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CRF acts in the midbrain to attenuate accumbens dopamine release to rewards but not their predictors

Matthew J Wanat[1,2], Antonello Bonci[3–5] & Paul E M Phillips[1,2]

Stressors affect dopamine-dependent behaviors such as motivation, although the underlying neurobiological mechanism is not well defined. We report that corticotropin-releasing factor (CRF) acts in the ventral tegmental area (VTA) to reduce the motivation to work for food rewards. CRF in the VTA regulates dopamine output in a stimulus- and pathway-specific manner, offering a mechanism by which acute stress selectively regulates information transmission via the VTA to reprioritize motivated behavior.

Stress can exacerbate the motivational disturbances found in psychiatric disorders such as drug addiction and depression[1,2], but the neural mechanisms by which stress influences goal-directed behavior are not well characterized. A wealth of experimental evidence indicates that motivated behavior is facilitated by activity of the mesolimbic dopamine projections from the VTA to the nucleus accumbens core (NAcc)[3]. Dopamine in the NAcc is elevated during appetitive behavior[4,5] and also in response to a variety of stressors[6,7], and thus mesolimbic VTA dopamine neurons are well positioned to mediate the interaction between stress and motivation. During stressor exposure, the neuropeptide CRF activates the hypothalamic-pituitary-adrenal axis, but it is also released into the VTA in an activity-dependent manner[8]. Electrophysiological studies demonstrate a functional diversity in CRF's postsynaptic effects on VTA dopamine neurons, with both inhibitory[9] and excitatory[10,11] actions. However, it is unknown whether CRF acts in the VTA *in vivo* to mediate the effect of acute stress on the motivation to work for natural rewards. We addressed this question and investigated the net effect of CRF in the VTA on mesolimbic dopamine transmission *in vivo* with fast-scan cyclic voltammetry during motivated behavior.

We assessed motivation by determining the amount of work (breakpoint) that rats would exert to obtain food rewards in an operant task (**Fig. 1a**) under a progressive-ratio reinforcement schedule, using a training regimen that elicits stable behavior across multiple days of testing[12] (**Supplementary Table 1**). To examine whether acute stress modulates the motivation to work for food rewards in a CRF-dependent manner, we bilaterally injected a CRF receptor antagonist (500 ng α-helical CRF) or vehicle control into the VTA and stressed the rats with 20 min of acute restraint before assessing motivation in a progressive-ratio session. Stressor exposure significantly reduced the breakpoint relative to baseline sessions, an effect that was blocked by administering the CRF receptor antagonist into the VTA (two-way ANOVA, stress × drug interaction: $F_{1,43} = 4.4$, $P < 0.05$; *post hoc* Bonferroni *t*-test, effect of stress: $t_{43} = 2.4$, $P < 0.05$; $n = 11$ rats for stress-vehicle group and $n = 12$ for other groups; **Fig. 1b**). Furthermore, a bilateral microinjection of exogenous CRF into the VTA reduced the breakpoint in a dose-dependent manner (Kruskal-Wallis $H_4 = 23.2$, $P < 0.001$; *post hoc* Mann-Whitney comparisons relative to vehicle, $n = 18$; 0.1 μg CRF, $U_{18,5} = 33$, $P > 0.05$, $n = 5$; 0.2 μg CRF, $U_{18,5} = 11.5$, $P < 0.05$, $n = 5$; 1 μg CRF, $U_{18,4} = 7$, $P < 0.05$, $n = 4$; and 2 μg CRF, $U_{18,23} = 45.5$, $P < 0.001$, $n = 23$; **Fig. 1c**). Neither stress nor CRF administration elicited gross motor impairments (**Supplementary Fig. 1**). We also observed this action of CRF in the VTA in suppressing the motivation to obtain food rewards after unilateral microinjections (**Supplementary Fig. 2**), but it was absent in

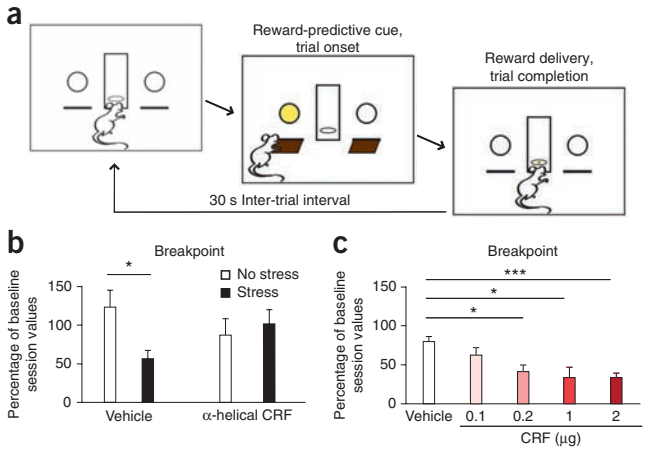

Figure 1 Effect of stress and CRF in the VTA on motivation to work for food rewards during progressive-ratio sessions. (**a**) Schematic of operant task. At trial onset the retractable levers extend and the cue light (yellow) denoting the active lever is illuminated. Completion of the correct number of lever presses leads to the delivery of food reward, retraction of the levers and the cue light turning off for a 30-s inter-trial interval. (**b**) Acute restraint stress reduced the breakpoint in progressive-ratio sessions, which was blocked by intra-VTA injections of α-helical CRF; *post hoc* Bonferroni *t*-test, $^*P < 0.05$). (**c**) The breakpoint in progressive-ratio sessions was dose-dependently attenuated by intra-VTA CRF injections; *post hoc* Mann-Whitney test relative to vehicle treatment, $^*P < 0.05$, $^{***}P < 0.001$. Data presented as mean + s.e.m.

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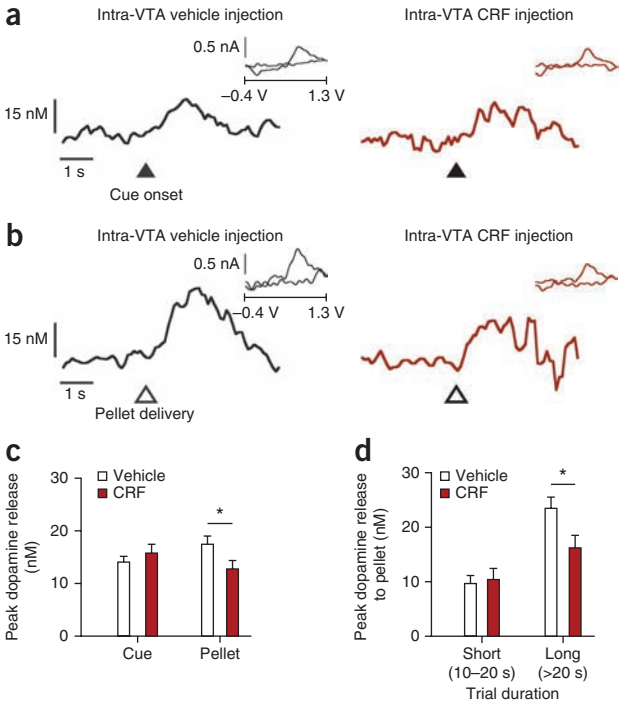

Figure 2 CRF in the VTA attenuates NAcc dopamine release to rewards but not to reward-predictive cues. (**a**,**b**) Representative change in extracellular dopamine concentration in response to the presentation of reward-predictive cues (**a**) or reward delivery (**b**) in progressive-ratio sessions after receiving intra-VTA injections of vehicle (left) or 2 µg CRF (right). Insets present cyclic voltammograms illustrating oxidation and reduction peaks that identify the detected electrochemical signal as dopamine. (**c**) Intra-VTA injections of CRF did not affect the average release in response to reward-predictive cues per trial, although they significantly attenuated the average dopamine release in response to reward delivery per trial (unpaired *t*-test, $^*P < 0.05$). (**d**) Intra-VTA CRF injections principally affected reward-evoked dopamine release in long trials (*post hoc* Bonferroni *t*-test, long trials $^*P < 0.05$). Data presented as mean + s.e.m.

rats in which the cannula placement missed the VTA (**Supplementary Fig. 3**). Collectively, these results demonstrate that CRF acts in the VTA to attenuate the motivation to work for natural rewards.

We next ascertained whether there was a corollary change in mesolimbic dopamine transmission during the suppression of motivation by CRF (**Fig. 2**). We used fast-scan cyclic voltammetry in rats during progressive-ratio sessions to monitor dopamine release in the NAcc in response to rewards and reward-predictive cues, both of which are sensitive to manipulations of reward magnitude (**Supplementary Fig. 4**). Intra-VTA injections of CRF (2 µg) reduced motivation in the rats used for voltammetry experiments (**Supplementary Fig. 5**) but, notably, had no effect on dopamine release to reward-predictive cues (unpaired *t*-test with Welch's correction, $t_{149} = 0.9$, $P > 0.05$, $n = 9$ rats with 116 trials for vehicle injection and 95 trials for CRF injection; **Fig. 2a**,**c**). In contrast, this manipulation significantly inhibited dopamine release in response to reward delivery (unpaired *t*-test, $t_{77} = 2.0$, $P < 0.05$; $n = 9$ rats with 43 trials for vehicle injection and 36 trials for CRF injection; **Fig. 2b**,**c**). Consequently, the effect of CRF in the VTA on reward-evoked dopamine release was significant for longer trials, where dopamine release is greatest (two-way ANOVA: trial duration $F_{1,75} = 24.7$, $P < 0.001$; trial duration × drug interaction $F_{1,75} = 4.2$, $P < 0.05$; *post hoc* Bonferroni *t*-test: long duration trials $t_{75} = 2.5$, $P < 0.05$; **Fig. 2d**). Intra-VTA injections of CRF also attenuated dopamine release in response to an unexpected food pellet delivery given at the end of the progressive-ratio session (**Supplementary Fig. 6**). These data demonstrate that when CRF acts in the VTA to reduce motivation to work for food rewards, it produces a selective abrogation of dopamine release in response to rewards, without affecting dopamine release in response to reward-predictive cues.

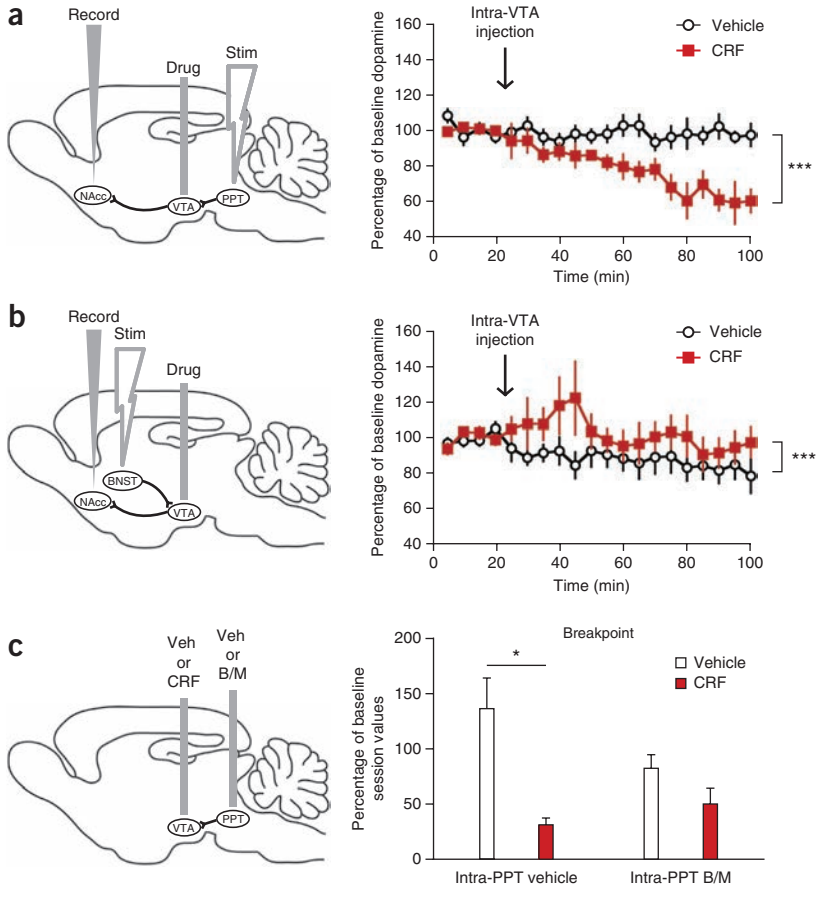

Figure 3 CRF in the VTA affects NAcc dopamine release in a pathway-specific manner. (**a**) Schematic of experimental procedure (left). Stim, stimulation. Intra-VTA CRF (2 µg) injections decreased dopamine release in the NAcc core when stimulating the PPT (two-way ANOVA drug effect, $^{***}P < 0.001$; right). (**b**) Schematic of experimental procedure (left). Intra-VTA CRF injections increased dopamine release in the NAcc core when stimulating the BNST (two-way ANOVA drug effect, $^{***}P < 0.001$; right). (**c**) Schematic of experimental procedure (left). Intra-VTA injections of 2 µg CRF did not alter motivation after inactivation of the PPT with injections of B/M (*post hoc* unpaired *t*-test, $^*P < 0.05$; right). Veh, vehicle. Data presented as mean + s.e.m.

The ability of CRF in the VTA to affect phasic dopamine release in a stimulus-specific manner suggests that CRF regulates information transmitted through a subset of synaptic inputs to the VTA. To probe this hypothesis, we assessed how CRF in the VTA affected dopamine release in the NAcc in response to stimulation of the pedunculopontine tegmental nucleus (PPT) or the bed nucleus of the stria terminalis (BNST). Activation of either the PPT or the BNST evokes phasic dopamine release in a VTA-dependent manner[13–15] (**Supplementary Fig. 7**). Intra-VTA CRF injections decreased dopamine release in the NAcc when stimulating the PPT (two-way ANOVA: drug $F_{1,170} = 88.8$, $P < 0.001$; time $F_{15,170} = 2.0$, $P < 0.05$; drug × time interaction $F_{15,170} = 1.9$, $P < 0.05$, $n = 6$ and 7 rats for vehicle and CRF groups, respectively; **Fig. 3a**) but increased dopamine when stimulating the BNST (two-way ANOVA: drug $F_{1,224} = 18.7$, $P < 0.001$, $n = 8$ rats for both groups; **Fig. 3b**), together illustrating the pathway-selective effects of CRF on dopamine release (**Supplementary Fig. 7**).

We next assessed whether the behavioral effect of CRF in the VTA on motivation could be occluded by inactivating the PPT with the GABA receptor agonists baclofen and muscimol (B/M; 0.3 nmol and 0.03 nmol, respectively) (**Fig. 3c**). CRF infusions into the VTA reduced motivation after vehicle infusions into the PPT, but this effect was blocked when the PPT was inactivated with B/M injections (two-way ANOVA: CRF $F_{1,34} = 16.9$, $P < 0.001$; CRF × B/M interaction $F_{1,34} = 4.7$, $P < 0.05$; *post hoc* unpaired *t*-test adjusted for planned comparisons with Welch's correction: effect of CRF, $t_8 = 3.6$, $P < 0.05$; $n = 9$ rats for vehicle-vehicle and B/M-CRF groups, and $n = 10$ rats for vehicle-CRF and B/M-vehicle groups; **Fig. 3c**). Reducing motivation through overnight *ad libitum* food access did not block the behavioral effect of intra-VTA CRF injections (**Supplementary Fig. 8**), suggesting that the occlusion by PPT inactivation was not due to a nonspecific manipulation of motivation. These results collectively highlight the involvement of PPT activity in the avolition elicited by CRF acting in the VTA.

Stress can reduce reward-seeking behaviors[16] and alter decision-making processes[17], which illustrates a reprioritization of behavior thought to arise from a reduction in dopamine transmission[18]. Here we demonstrate that the motivational suppressant effects of acute stress are mediated by endogenous CRF acting in the VTA and that exogenous VTA application of CRF can recapitulate these effects. Notably, in contrast to its effects in the VTA, CRF acts in the NAcc of stress-naive mice to increase dopamine release and promote appetitive behavior[19]. Moreover, CRF positively affects drug-seeking after an experience-dependent neuroadaptation in CRF's capacity to regulate glutamate release in the VTA[8,20]. Taken together, these studies illustrate the diverse effects of CRF on behavior and highlight the involvement of CRF in models of psychiatric disorders. Collectively, our results demonstrate that CRF selectively gates afferent inputs to the VTA in a stimulus- and pathway-specific manner, as well as offer a mechanism by which acute stress selectively regulates information transmission via the VTA to reprioritize motivated behavior.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

M.J.W., A.B. and P.E.M.P. designed the experiments. M.J.W. collected and analyzed the data. M.J.W. and P.E.M.P. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Subjects and surgery. All procedures were approved by the University of Washington Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Charles River, CA) were pair-housed upon arrival, given *ad libitum* access to water and laboratory chow, and maintained on a 12-h light/dark cycle. Recovery surgeries were performed under isoflurane anesthesia on rats weighing 300–350 g (~60–70 days old), after which the rats were single-housed. Carbon-fiber electrodes targeting the NAcc (relative to bregma: 1.3 mm anterior, ± 1.3 mm lateral, 7.0 mm ventral) and a Ag/AgCl reference electrode were implanted for voltammetry experiments. Implantation of guide cannulas for microinjection experiments targeted the VTA (relative to bregma: 5.6 mm posterior, 0.5 mm lateral, 7.0 mm ventral) and/or the PPT (relative to bregma: 8.0 mm posterior, 1.5 mm lateral, 5.8 mm ventral). For non-recovery voltammetry surgeries, rats were anesthetized with urethane (1.5 g/kg), additional holes were drilled above the PPT (relative to bregma: 8.0 mm posterior, 2.0 mm lateral) or the BNST (relative to bregma: 0.3 mm posterior, 1.5 mm lateral), and only the guide cannula above the VTA was cemented into place.

Behavioral training. After at least 2 weeks of recovery from surgery, rats were placed and maintained on mild food restriction (~15 g/day of standard lab chow) to target 90% free-feeding weight, allowing for an increase in weight of 1.5% per week. Operant training was performed as described previously[12]. Behavioral sessions were performed in operant chambers (Med Associates, VT) that had sloped floors and a house light, and contained a food tray and two cue lights above two retractable levers on a single wall. The cue lights and their corresponding levers were located on either side of the food tray. Rats were exposed to progressive-ratio or fixed-ratio (FR) experimental sessions (one session per day) according to the schedule presented in **Supplementary Table 1**, which was previously shown to elicit stable behavior[12] and was designed to minimize inflexible behaviors by alternating the side of the active lever across sessions. This behavioral schedule also accommodated at least 2 d of recovery time between intra-VTA pharmacological manipulations. Behavioral sessions began with both levers extending, and illumination of the house light and the cue light over the active lever. Completion of the correct number of lever presses led to the delivery of food rewards (45-mg food pellets, BioServ, NJ), retraction of the levers, and the cue and house lights turning off for a 30-s inter-trial interval (ITI). Food rewards were earned on an FR4 reinforcement schedule during FR sessions that consisted of 60 trials. Progressive-ratio sessions were identical to FR4 sessions except that the operant requirement on each trial (T) was the integer (rounded down) of $1.4^{(T-1)}$ lever presses, starting at 1 lever press (that is, 1, 1, 1, 2, 3, 5, 7, 10, 14, 20, 28, 40, 56, 79, 111, 155, 217, 304, 426). Progressive-ratio sessions ended after 15 min elapsed without completion of the response requirement in a trial. Rats completed at least two baseline progressive-ratio sessions before the reward magnitude was changed or drugs were administered intracerebrally. Acute stress was administered by placing the rat in a tail vein restrainer for 20 min. No experimental manipulation was performed on the progressive-ratio session after exposure to restraint stress. All manipulations were performed in a counterbalanced manner and only during progressive-ratio sessions.

Microinjections. Intracerebral injectors extended 1 mm past the end of the guide cannula, targeting a final depth below the skull surface of 8.0 mm for intra-VTA injections and 6.8 mm for intra-PPT injections. Infusions were performed 15 min before the start of the behavioral session and were visually monitored to ensure successful infusion. Injectors remained in place for 1 min after injection to minimize backflow of the drug. Injections of α-helical CRF (500 ng in 1% acetic acid in saline vehicle) and CRF (100 ng, 200 ng, 1 μg or 2 μg in artificial cerebrospinal fluid) were administered into the VTA in a volume of 0.5 μl. Doses of CRF were based upon previous work using site-specific injections[21]. Intra-PPT injections of baclofen and muscimol (0.3 nmol and 0.03 nmol, respectively) were delivered in saline vehicle at a volume of 0.3 μl. All drug treatments were administered in a counterbalanced manner across progressive-ratio sessions. Drugs were purchased from Bachem (CRF), Tocris (baclofen and muscimol) and Sigma (α-helical CRF).

Voltammetry recording sessions. During experimental recording sessions in behaving rodents, the chronically implanted carbon-fiber microelectrodes were connected to a head-mounted voltammetric amplifier for dopamine detection by fast-scan cyclic voltammetry as described elsewhere[22]. The potential applied to the carbon fiber was ramped from −0.4 V (versus Ag/AgCl) to +1.3 V and back at a rate of 400 V/s during a voltammetric scan and held at −0.4 V between scans at a frequency of 10 Hz. To confirm that electrodes were capable of detecting dopamine, unexpected food pellets were delivered before and after a recording session to elicit dopamine release. Chemical verification of dopamine was achieved by obtaining high correlation of the cyclic voltammogram (electrochemical signature) to that of a dopamine standard (correlation coefficient $r^2 \geq 0.75$ by linear regression). The voltammetry data for a session were not analyzed if food pellet delivery did not elicit dopamine release that satisfied the chemical verification criteria. For anesthetized experiments, dopamine release was evoked by stimulating the PPT (relative to bregma: 8.0 mm posterior, 2.0 mm lateral, 6.5–7.5 mm ventral) or the BNST (relative to bregma: 0.3 mm posterior, 1.5 mm lateral, 6.5–7.5 mm ventral) with a bipolar stimulating electrode (60 pulses delivered at 60 Hz, ≤200 μA). Stimulations were performed every 5 min until a stable baseline for 20 min was achieved (<10% deviation from the mean peak response of dopamine). Intra-VTA drug injections were performed as described above, and stimulations commenced immediately after completion of the infusion.

Data analysis. Dopamine was isolated from the voltammetric signal using chemometric analysis[23] with a standard training set of stimulated dopamine release detected by chronically implanted electrodes, as has been previously reported[12]. Dopamine concentration was estimated on the basis of the average postimplantation sensitivity of electrodes[22]. A within-animal design was used for voltammetry data analysis, so that data were included only from rats where dopamine release satisfied the chemical verification criteria on both the control and treatment sessions. Voltammetric data analysis was carried out using software written in LabVIEW and low-pass filtered at 2,000 Hz. Data were smoothed using a 0.5-s moving average. Analysis of extracellular dopamine concentration was restricted to a period of 3 s after cue onset or reward delivery. The analysis of cue-evoked dopamine release omitted the first trial of a session; that is, before the first reward in the session. Reward-evoked dopamine release in individual trials was analyzed only for trials lasting more than 10 s in order to minimize any carryover contribution of cue-evoked dopamine release in the detected signal, as described previously[12]. The number of animals used per experiment was determined by a power analysis with an α of 0.05 and power of 0.8, using the effect size and variance estimated from preliminary data. To assess normality, the Kolmogorov-Smirnov test was performed on the residuals after data were fitted to a Gaussian curve with the mean and s.d. of each data set. If data failed this test, nonparametric statistical tests were performed. The Welch's correction was used for *post hoc* tests under conditions with unequal variances between groups. Statistical analyses of voltammetry data used unpaired Student's *t*-tests, or two-way ANOVAs with repeated measures when appropriate, followed by *post hoc t*-tests. Statistical analyses of behavioral data used Kruskal-Wallis tests followed by Mann-Whitney *post hoc* tests corrected for inflated α, Student's *t*-tests (unpaired or paired, as appropriate) or two-way ANOVAs, followed by *post hoc t*-tests. Data were normalized to the behavior observed on the baseline sessions before experimental manipulations to reduce inter-animal variability. Rats were also excluded from behavioral studies if they did not complete >50% of planned experiments owing to technical complications. Rats were excluded for analysis if cannulas did not target the region of interest, save for those illustrating the lack of an effect of CRF when administered outside of the VTA (**Supplementary Fig. 3**). General motor activity was assessed using the rate of head entries into the food tray. Data were analyzed using Excel, Prism and SPSS.

Histology. After completion of the experimental sessions, rats were anesthetized with ketamine and xylazine (100 mg/kg and 20 mg/kg, respectively) and the recording site was marked by making a small electrolytic lesion at the electrode tip by passing a current (~70 μA) through the carbon fiber microelectrode for 20 s. Animals were subsequently perfused transcardially with 4% paraformaldehyde in phosphate-buffered saline at pH = 7.4 before the brains were removed and postfixed in the paraformaldehyde solution. The brains were then placed in 30% sucrose solution in phosphate-buffered saline for 48 h, flash frozen and sectioned coronally (60 μm). All sections were mounted and stained with cresyl violet. Histology is presented in **Supplementary Figure 9**.

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SUPPLEMENTARY INFORMATION

CRF acts in the midbrain to attenuate accumbens dopamine release to rewards but not their predictors

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Session number	1	2	3	4	5	6	7	8	9	10
Session type	FR4	PR	FR4	FR4	PR	FR4	PR	FR4	FR4	PR
Active lever	Left	Left	Left	Right	Right	Right	Right	Right	Left	Left

Supplementary Table. Training schedule. Training schedule repeats until the experiment is completed. Only one session was performed per day. Manipulations of reward size or intra-VTA drug injections were performed only on progressive ratio (PR) sessions. No manipulations were performed fixed ratio – 4 (FR4) sessions.

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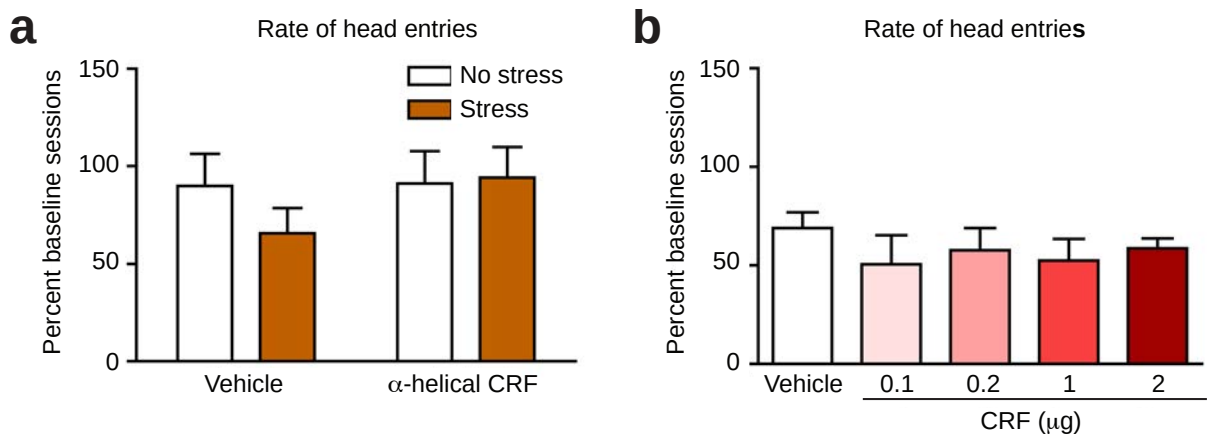

Supplementary Figure 1. Acute restraint stress and intra-ventral tegmental area (VTA) injections of corticotropin-releasing factor (CRF) did not elicit gross impairments of motor function. (**a**) The rate of head entries into the food tray was unperturbed by acute stress or intra-VTA injections of the CRF receptor antagonist, 500 ng α-helical CRF (two-way ANOVA: stress $F_{1,43} = 0.5$, $P > 0.05$; drug $F_{1,43} = 0.9$, $P > 0.05$; stress x drug interaction: $F_{1,43} = 0.7$, $P > 0.05$; $n = 11$ for Stress-Vehicle group and $n = 12$ rats for all other groups). (**b**) Intra-VTA injections of CRF did not alter the rate of head entries into the food tray (one-way ANOVA, $F_{4,50} = 0.7$, $P > 0.05$; vehicle: $n = 18$; 0.1 μg CRF: $n = 5$; 0.2 μg CRF: $n = 5$; 1 μg CRF: $n = 4$; 2 μg CRF: $n = 23$). Data presented as mean + s.e.m.

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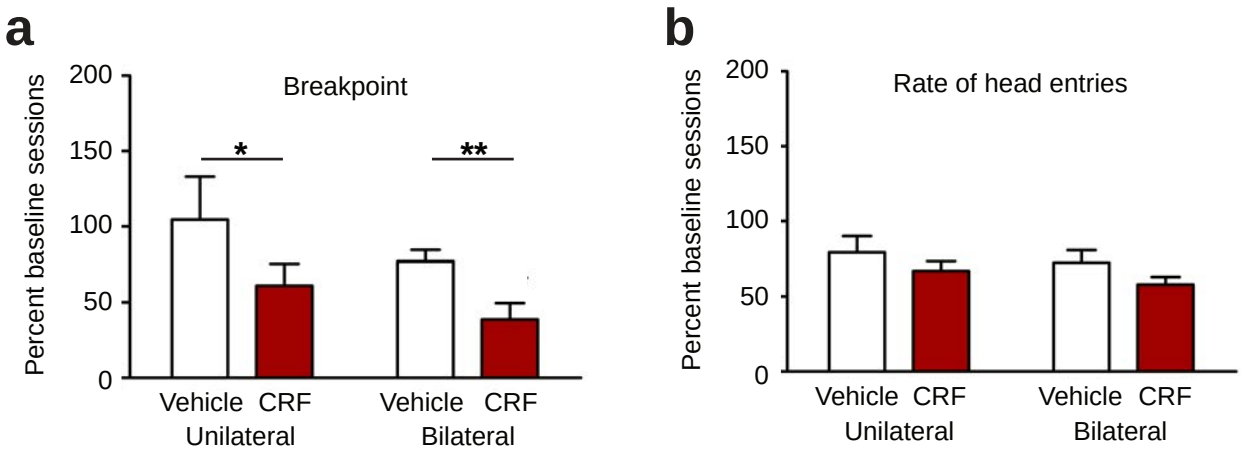

Supplementary Figure 2. Behavioral effects of unilateral or bilateral injections of CRF on motivated behavior during PR sessions. (**a**) Both unilateral (n = 9 rats) and bilateral (n = 16 rats) injections of 2 μg CRF into the VTA reduced the breakpoint (two-way ANOVA: drug $F_{1,46} = 7.8$, $P < 0.01$; injection type $F_{1,46} = 2.9$, $P > 0.05$; drug x injection type $F_{1,46} = 0.0$, $P > 0.05$; post-hoc paired t-tests of drug effect, unilateral: $t_8 = 2.7$, * $P < 0.05$; bilateral: $t_{15} = 3.0$, ** $P < 0.01$). (**b**) There was no effect of unilateral or bilateral intra-VTA CRF injections on the rate of head entries during PR sessions (two-way ANOVA: drug $F_{1,46} = 2.8$, $P > 0.05$; injection type $F_{1,46} = 1.0$, $P > 0.05$; drug x interaction type $F_{1,46} = 0.0$, $P > 0.05$; post-hoc paired t-tests of drug effect $P > 0.05$). Data presented as mean + s.e.m.

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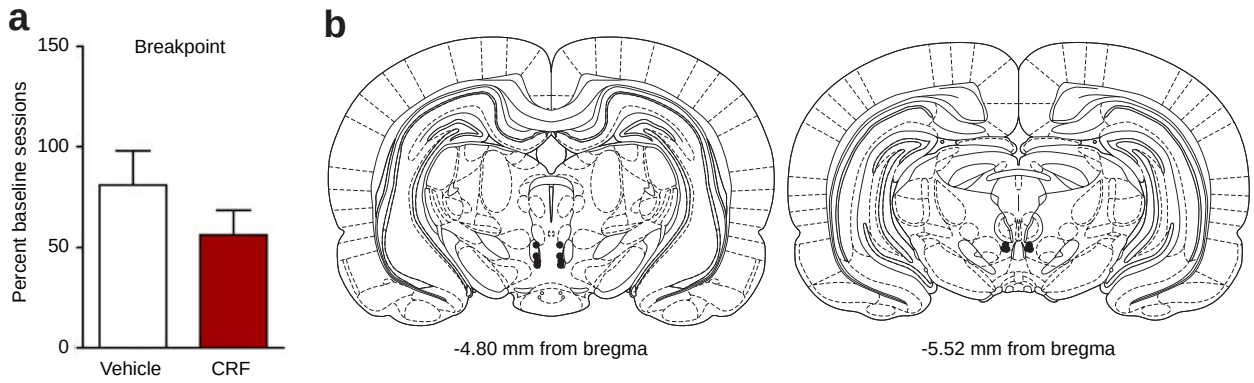

Supplementary Figure 3. The effect of CRF on motivated behavior was absent when guide cannula did not target the VTA. (**a**) In rats with cannula placements outside of the VTA (n = 7 rats), CRF (2 μg) injections had no effect on the breakpoint relative to vehicle injections (paired t-test, $t_6 = 1.3$, $P > 0.05$). (**b**) Injection sites that were outside of the VTA are denoted by black circles. Data presented as mean + s.e.m.

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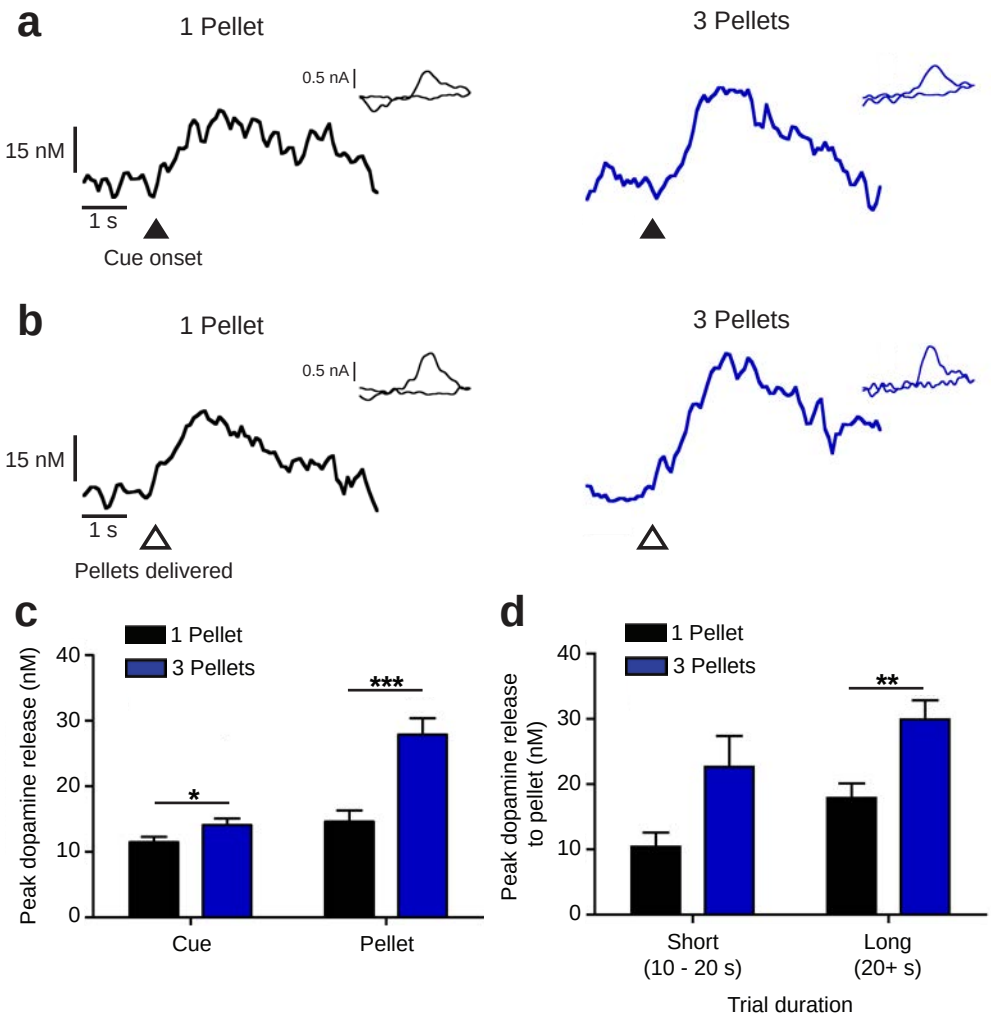

Supplementary Figure 4. NAcc dopamine release in response to rewards and their predictors is sensitive to reward size in PR sessions. Representative change in extracellular dopamine concentration in response to the presentation of (**a**) reward-predictive cues or (**b**) reward delivery when working for a single food pellet per trial (left) or three food pellets per trial (right) in PR sessions. Insets present cyclic voltammograms identifying the detected electrochemical signal as dopamine. (**c, left**) Average dopamine release to reward-predictive cues per trial was enhanced by increased reward size (unpaired t-test with Welch's correction, $t_{201} = 2.0$, * $P < 0.05$, $n = 8$ rats with 103 trials from the 1 pellet condition and 109 trials from the 3 pellet condition). (**c, right**) Average dopamine release to reward delivery per trial was enhanced by increased reward size (unpaired t-test with Welch's correction, $t_{71} = 4.4$, *** $P < 0.001$, $n = 8$ rats with 39 trials from the 1 pellet condition and 43 trials from the 3 pellet condition). (**d**) Dopamine release to rewards as a function of trial duration was sensitive to reward size (two-way ANOVA: trial duration $F_{1,78} = 5.3$, $P < 0.05$; reward size $F_{1,78} = 14.4$, $P < 0.001$; trial duration x reward size $F_{1,78} = 0.0$, $P > 0.05$; planned post-hoc t-tests with Welch's correction, long duration trials, $t_{50} = 3.3$, ** $P < 0.01$). Data presented as mean + s.e.m.

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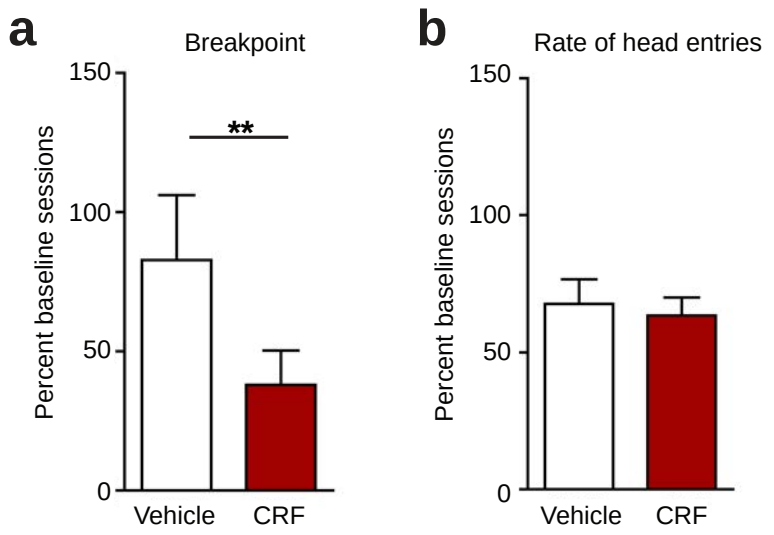

Supplementary Figure 5. Behavioral data from the rats included in the voltammetry experiments assessing the effect of CRF in the VTA on motivation. During PR sessions, intra-VTA injections of 2 μg CRF (**a**) significantly reduced the breakpoint (paired t-test, $t_8 = 3.8$, ** $P < 0.01$, $n = 9$ rats), (**b**) without affecting the rate of head entries (paired t-test, $t_8 = 0.3$, $P > 0.05$). These data from the voltammetry subset of rats are in agreement with the entire set of animals (**Fig. 1c**). Data presented as mean + s.e.m.

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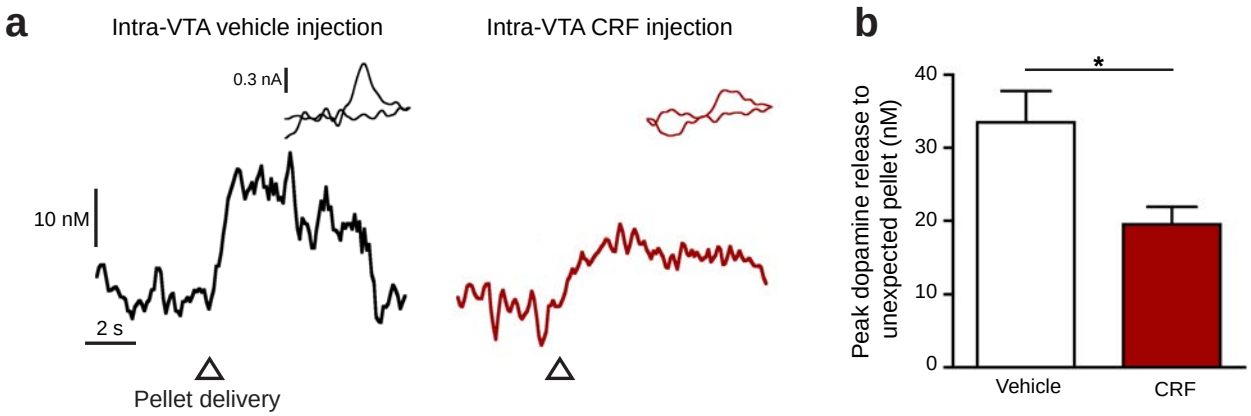

Supplementary Figure 6. CRF in the VTA reduced dopamine release to unexpected reward delivery after completion of the PR session. (**a**) Representative change in extracellular dopamine concentration in response to the delivery of a single unexpected food pellet following completion of the PR session in rats that had received intra-VTA injections of vehicle (left) or 2 μg CRF (right). Insets present cyclic voltammograms identifying the detected electrochemical signal as dopamine. (**b**) Average dopamine release to an unexpected food pellet was attenuated by intra-VTA CRF injections (unpaired t-test with Welch's correction, $t_{27} = 2.7$, * $P < 0.01$, $n = 19$ for Vehicle group and $n = 18$ for CRF group from 9 rats). Data presented as mean + s.e.m.

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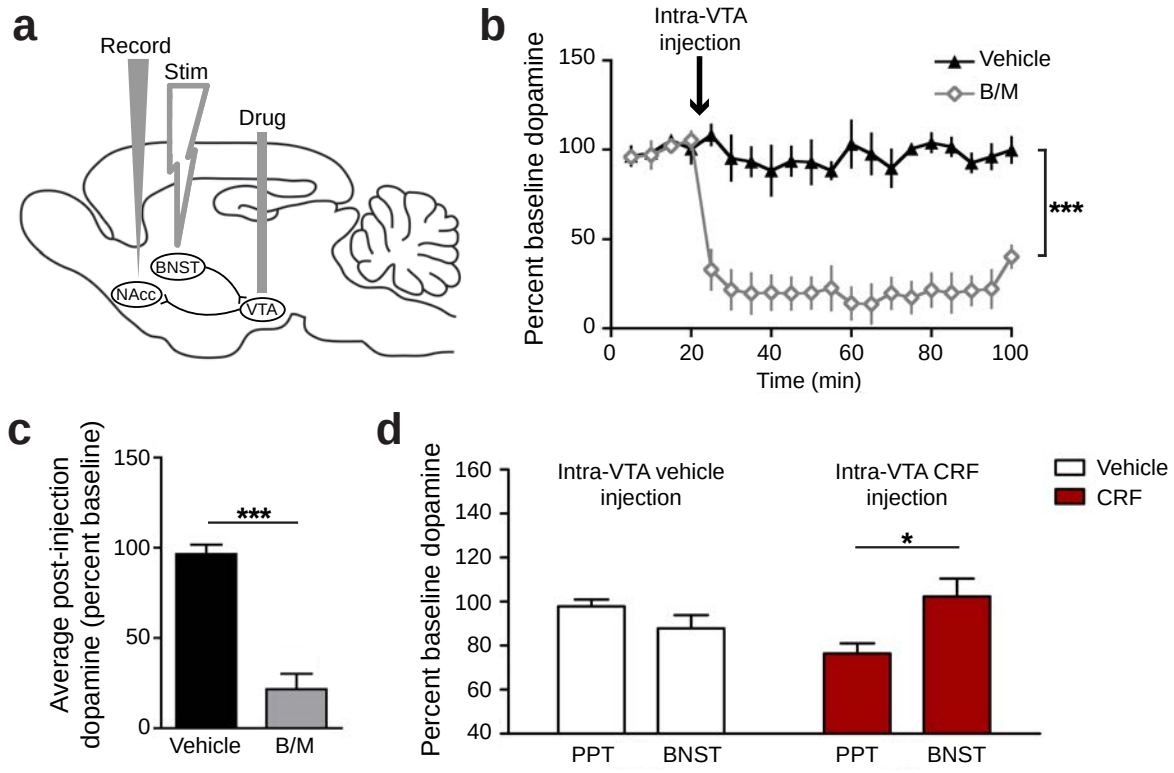

Supplementary Figure 7. Dopamine release in the NAcc elicited by stimulating VTA afferent pathways and its modulation by CRF. (**a**) Schematic of experimental procedure to assess if silencing the VTA with GABA receptor agonists baclofen (0.3 nmol) and muscimol (0.03 nmol) (B/M) would alter dopamine release in the NAcc induced by bed nucleus of the stria terminalis (BNST) electrical stimulation. **(b**) Intra-VTA B/M injections decreased dopamine release in the NAcc evoked by stimulating the BNST (two-way ANOVA: drug effect $F_{1,112}$ = 484.4, *** P < 0.001; trial effect $F_{1,15}$ = 0.5, P > 0.05; drug x trial interaction $F_{1,15}$ = 0.3, P > 0.05; n = 4 rats for Vehicle group, n = 5 rats for B/M group). (**c**) Average dopamine release after the intra-VTA injection was significantly attenuated by B/M (unpaired t-test, t_7 = 7.0, *** P < 0.001; n = 4 rats for Vehicle group, n = 5 rats for B/M group). (**d**) The effect of CRF in the VTA on stimulated dopamine release was pathway-selective (average dopamine release after intra-VTA injection; two-way ANOVA: drug x region effect $F_{1,25}$ = 8.4, P < 0.01; post-hoc Bonferroni t-test, effect of CRF, t_{25} = 3.0, P < 0.05; n = 6/7 rats for Vehicle/CRF groups from the pedunculopine tegmental nucleus (PPT) stimulation and n = 8 rats for both groups from the BNST stimulation). Data presented as mean ± s.e.m.

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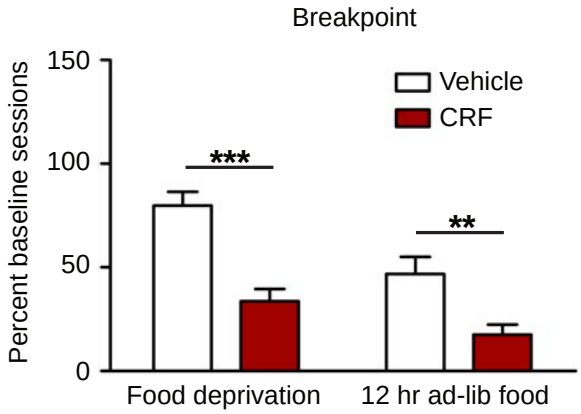

Supplementary Figure 8. Intra-VTA injections of CRF further attenuated motivation in rats in a reduced motivational state. Overnight ad libitum access to food reduced motivation but this did not prevent a further attenuation in motivation elicited by intra-VTA 2 μg CRF injections (two-way ANOVA: food deprivation effect $F_{1,61}$ = 13.1, P < 0.001; drug effect $F_{1,61}$ = 30.7, P < 0.001; food deprivation x drug interaction $F_{1,61}$ = 1.5, P > 0.05; post hoc unpaired t-tests, 12 hr ad-lib: t_{11} = 3.9, ** P < 0.01; Food deprivation: t_{39} = 5.2, *** P < 0.001; n = 18 for Food deprivation-Vehicle group, n = 23 for Food deprivation-CRF group and n = 12 rats for both 12 ad-lib food groups,). Data presented as mean + s.e.m.

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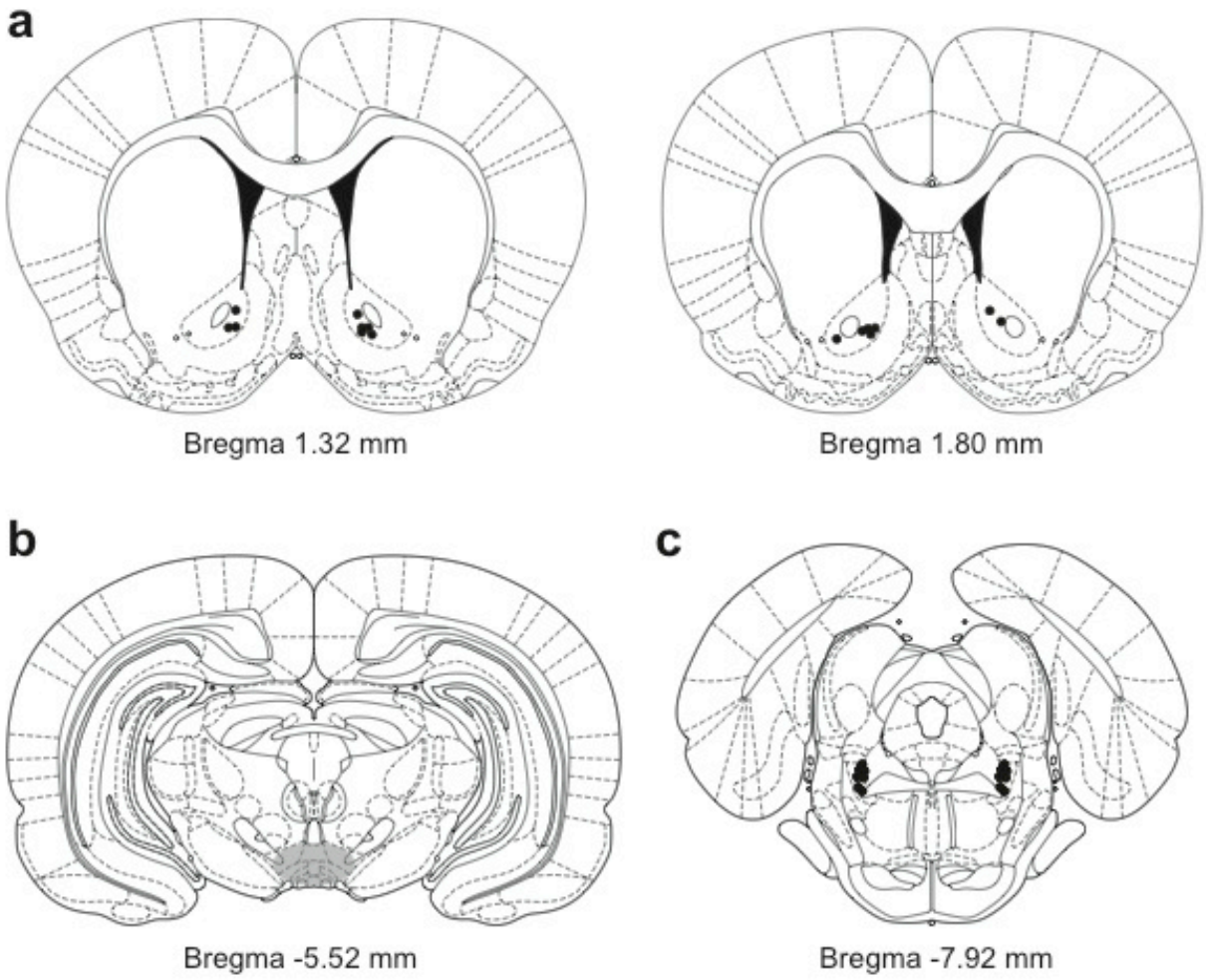

Supplementary Figure 9. Histology. (**a**) Black circles identify the sites of electrolytic lesions that denote the location of voltammetry electrodes used in this study. (**b**) Grey region denotes the location of intra-VTA microinjections included in the study. (**c**) Black circles identify the sites of intra-PPT microinjections. The location of guide cannula that missed the VTA is presented in **Supplementary Fig. 3**.

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