

Rats were additionally tested for self-stimulation using a second place-based task, where entering and staying in a designated chamber earned laser (cycling 3 s on/4 s off; 15 min).

NAc and CeA Place-Based Self-stimulation. NAc ChR2 rats spent >150% more time in the laser-delivering chamber than in the no-laser chamber ($F_{1,6}$ = 6.664; p = .042) (Figure 9A). NAc ChR2 rats also spent 150% longer in the laser-delivering chamber than they had during previous baseline tests without laser (t_7 = 3.376; p = .012; 95% CI, 56–318; d = 1.21) and more time in the laser-delivering chamber than inactive eYFP control rats (t_{11} = 2.318; p = .041; 95% CI, 9–353; d = 1.05). Both female (n = 2) and male (n = 6) NAc ChR2 rats spent comparably more time in the laser-delivering chamber (female: 160 ± 20%; males: 140 ± 10%), and 10 Hz (n = 3; 160 ± 20%) and 40 Hz (n = 5; 160 ± 20%) frequencies were equally effective.

CeA ChR2 rats (n = 8) demonstrated robust place-based self-stimulation of CeA CRF-containing neurons, spending ∼200% more time in the laser-delivering than the no-laser chamber ($F_{1,6}$ = 21.085; p = .004). CeA ChR2 rats also spent 200% longer in the laser-delivering chamber than they had during previous baseline tests without laser (t_7 = 3.038; p = .019; 95% CI, 63–509; d = 1.41) and more than CeA eYFP control rats (t_{11} = 2.062; p = .011; 95% CI, 57–484; d = 1.20). Both female (n = 2; 160 ± 20% more laser-delivering time) and male (n = 3; 200 ± 20%) CeA ChR2 rats showed place-based

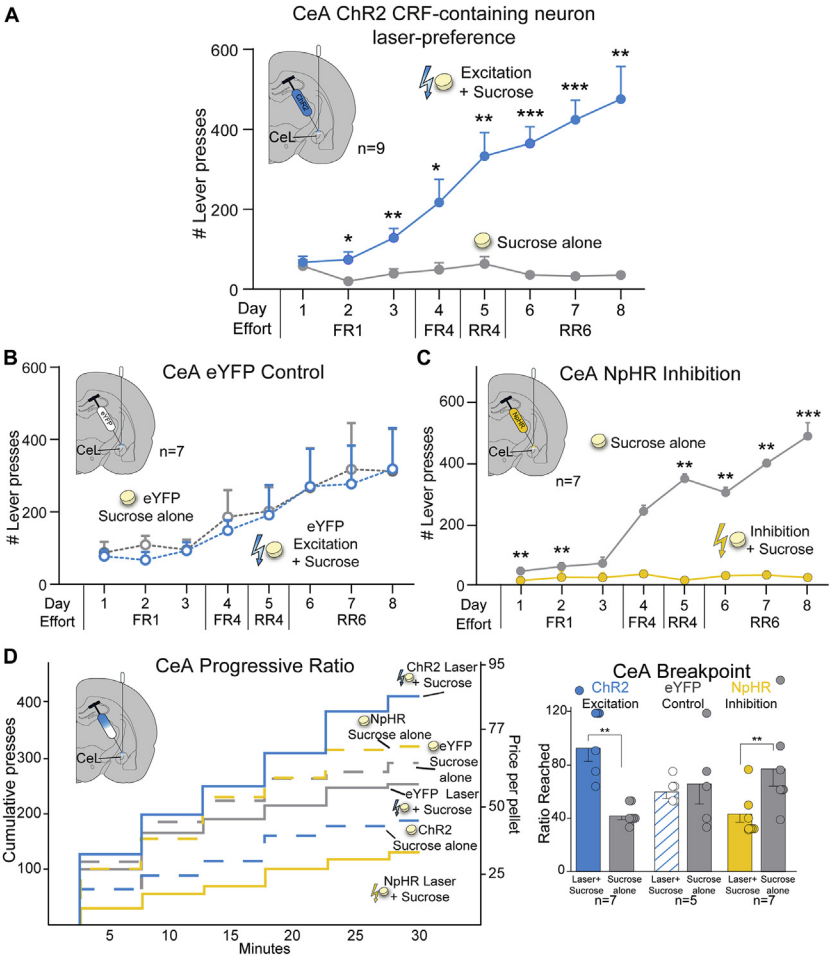

Figure 6. CRF-containing neuron stimulation in the CeA biases and amplifies sucrose motivation. **(A)** CeA ChR2 excitation of CRF-containing neurons caused near-exclusive preference for laser+sucrose over sucrose-alone rewards in two-choice test (n = 4 female, n = 5 male), reaching a 10:1 ratio by day 8. **(B)** CeA eYFP control rats chose equally between sucrose options (n = 7) and differed from CeA ChR2 rats. **(C)** CeA NpHR inhibition of CRF-containing neurons caused laser+sucrose avoidance and sucrose-alone preference (n = 2 female, n = 5 male). **(D)** In progressive ratio test, CeA CRF-containing neuron excitation enhanced incentive motivation for sucrose breakpoint (n = 3 female, n = 4 male). Laser did not affect CeA eYFP control breakpoint (n = 5), which differed from ChR2 rats. CeA NpHR inhibition of CRF-containing neurons reduced laser+sucrose breakpoint (n = 2 female, n = 5 male). Mean and SEM reported; *$p < .05$; **$p < .01$; ***$p < .001$. CeA, central nucleus of amygdala; CeL, lateral central amygdala; ChR2, channelrhodopsin-2; CRF, corticotropin-releasing factor; eYFP, enhanced yellow fluorescent protein; FR, fixed ratio; NpHR, halorhodopsin; RR, random ratio.

self-stimulation, and 10 Hz (n = 5) and 40 Hz (n = 3) laser frequencies were both effective (10 Hz: 200 ± 10%; 40 Hz: 150 ± 30%).

BNST Induces Place-Avoidance. BNST ChR2 rats mildly avoided the laser-delivering chamber that stimulated CRF-containing neurons in BNST, spending only <75% as much time there as in the no-laser chamber (n = 10; $F_{1,8}$ = 6.593; p = .033; Figure 9C). ChR2 BNST rats also spent less time in the laser-delivering chamber than they had during baseline tests without laser (t_9 = 3.188; p = .011; 95% CI, 67–397; d = 1.25) and less time than eYFP control rats (t_{13} = 2.737; p = .017; 95% CI, 49–415; d = 1.76). Both female (n = 5) and male (n = 5) BNST ChR2 rats showed avoidance of the laser-delivering chamber (female: <85 ± 10%; males: <50 ± 10%), and both 10 Hz (n = 6; <65 ± 10%) and 40 Hz (n = 4; <70 ± 10%) frequencies induced place-based avoidance.

DISCUSSION

Our results demonstrate that optogenetic excitation of CRF-containing neural systems in both the lateral CeA and the medial NAc shell focused and increased incentive motivation or "wanting" for sucrose and carried positive valence by itself. ChR2 stimulation of CRF-containing neurons in the CeA and NAc 1) focused intense incentive motivation on the laser+sucrose option over an alternative sucrose-alone option in the two-choice task, 2) amplified incentive motivation, measured as breakpoint effort for sucrose reward, and 3) was actively sought by itself as laser self-stimulation. Simultaneously, ChR2 stimulation of CRF-expressing neurons in the CeA and NAc recruited reward-related mesolimbic circuitry, reflected as Fos increases in the VTA, NAc, VP, and LH.

In contrast, BNST optogenetic excitation of CRF-containing neurons produced aversive motivation. BNST CRF-containing neuronal excitation here caused avoidance of the laser+sucrose option and of laser by itself; suppressed breakpoint of sucrose motivation; and recruited increased Fos in the PVN and periaqueductal gray, structures associated with negative avoidance or distress.

Our NAc and CeA incentive effects are consistent with previous reports that CRF systems in the CeA or NAc can contribute positively to reward motivation (11–15,36). NAc CRF microinjections increase bursts of cue-triggered "wanting" for sucrose rewards in rats and cause

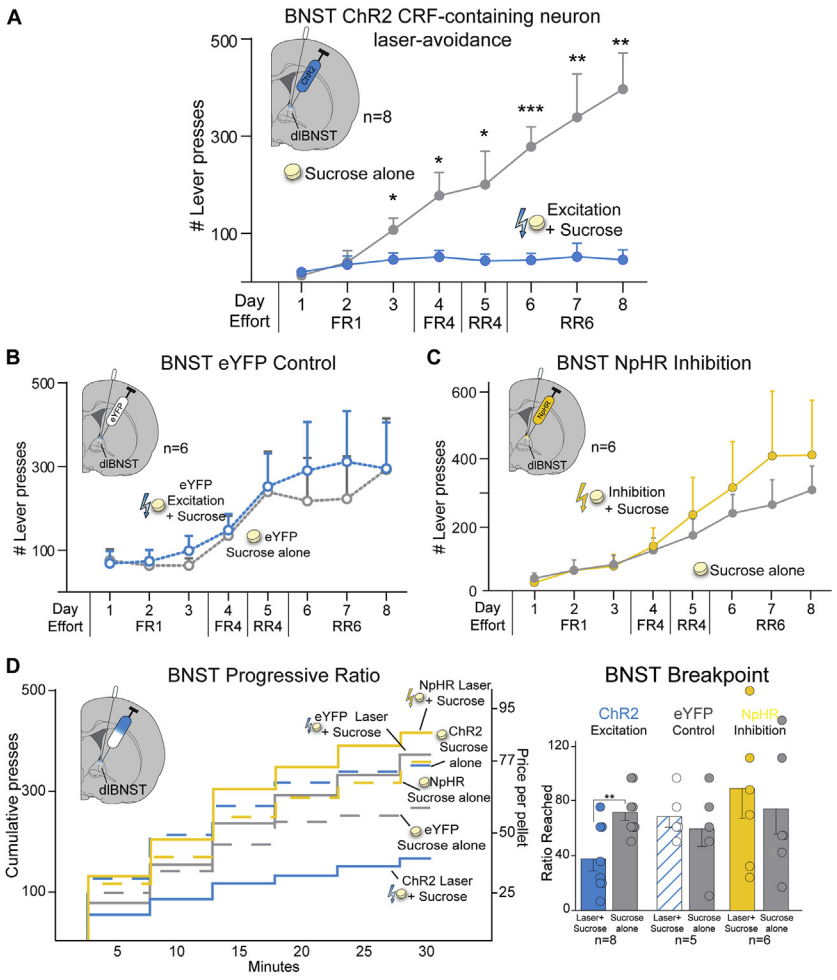

Figure 7. CRF-containing neuron stimulation in the BNST is avoided and suppresses sucrose motivation. **(A)** ChR2 rats avoided laser+sucrose that stimulated CRF-expressing neurons in BNST in two-choice test (n = 5 female, n = 3 male). BNST ChR2 laser+sucrose avoidance rose to an 8:1 opposite preference for sucrose alone by day 8. **(B)** Control eYFP BNST rats chose equally between sucrose options (n = 6). **(C)** BNST NpHR rats (n = 3 female, n = 3 male) showed no significant difference between inhibitory laser+sucrose and sucrose alone. **(D)** BNST ChR2 excitation of CRF-containing neurons suppressed breakpoint effort for sucrose in progressive ratio tests (n = 5 female, n = 3 male). Laser did not affect BNST eYFP control breakpoint, and so eYFP rats significantly differed from BNST ChR2 rats in laser effects on breakpoint. NpHR inhibition of BNST CRF-containing neurons did not statistically alter sucrose breakpoint, despite a nonsignificant trend toward increased motivation (n = 3 female, n = 3 male). Mean and SEM reported; $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. BNST, bed nucleus of stria terminalis; ChR2, channelrhodopsin-2; CRF, corticotropin-releasing factor; dlBNST, dorsolateral BNST; eYFP, enhanced yellow fluorescent protein; FR, fixed ratio; NpHR, halorhodopsin; RR, random ratio.

conditioned place preference and increase NAc dopamine release in nonstressed mice (13–15).

CRF systems mobilize bio/behavioral responses to changing events (9–11) and can be responsive to either positive or negative events. For instance, it has long been known that CRF systems in the CeA respond to positive reward stimuli, such as food cues, not only to aversive stimuli (11). Indicating positively valenced roles, mice optogenetically self-stimulate CRF-containing neurons in the CeA (12). Our results confirm CeA CRF-containing neuronal self-stimulation in rats and extend CRF neuronal self-stimulation to NAc. They further demonstrate that NAc and CeA activations potentiate and focus incentive motivation for natural sucrose reward. Conversely, CRF-containing neuronal stimulation in BNST produced opposite negative motivational effects.

Future studies could identify the specific projections from the CeA, NAc, and BNST that mediate these effects. For example, CeA CRF-containing neurons project to the LH, VP, VTA, and BNST (21,52–56). CeA-BNST CRF-containing projections may reliably mediate aversive motivation (35,53–55), implying that projections to the LH, VP, VTA, or elsewhere may mediate incentive motivation effects. ChR2 stimulation here likely activated these CeA-BNST projections too, implying that other positively valenced CeA outputs may overpower BNST aversive effects when simultaneously activated. For the NAc, local connections of CRF-containing neurons may mediate incentive motivation effects, such as intra-NAc connections to cholinergic interneurons, which may modulate dopamine release in NAc (14–16). Neuroanatomically, it would be of interest to additionally investigate the motivational effects of dense CRF-containing neuronal projections from the hypothalamic PVN. However, PVN CRF-containing neurons may co-release glutamate, whereas the *Crh*-Cre rat line used here may primarily target CRF-expressing neurons that co-release GABA (21,57).

Neurochemically, it would be useful in future studies to examine the roles in these motivational effects of CRF release versus other neurotransmitters co-released by CRF-expressing neurons, such as GABA, dynorphin, neurotensin, and somatostatin (21,47,58–60). Co-release might be related to why CRFR1 antagonists may fail to block stress-induced craving in clinical models (61–64).

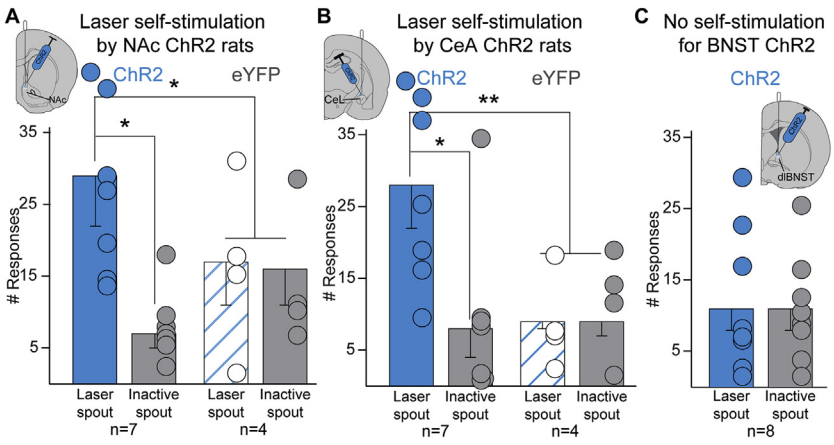

Figure 8. Laser is self-stimulated by NAc *Crh*-Cre+ rats and CeA *Crh*-Cre+ rats in spout-touch task but not by BNST *Crh*-Cre+ rats. **(A)** NAc ChR2 rats self-stimulated ~25–35 times on average (n = 5 female, n = 2 male), whereas NAc eYFP control rats (n = 4) touched both spouts equally about 15 times. **(B)** CeA ChR2 rats similarly self-stimulated (n = 2 female, n = 5 male), whereas eYFP control rats did not (n = 4). **(C)** BNST ChR2 rats failed to self-stimulate for laser in BNST corticotropin-releasing factor–containing neurons (n = 3 female, n = 5 male). See Figures S4 and S6D. Means ± SEM, and individual scores shown; $^{*}p < .05$; $^{**}p < .01$. BNST, bed nucleus of stria terminalis; ChR2, channelrhodopsin-2; CeA, central nucleus of amygdala; CeL, lateral central amygdala; dlBNST, dorsolateral BNST; eYFP, enhanced yellow fluorescent protein; NAc, nucleus accumbens.

Positive NAc and CeA Versus Negative BNST: Anatomical Differences in Motivational Valence

Why did CRF-containing neuron activations have positively valenced effects in the NAc and CeA but negatively valenced effects in the BNST? The NAc and CeA are both striatal-level structures in cortico-striatal-pallidal macrosystem frameworks of telencephalon organization, having neuronal, connectivity, neurochemical, and embryological features shared with the neostriatum (65–67). For example, the CeA and NAc contain mostly GABAergic neurons that receive descending cortical-type glutamatergic inputs and ascending mesotelencephalic dopaminergic inputs, and both send GABAergic outputs to pallidal-level structures of the BNST or VP (65–68). In the same frameworks, the BNST is a pallidal-level structure with descending outputs to the hypothalamus and brainstem, plus ascending re-entrant projections back to thalamo-cortico-striatal-pallidal loops (22–24,65–68).

Hypothesized Roles of CRF-Containing Systems in Addiction

Traditionally, CRF-containing neurons have been hypothesized to generate aversive states such as anxiety and drug withdrawal, although CRF systems also have wider roles in affective appraisals of incentives that mobilize motivational states (9–11). Our study helps put this in perspective.

Regarding the role of CRF in anxiety and addiction, the allostatic theory of addiction posits that CRF-containing neuronal activation in CeA and BNST components of extended amygdala cause aversive drug withdrawal, which is hypothesized to promote relapse through efforts to hedonically self-medicate via consumption of drug rewards (27–29,32–34).

Our results call into question some of these assumptions. Indeed, the hypothesis that CRF-containing neurons in CeA and BNST (i.e., extended amygdala) necessarily generate negatively valenced states may not apply to CeA. Instead, our results indicate that CRF-expressing neuronal activation in both the CeA and NAc increases reward pursuit and produces positively valenced incentive states that rats actively worked to induce. Conversely, in partial support of the allostatic model, BNST CRF-containing neural activation did cause aversive motivational states. However, the aversive state induced by stimulating BNST CRF-expressing neurons failed to increase reward seeking, instead suppressing sucrose pursuit.

This suggests that hedonic self-medication of aversion may not be the primary mechanism by which CRF-containing neurons promote reward pursuit and consumption for any of these structures. Instead, CRF-expressing neurons in the CeA and NAc amplify "wanting" to pursue and consume rewards without aversive states, whereas BNST CRF-expressing neuronal excitation may actually impede reward pursuit and consumption. This may be why drug withdrawal is not as effective for reinstatement of drug taking as stress or drug priming (25,37–39). Although brainwide CRF activation may cause aversive withdrawal states through BNST CRF-containing neurons, our results suggest that any accompanying increases in reward pursuit or addictive relapse might predominantly be due to coactivation of CRF incentive motivation systems in the NAc and CeA.

Valence Flips

Motivational valence induced by CeA optogenetic stimulation can switch depending on environmental situation, and therefore the valence of our CRF-containing neuron stimulation could potentially switch in certain circumstances (14,45). If so, CRF systems could be quite labile in their functional role in motivated behaviors depending on context and need, which deserves further investigation.

Clinical Implications

Activation of CRF systems during stress or emotional excitement may promote relapse in addiction, binge eating, and other excessive consumption. The dominant perspective relied solely on the postulated aversiveness of CRF-expressing neural activation. However, our results indicate that incentive motivation roles of CRF-containing neurons in the NAc and CeA predominate under tested conditions and promote intense reward pursuit without aversive distress (12–14). This could explain why even positively valenced stressors (e.g., new

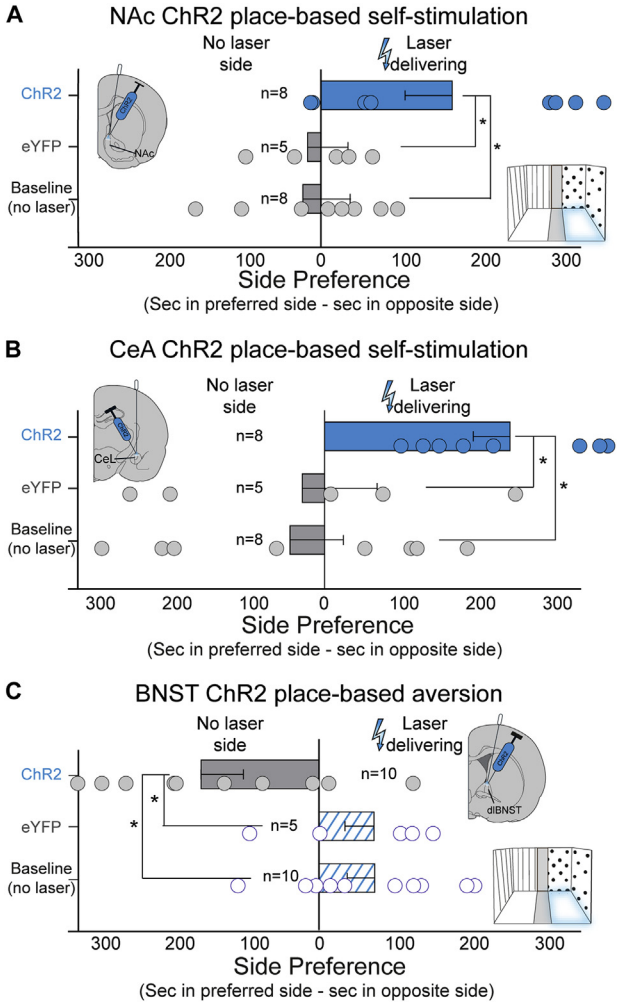

Figure 9. Place-based self-stimulation and place-based aversion following CRF-containing neuron stimulation. **(A)** The NAc supported ChR2 place-based laser self-stimulation of CRF-containing neurons. NAc ChR2 rats (n = 2 female, n = 6 male) spent more time in the laser-delivering chamber than in the no-laser chamber, more time in the laser-delivering chamber than NAc eYFP control rats (n = 5), and more than they previously spent in same chamber during no-laser baseline preference tests. **(B)** The CeA also supported place-based self-stimulation of CRF-containing neurons. CeA ChR2 rats (n = 5 female, n = 3 male) spent more time in the laser-delivering chamber than in the no-laser chamber, more time in the laser-delivering chamber than CeA eYFP control rats (n = 5), and more than they previously spent in identical chamber during no-laser baseline tests. **(C)** Conversely, BNST produced avoidance of the laser-delivering chamber. BNST ChR2 rats (n = 5 female, n = 5 male) spent less time in the laser-delivering chamber than eYFP control rats (n = 5) and less time than they spent in the same chamber during no-laser baseline tests. See Figures S5 and S6E. Mean and SEM reported; *$p < .05$. BNST, bed nucleus of stria terminalis; CeA, central nucleus of amygdala; CeL, lateral central amygdala; ChR2, channelrhodopsin-2; CRF, corticotropin-releasing factor; dlBNST, dorsolateral BNST; eYFP, enhanced yellow fluorescent protein; NAc, nucleus accumbens.

relationships, winning the lottery) can be triggers of addictive relapse and binge eating (69–72). Conversely, aversive motivation induced by BNST CRF-containing neurons contributed little to reward pursuit. Ultimately, CRF-containing systems have diverse motivational roles. Further clarification of negatively valenced versus positively valenced motivation roles of CRF systems will be important to understand how they promote excessive consumption in addiction and related disorders.

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HMB designed, collected, analyzed data, and wrote the paper. KCB designed, analyzed data, and wrote the paper. JS designed and wrote the paper.

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The data that support the findings of this study (Figures 1–9 and the Supplement) are made available through NIH figshare public repository, doi: https://doi.org/10.6084/m9.figshare.12762383. Code for MedPC software for two-choice, progressive ratio, and spout self-stimulation tasks is available through NIH figshare public repository, doi: https://doi.org/10.6084/m9.figshare.12762389.v1.

The authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

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REFERENCES

1. Vale W, Spiess J, Rivier C, Rivier J (1981): Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213:1394–1397.
2. Hupalo S, Bryce CA, Bangasser DA, Berridge CW, Valentino RJ, Floresco SB (2019): Corticotropin-releasing factor (CRF) circuit modulation of cognition and motivation. Neurosci Biobehav Rev 103:50–59.
3. McEwen BS, Akil H (2020): Revisiting the stress concept: Implications for affective disorders. J Neurosci 40:12–21.
4. Stewart J (2000): Pathways to relapse: The neurobiology of drug- and stress-induced relapse to drug-taking. J Psychiatry Neurosci 25:125–136.
5. Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, Houshyar H, *et al.* (2003): Chronic stress and obesity: A new view of "comfort food". Proc Natl Acad Sci U S A 100:11696–11701.
6. Koob GF, Bloom FE (1985): Corticotropin-releasing factor and behavior. Fed Proc 44:259–263.
7. Dunn AJ, Berridge CW (1990): Physiological and behavioral responses to corticotropin-releasing factor administration: Is CRF a mediator of anxiety or stress responses? Brain Res Brain Res Rev 15:71–100.
8. Bale TL, Vale WW (2003): Increased depression-like behaviors in corticotropin-releasing factor receptor-2-deficient mice: Sexually dichotomous responses. J Neurosci 23:5295–5301.

9. Schulkin J (2017): The CRF Signal: Uncovering an Information Molecule. Oxford, United Kingdom: Oxford University Press.
10. Merali Z, McIntosh J, Anisman H (2004): Anticipatory cues differentially provoke in vivo peptidergic and monoaminergic release at the medial prefrontal cortex. Neuropsychopharmacology 29:1409–1418.
11. Merali Z, McIntosh J, Kent P, Michaud D, Anisman H (1998): Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. J Neurosci 18:4758–4766.
12. Kim J, Zhang X, Muralidhar S, LeBlanc SA, Tonegawa S (2017): Basolateral to central amygdala neural circuits for appetitive behaviors. Neuron 93:1464–1479.e5.
13. Peciña S, Schulkin J, Berridge KC (2006): Nucleus accumbens corticotropin-releasing factor increases cue-triggered motivation for sucrose reward: Paradoxical positive incentive effects in stress? BMC Biol 4:8.
14. Lemos JC, Wanat MJ, Smith JS, Reyes BAS, Hollon NG, Van Bockstaele EJ, *et al.* (2012): Severe stress switches crf action in the nucleus accumbens from appetitive to aversive. Nature 490:402–406.
15. Lemos JC, Alvarez VA (2020): The upside of stress: A mechanism for the positive motivational role of corticotropin releasing factor. Neuropsychopharmacology 45:219–220.
16. Lemos JC, Shin JH, Alvarez VA (2019): Striatal cholinergic interneurons are a novel target of corticotropin releasing factor. J Neurosci 39:5647–5661.
17. Swanson LW, Simmons DM (1989): Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: A hybridization histochemical study in the rat. J Comp Neurol 285:413–435.
18. Makino S, Gold PW, Schulkin J (1994): Effects of corticosterone on CRH mRNA and content in the bed nucleus of the stria terminalis; comparison with the effects in the central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus. Brain Res 657:141–149.
19. Makino S, Gold PW, Schulkin J (1994): Corticosterone effects on corticotropin-releasing hormone mRNA in the central nucleus of the amygdala and the parvocellular region of the paraventricular nucleus of the hypothalamus. Brain Res 640:105–112.
20. Itoga CA, Chen Y, Fateri C, Echeverry PA, Lai JM, Delgado J, *et al.* (2019): New viral-genetic mapping uncovers an enrichment of corticotropin-releasing hormone-expressing neuronal inputs to the nucleus accumbens from stress-related brain regions. J Comp Neurol 527:2474–2487.
21. Pomrenze MB, Millan EZ, Hopf FW, Keiflin R, Maiya R, Blasio A, *et al.* (2015): A transgenic rat for investigating the anatomy and function of corticotrophin releasing factor circuits. Front Neurosci 9:487.
22. Gray TS, Magnuson DJ (1992): Peptide immunoreactive neurons in the amygdala and the bed nucleus of the stria terminalis project to the midbrain central gray in the rat. Peptides 13:451–460.
23. Giardino WJ, Eban-Rothschild A, Christoffel DJ, Li SB, Malenka RC, de Lecea L (2018): Parallel circuits from the bed nuclei of stria terminalis to the lateral hypothalamus drive opposing emotional states. Nat Neurosci 21:1084–1095.
24. Dabrowska J, Martinon D, Moaddab M, Rainnie DG (2016): 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 28.
25. Mantsch JR, Baker DA, Funk D, Lê AD, Shaham Y (2016): Stress-induced reinstatement of drug seeking: 20 years of progress. Neuropsychopharmacology 41:335–356.
26. Grilo CM, Pagano ME, Stout RL, Markowitz JC, Ansell EB, Pinto A, *et al.* (2012): Stressful life events predict eating disorder relapse following remission: Six-year prospective outcomes. Int J Eat Disord 45:185–192.
27. Koob GF, Schulkin J (2019): Addiction and stress: An allostatic view. Neurosci Biobehav Rev 106:245–262.
28. Koob GF (2013): Addiction is a reward deficit and stress surfeit disorder. Front Psychiatry 4:72.
29. Roberto M, Spierling SR, Kirson D, Zorrilla EP (2017): Corticotropin-releasing factor (crf) and addictive behaviors. Int Rev Neurobiol 136:5–51.
30. Solomon RL, Corbit JD (1978): An opponent-process theory of motivation. Am Econ Rev 68:12–24.
31. Solomon RL (1980): The opponent-process theory of acquired motivation: The costs of pleasure and the benefits of pain. Am Psychol 35:691–712.
32. Koob GF, Le Moal M (1997): Drug abuse: Hedonic homeostatic dysregulation. Science 278:52–58.
33. Funk CK, O'Dell LE, Crawford EF, Koob GF (2006): Corticotropin-releasing factor within the central nucleus of the amygdala mediates enhanced ethanol self-administration in withdrawn, ethanol-dependent rats. J Neurosci 26:11324–11332.
34. Zorrilla EP, Logrip ML, Koob GF (2014): Corticotropin releasing factor: A key role in the neurobiology of addiction. Front Neuroendocrinol 35:234–244.
35. de Guglielmo G, Kallupi M, Pomrenze MB, Crawford E, Simpson S, Schweitzer P, *et al.* (2019): Inactivation of a crf-dependent amygdalofugal pathway reverses addiction-like behaviors in alcohol-dependent rats. Nat Commun 10:1238.
36. Lim MM, Liu Y, Ryabinin AE, Bai Y, Wang Z, Young LJ (2007): CRF receptors in the nucleus accumbens modulate partner preference in prairie voles. Horm Behav 51:508–515.
37. Shaham Y, Funk D, Erb S, Brown TJ, Walker CD, Stewart J (1997): Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. J Neurosci 17:2605–2614.
38. Shaham Y, Stewart J (1995): Stress reinstates heroin-seeking in drug-free animals: An effect mimicking heroin, not withdrawal. Psychopharmacology (Berl) 119:334–341.
39. Erb S, Petrovic A, Yi D, Kayyali H (2006): Central injections of CRF reinstate cocaine seeking in rats after postinjection delays of up to 3 h: An influence of time and environmental context. Psychopharmacology (Berl) 187:112–120.
40. Refojo D, Schweizer M, Kuehne C, Ehrenberg S, Thoeringer C, Vogl AM, *et al.* (2011): Glutamatergic and dopaminergic neurons mediate anxiogenic and anxiolytic effects of CRHR1. Science 333:1903–1907.
41. Wang B, Shaham Y, Zitzman D, Azari S, Wise RA, You ZB (2005): Cocaine experience establishes control of midbrain glutamate and dopamine by corticotropin-releasing factor: A role in stress-induced relapse to drug seeking. J Neurosci 25:5389–5396.
42. Vranjkovic O, Van Newenhizen EC, Nordness ME, Blacktop JM, Urbanik LA, Mathy JC, *et al.* (2018): Enhanced CRFR1-dependent regulation of a ventral tegmental area to prelimbic cortex projection establishes susceptibility to stress-induced cocaine seeking. J Neurosci 38:10657–10671.
43. Robinson MJF, Warlow SM, Berridge KC (2014): Optogenetic excitation of central amygdala amplifies and narrows incentive motivation to pursue one reward above another. J Neurosci 34:16567–16580.
44. Baumgartner HM, Cole SL, Olney JJ, Berridge KC (2020): Desire or dread from nucleus accumbens inhibitions: Reversed by same-site optogenetic excitations. J Neurosci 40:2737–2752.
45. Warlow SM, Naffziger EE, Berridge KC (2020): The central amygdala recruits mesocorticolimbic circuitry for pursuit of reward or pain. Nat Commun 11:2716.
46. Fadok JP, Krabbe S, Markovic M, Courtin J, Xu C, Massi L, *et al.* (2017): A competitive inhibitory circuit for selection of active and passive fear responses. Nature 542:96–100.
47. Torruella-Suárez ML, Vandenberg JR, Cogan ES, Tipton GJ, Teklezghi A, Dange K, *et al.* (2020): Manipulations of central amygdala neurotensin neurons alter the consumption of ethanol and sweet fluids in mice. J Neurosci 40:632–647.
48. Soares-Cunha C, Coimbra B, David-Pereira A, Borges S, Pinto L, Costa P, *et al.* (2016): Activation of D2 dopamine receptor-expressing neurons in the nucleus accumbens increases motivation. Nat Commun 7:11829.
49. Warlow SM, Robinson MJF, Berridge KC (2017): Optogenetic central amygdala stimulation intensifies and narrows motivation for cocaine. J Neurosci 37:8330–8348.
50. Paxinos G, Watson C (2007): The Rat Brain in Stereotaxic Coordinates, 6th ed. Amsterdam, the Netherlands: Academic Press.

51. Wang F, Flanagan J, Su N, Wang LC, Bui S, Nielson A, *et al.* (2012): RNAscope: A novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J Mol Diagn 14:22–29.
52. Rodaros D, Caruana DA, Amir S, Stewart J (2007): Corticotropin-releasing factor projections from limbic forebrain and paraventricular nucleus of the hypothalamus to the region of the ventral tegmental area. Neuroscience 150:8–13.
53. Asok A, Draper A, Hoffman AF, Schulkin J, Lupica CR, Rosen JB (2018): Optogenetic silencing of a corticotropin-releasing factor pathway from the central amygdala to the bed nucleus of the stria terminalis disrupts sustained fear. Mol Psychiatry 23:914–922.
54. Pomrenze MB, Tovar-Diaz J, Blasio A, Maiya R, Giovanetti SM, Lei K, *et al.* (2019): A corticotropin releasing factor network in the extended amygdala for anxiety. J Neurosci 39:1030–1043.
55. Ventura-Silva AP, Borges S, Sousa N, Rodrigues AJ, Pêgo JM (2020): Amygdalar corticotropin-releasing factor mediates stress-induced anxiety. Brain Res 1729:146622.
56. Erb S, Salmaso N, Rodaros D, Stewart J (2001): A role for the CRF-containing pathway from central nucleus of the amygdala to bed nucleus of the stria terminalis in the stress-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 158:360–365.
57. Dabrowska J, Hazra R, Guo JD, Dewitt S, Rainnie DG (2013): 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 7:156.
58. Pomrenze MB, Giovanetti SM, Maiya R, Gordon AG, Kreeger LJ, Messing RO (2019): Dissecting the roles of GABA and neuropeptides from rat central amygdala CRF neurons in anxiety and fear learning. Cell Rep 29:13–21.e4.
59. Partridge JG, Forcelli PA, Luo R, Cashdan JM, Schulkin J, Valentino RJ, Vicini S (2016): Stress increases GABAergic neurotransmission in CRF neurons of the central amygdala and bed nucleus stria terminalis. Neuropharmacology 107:239–250.
60. Shimada S, Inagaki S, Kubota Y, Ogawa N, Shibasaki T, Takagi H (1989): Coexistence of peptides (corticotropin releasing factor/neurotensin and substance P/somatostatin) in the bed nucleus of the stria terminalis and central amygdaloid nucleus of the rat. Neuroscience 30:377–383.
61. Schwandt ML, Cortes CR, Kwako LE, George DT, Momenan R, Sinha R, *et al.* (2016): The CRF1 antagonist verucerfont in anxious alcohol-dependent women: Translation of neuroendocrine, but not of anti-craving effects. Neuropsychopharmacology 41:2818–2829.
62. Kwako LE, Spagnolo PA, Schwandt ML, Thorsell A, George DT, Momenan R, *et al.* (2015): The corticotropin releasing hormone-1 (CRH1) receptor antagonist pexacerfont in alcohol dependence: A randomized controlled experimental medicine study. Neuropsychopharmacology 40:1053–1063.
63. Grillon C, Hale E, Lieberman L, Davis A, Pine DS, Ernst M (2015): The CRH1 antagonist GSK561679 increases human fear but not anxiety as assessed by startle. Neuropsychopharmacology 40:1064–1071.
64. Shaham Y, de Wit H (2016): Lost in translation: CRF1 receptor antagonists and addiction treatment. Neuropsychopharmacology 41:2795–2797.
65. Swanson LW (2005): Anatomy of the soul as reflected in the cerebral hemispheres: Neural circuits underlying voluntary control of basic motivated behaviors. J Comp Neurol 493:122–131.
66. Heimer L, Van Hoesen GW, Trimble M, Zahm DS (2008): Anatomy of Neuropsychiatry: The New Anatomy of the Basal Forebrain and Its Implications for Neuropsychiatric Illness. San Diego: Academic Press.
67. Zahm DS (2006): The evolving theory of basal forebrain functional-anatomical 'macrosystems'. Neurosci Biobehav Rev 30:148–172.
68. Heimer L, Van Hoesen GW (2006): The limbic lobe and its output channels: Implications for emotional functions and adaptive behavior. Neurosci Biobehav Rev 30:126–147.
69. Larimer ME, Palmer RS, Marlatt GA (1999): Relapse prevention. An overview of Marlatt's cognitive-behavioral model. Alcohol Res Health 23:151–160.
70. Maisto SA, O'Farrell TJ, Connors GJ, McKay JR, Pelcovits M (1988): Alcoholics' attributions of factors affecting their relapse to drinking and reasons for terminating relapse episodes. Addict Behav 13:79–82.
71. Kaundal P, Sharma I, Jha T (2016): Assessment of psychosocial factors associated with relapse in patients with alcohol dependence: A retrospective observational study. Int J Basic Clin Pharmacol 5:969–974.
72. Annis HM, Graham JM (1995): Profile types on the inventory of drinking situations: Implications for relapse prevention counseling. Psychol Addict Behav 9:176–182.