

Effects of food restriction on expression of place conditioning and biochemical correlates in rat nucleus accumbens

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Abstract

Rationale When ad libitum-fed rats undergo cocaine place preference conditioning (CPP) but are switched to food restriction for testing, CPP becomes resistant to extinction and correlates with phosphorylation of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor GluA1 at Ser845 in nucleus accumbens (NAc) core.

Objectives This study tested whether food restriction increases persistence of morphine CPP and conditioned place aversions (CPA) induced by LiCl and naloxone-precipitated morphine withdrawal.

Materials and methods Ad libitum-fed rats were conditioned with morphine (6.0 mg/kg, i.p.), LiCl (50.0/75.0 mg/kg, i.p.), or naloxone (1.0 mg/kg, s.c.) 22 h post-morphine (20.0 mg/kg, s.c.). Half of the subjects were then switched to food restriction. Daily testing resumed 3 weeks later, and brains were harvested when one diet group met extinction criterion. Western analyses probed for pSer845-GluA1, pERK1, and pERK2 in NAc.

Results Food restriction increased persistence of morphine CPP and preference scores correlated with pSer845-GluA1 in NAc core and shell. LiCl CPA was curtailed by food restriction, yet pSer845-GluA1 and pERK2 were elevated in NAc core of food-restricted rats. Food restriction increased persistence of naloxone CPA and elevated pSer845-GluA1 in NAc

core and shell, and aversion scores were negatively correlated with pERK1 and pERK2 in NAc core.

Conclusions These results suggest that food restriction prolongs responsiveness to environmental contexts paired with subjective effects of both morphine and morphine withdrawal. A mechanistic scheme, attributing these effects to upregulation of pSer845-GluA1, but subject to override by CPA-specific, pERK2-mediated extinction learning, is explored to accommodate opposite effects of food restriction on LiCl and naloxone CPA.

Keywords Food restriction · Nucleus accumbens · Conditioned place preference · Conditioned place aversion · GluA1 · ERK

An early guiding hypothesis in drug abuse research, now well supported by several decades of research, is that drugs of abuse target and appropriate the neural substrates for appetitive motivation and reward. With a primary focus on mechanisms of energy homeostasis and ingestive behavior, CNS and behavioral effects of a wide array of abused drugs have been shown to be modulated by diet and patterns of ingestive behavior (Carroll et al. 1979; Cabeza de Vaca and Carr 1998; Wellman et al. 2007; Thanos et al. 2010; Puhl et al. 2011; Collins et al. 2015; Sedki et al. 2015). Most of this research has employed drug abuse models that involve acute drug challenge or acquisition/maintenance of self-administration behavior.

A hallmark characteristic of addiction, which undercuts the efforts of clinicians and abstinent drug users, is the craving and relapse induced by several identified triggers, one of which is environmental cues/contexts associated with previous drug use (Childress et al. 1988; O'Brien et al. 1992). The modulatory effects of diet, energy balance, and associated

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neural and endocrine factors on responsiveness to drug-associated cues and contexts have received little experimental attention. Using a conditioned place preference (CPP) paradigm, it has been shown that a cocaine CPP is more robust and resistant to extinction if subjects are switched from ad libitum feeding to chronic food restriction in the 3-week period that intervenes between conditioning and testing (Zheng et al. 2012). This observation is concordant with reports that food restriction enhances reinstatement of cocaine (Carroll 1985) and heroin seeking (D’Cunha et al. 2013) in self-administration protocols. The cocaine CPP studies have also revealed that the persistent CPP in food-restricted subjects is not affected by the temporal relation between the most recent meal and testing, or meal-entrained levels of blood-borne metabolic hormones (Zheng et al. 2013), but correlates with phosphorylation of the glutamatergic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor GluA1 subunit at Ser845 in nucleus accumbens core and is blocked by accumbens core microinjection of Naspmp, a selective antagonist of calcium-permeable AMPA receptors (Zheng et al. 2013; Zheng et al. 2015). This parallels findings relating to increased unconditioned drug reward in food-restricted rats, though the accumbens subdivision implicated is the shell (Carr et al. 2010; Peng et al. 2014).

The purpose of the present study was to more fully characterize the effect of food restriction on conditioned responding to contexts. To test generality of the enhancing effect of food restriction on CPP expression, experiment 1 compared morphine CPP expression in ad libitum-fed and food-restricted rats. Following observation of an enhancing effect similar to that seen in the cocaine CPP experiments, two additional experiments were conducted to determine whether the effect of food restriction is confined to reward-related behavior or extends to other forms of place conditioning and therefore might reflect alteration of a general function such as memory retrieval or extinction learning. Consequently, conditioned place aversions (CPA) induced by LiCl and naloxone-precipitated morphine withdrawal were examined. In all experiments, the association between diet, behavior, pSer845-GluA1, as well as pERK1/2, was examined in NAc core and shell.

Materials and methods

Subjects and food restriction

Experimental procedures were approved by the Institutional Animal Care and Use Committee at the New York University School of Medicine and were consistent with the *Principles of Laboratory Care* (NIH Publication no. 85-23). All subjects were mature male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 300–400 g (10–12 weeks old) at the start of each experiment. Rats were housed on a 12-h

light/dark photoperiod with lights on between 0600 and 1800 in a central animal facility. All subjects were singly housed in individual plastic cages with bedding and free access to water.

Ad libitum-fed animals had free access to standard lab pellets (PicoLab Rodent Diet 20, LabDiet, St. Louis, MO). Food-restricted animals followed a feeding regimen described previously (e.g., Cabeza de Vaca and Carr 1998), in which rats received 40 % of ad libitum chow intake (10 g) daily, delivered to the home cage at 1700, until body weight was reduced by 20 % (~2 weeks). Daily feeding was then titrated to maintain the new body weight for 1 week before resuming behavioral testing. Restricted feeding and body weight were maintained until the end of the experiment.

Place conditioning apparatus

Each conditioning apparatus consisted of two main cubicles (25.4 × 30.5 × 30.5 cm) separated by a center compartment (10.2 × 30.5 × 30.5 cm). Removable partitions separating the main cubicles from the center compartment had walls matching their respective cubicles and a small opening (12.5 × 15 cm) allowing movement between cubicles. Partitions without openings, but still matching the respective walls, were used to isolate subjects within a cubicle during conditioning. Automated data collection was accomplished through 24 infrared photo-beam detectors along the length of the test chamber (VersaMax System, Accuscan, Columbus, OH) and provided a record of horizontal activity and time spent in each compartment.

Habituation and pre-conditioning

All rats were habituated to transport and handling on at least five occasions. On the first experimental day, each rat was pre-exposed to the place conditioning apparatus. Pre-exposure involved placing the rat in the center compartment and allowing free movement for 20 min. Time spent in each compartment was recorded. Based on the absence of initial preference for either conditioning compartment, rats were randomly assigned to receive the experimental treatment prior to placement in one of the two compartments.

Experiment 1: morphine conditioning

At least 48 h after pre-exposure, rats underwent a series of eight consecutive daily conditioning sessions. Sessions were of 30 min duration, with partitions in place and animals confined to one side of the apparatus. On alternate days, rats were injected with morphine sulfate (6.0 mg/kg, i.p.) immediately prior to placement in the assigned compartment. On intervening days, rats were injected with saline vehicle (1.0 ml/kg, i.p.) before confinement to the opposite compartment.

Experiment 2

LiCl conditioning

At least 48 h after pre-exposure, rats underwent 12 conditioning sessions, of 20-min duration, over consecutive days except as described below. On alternate days, rats were injected with LiCl (50.0 mg/kg, i.p.) immediately prior to placement in the assigned compartment. On intervening days, rats received saline-vehicle injections (1.0 ml/kg, i.p.) before confinement to the opposite side compartment. However, a CPA test conducted after the first 8 days of conditioning did not reveal a statistically significant place aversion ($t(23) = 1.19, p = 0.12$). Consequently, rats were conditioned for four more days with 75.0 mg/kg (i.p.) LiCl alternating with saline control injections.

Experiment 3

Naloxone-precipitated morphine withdrawal conditioning

The conditioning phase of this experiment consisted of four cycles based on the method of Parker and Joshi (1998). On the first day of each cycle, rats were injected with saline vehicle (1.0 ml/kg, s.c.) 5 min before placement in the assigned compartment for 30 min. One hour after removal from the compartment, rats were injected with morphine sulfate at 20.0 mg/kg (s.c.) and remained in home cages. Twenty-two hours following morphine administration, rats were injected with naloxone (1.0 mg/kg, s.c.) 5 min before placement in the alternate compartment of the conditioning apparatus for 30 min. Cycles were separated by intervals of 48–72 h.

Testing

At least 48 h after the last conditioning session, rats were tested for CPP or CPA expression. On test days, rats were placed in the center compartment and allowed to move freely throughout the apparatus for 20 min. Additional tests were conducted after a 3-week interval, during which half the subjects had been switched from ad libitum feeding to a food restriction protocol, which was maintained until the end of the experiment. Daily testing continued until one diet group displayed extinction of conditioned place behavior. Extinction was defined as three consecutive test sessions in which time spent on the experimental treatment-paired side of the conditioning apparatus did not differ from time spent on the saline-paired side, as determined by *t* test. To ensure a conservative approach to establishing extinction, *t* tests were each one tailed with the α -level set at 0.05 (i.e., no correction for multiple tests); this maximized the likelihood of concluding a

difference between time spent on the two sides, and thus no extinction. This was followed by at least one additional test session, enabling brain harvesting and biochemical assay immediately after.

Drugs

Morphine sulfate was obtained from the National Institute on Drug Abuse (NIDA), Research Triangle Institute, Research Triangle Park, NC and dissolved in sterile 0.9 % saline for subcutaneous injection. Naloxone hydrochloride dihydrate and LiCl (Sigma-Aldrich, St. Louis, MO) were dissolved in sterile 0.9 % saline for subcutaneous and intraperitoneal injection, respectively.

Biochemical measures

Immediately following the final expression test in each behavioral experiment, subjects were briefly exposed to CO₂ and decapitated by guillotine. Brains were extracted and rapidly frozen in powdered dry ice. A series of 500- μ m sections were cut using an IEC Minotome cryostat, and NAc core and shell and caudate putamen were dissected using a combination of micropunch and microknife under an Olympus dissecting microscope. Tissue lysates were prepared as described previously (e.g., Zheng et al. 2013) and stored in sample buffer in aliquots at -80°C . The protein content was determined using the BCA reagent kit with bovine serum albumin as a standard (Pierce, Rockford, IL).

Western blotting

Proteins (6–10 μ g/lane) were separated by electrophoresis on precast 4–12 % sodium dodecyl sulfate polyacrylamide gels (Lonza, Rockland, ME). Precision Plus protein standard molecular weight markers (Bio-Rad) were loaded to estimate the size of the target proteins and to ensure complete transfer of proteins from gel to membrane. Proteins were electrophoretically transferred to Protran nitrocellulose membranes (Whatman, Mobile, AL) for 1.75 h at a constant voltage of 100 V.

Membranes were blocked for 60 min with Odyssey Blocking Buffer (LI-COR Biosciences, Lincoln, NE) with shaking at room temperature. Membranes were then probed overnight at 4°C using primary antibodies for target proteins or the protein loading control, α -tubulin. Primary antibodies were diluted in Odyssey blocking buffer with 0.2 % Tween 20. After probing with primary antibodies and washing with TBST buffer (3 \times 5 min), membranes were incubated for 1 h at room temperature with IRDye 680RD goat anti-mouse IgG (H + L) and IRDye 800CW goat anti-rabbit IgG (H + L) (1:16,000; LI-COR Biosciences) in Odyssey blocking buffer with

0.2 % Tween 20. Proteins were visualized using an Odyssey CLx infrared imaging system, and bands were quantified using the Image Studio software (LI-COR Biosciences).

Primary antibodies used included mouse monoclonal anti-GluA1 (1:1000; MAB2263, Millipore, Temecula, CA), rabbit polyclonal anti-phospho-Ser845-GluA1 (1:1000; AB5849, Millipore), mouse monoclonal anti-phospho-(Thr202/Tyr204)-p44/42 ERK1/2 (1:1000; 9106s, Cell Signaling), rabbit polyclonal anti-p42/44 ERK1/2 (1:5000; 9102s, Cell Signaling), and mouse monoclonal anti- α -tubulin (1:10,000; T6199, Sigma-Aldrich).

For data analysis, phospho-proteins (pERK1, pERK2, pSer845-GluA1) were normalized to corresponding total protein/tubulin and total proteins (ERK1, ERK2, GluA 1) were normalized to tubulin.

Results

Morphine CPP

Prior to conditioning (pre), the 18 rats in this experiment showed no unconditioned preference for either side of the place preference apparatus. In the first post-conditioning expression test, there was a marked preference for the morphine-paired side of the chamber ($t(17) = 4.64, p < .001$). This result was followed by establishment of two diet groups matched for CPP expression (group assigned to food restriction: $t(8) = 3.09, p < .01$; group assigned to ad libitum feeding: $t(8) = 3.29, p < .01$) and side of chamber associated with morphine and was followed by a 3-week hiatus during which the group assigned to food restriction attained and stabilized at target body weight. Following the resumption of testing, CPP extinguished in the ad libitum-fed group after the fifth test, as defined by absence of a morphine side preference on the 6th, 7th, 8th, and 9th test days. The food-restricted group continued to display a CPP throughout this period, including the 6th ($t(8) = 4.05, p < .01$), 7th ($t(8) = 3.78, p < .01$), 8th ($t(8) = 4.01, p < .01$), and 9th ($t(8) = 3.82, p < .01$) test days. In a direct comparison of the two feeding groups, based on CPP difference scores via two-way mixed design analysis of variance (ANOVA; diet \times day), the difference between diet groups in the final three tests was confirmed ($F(1, 16) = 15.3, p < .002$) with no effect of test day and no interaction between diet and test day. Results are shown in Fig. 1.

Considering that changes in the balance of time spent between the vehicle-paired side and the neutral center may contribute to the difference score as calculated above, and to facilitate comparison of the three experiments in this study, time spent in the morphine-paired compartment across test sessions was examined. Figure 2 (top) indicates that divergence between diet groups (final three test sessions: $F(1, 16) = 6.32,$

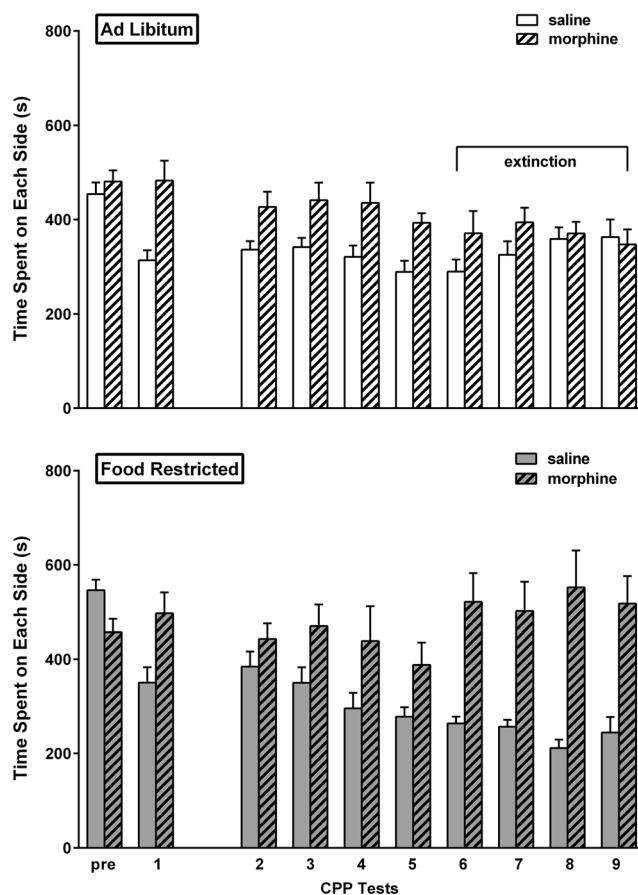
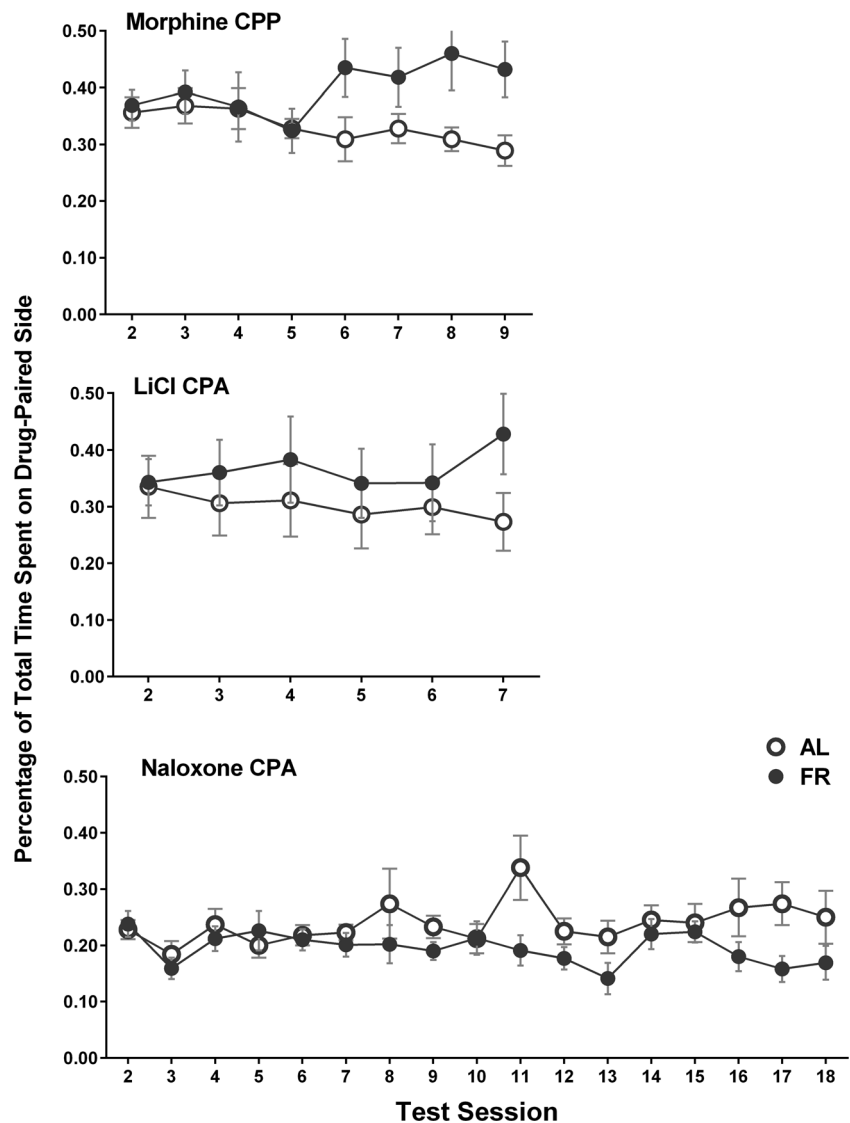


Fig. 1 Morphine CPP. Mean (\pm SEM) time spent (s) on the morphine- and saline-paired sides of the CPP apparatus during the pre-conditioning session (pre), the first post-conditioning test session (1), and all tests after the 3-week hiatus (2–9) in ad libitum-fed ($n = 9$; top) and food-restricted ($n = 9$; bottom) rats. Testing continued until the ad libitum-fed group satisfied extinction criterion (sessions 6–9 as indicated by horizontal bracket)

$p < .025$) results from a combination of progressive decrease in the ad libitum-fed group and increase in the food-restricted group. Interestingly, food-restricted subjects displayed a significant linear trend of increasing time spent on the morphine-paired side with repeated testing ($F(1, 56) = 17.3, p < .005$). This same trend was seen in food-restricted subjects expressing a cocaine CPP (Zheng et al. 2012).

Immediately following the final behavioral test, in which the food-restricted group displayed a robust CPP and the ad libitum-fed group did not, levels of pSer845-GluA1 in the NAc core and shell of food-restricted rats were higher than in ad libitum-fed rats (core: $t(16) = 2.39, p < .05$; shell: $t(16) = 2.62, p < .02$). This difference was not seen in CPU. There were no differences between diet groups in levels of total GluA1, pERK1, or pERK2 (Fig. 3). Among all subjects, regression analyses indicated a positive correlation between individual subjects' difference scores (time spent in the morphine-paired compartment – time spent in the saline-paired compartment) and phosphorylation of GluA1 at

Fig. 2 Time spent on the drug-paired side. Percentage of the total test period (mean \pm SEM) spent on the drug-paired side of the apparatus, across successive test sessions, by ad libitum-fed (*open circles*) and food-restricted (*filled circles*) subjects in the morphine CPP (*top*), LiCl CPA (*middle*), and naloxone CPA (*bottom*) experiments



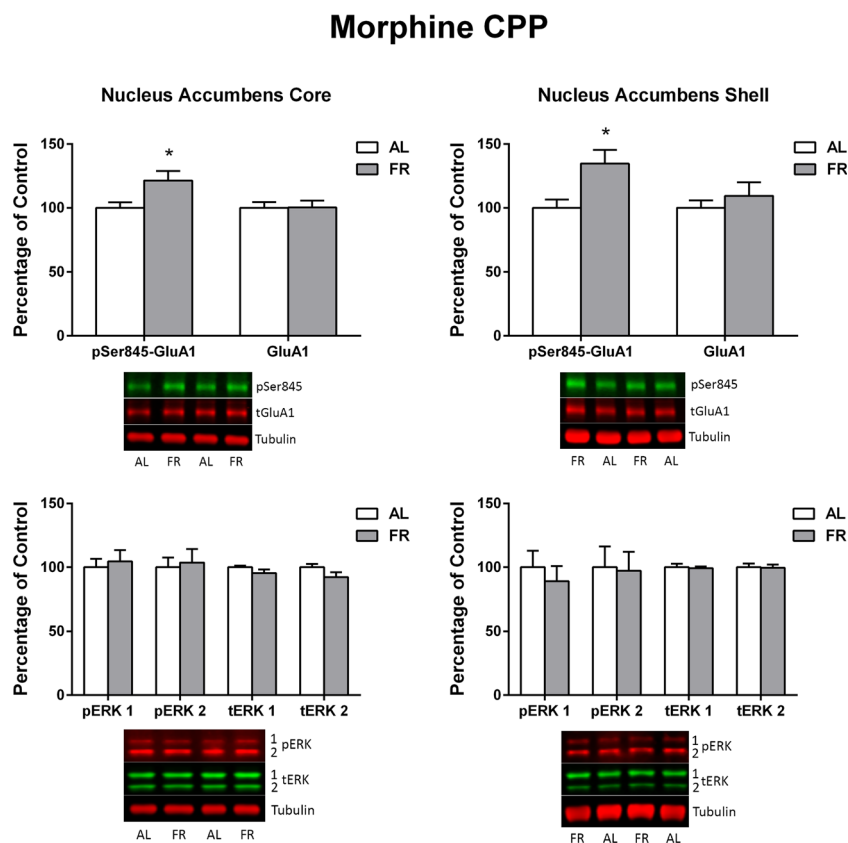
Ser845 in the core ($r = .58$; $t(16) = 2.83$, $p < .02$) and shell ($r = .56$; $t(16) = 2.7$, $p < .02$; Fig. 4). These correlations are mainly reflective of the diet group differences, in as much as regression analyses confined to individual diet groups were positive but nonsignificant. This differs from previous cocaine CPP studies in which this correlation in NAc core was significant within each diet group (Zheng et al. 2013, 2015). There were no significant correlations between total GluA1, pERK1, or pERK2 and behavior.

LiCl CPA

Prior to conditioning (pre), the 24 rats in this experiment showed no unconditioned preference for either side of the place preference apparatus. In the first post-conditioning expression test, rats displayed a strong aversion to the LiCl-paired side of the chamber ($t(23) = 3.65$, $p < 0.001$). Using these results, two groups were matched on CPA expression

and side of chamber associated with LiCl. The resulting groups both displayed CPA (ad libitum fed: $t(11) = 2.46$, $p < 0.05$; food restricted: $t(11) = 2.59$, $p < 0.05$). This was followed by a 3-week hiatus during which the group assigned to food restriction attained and stabilized at target body weight. Upon the resumption of testing, extinction criterion was met by the food-restricted group after the third test, defined by absence of a CPA on the 4th, 5th, and 6th test sessions. CPA was similarly absent in the subsequent test and then on the day of brain harvesting. In contrast, the ad libitum-fed group continued to display a CPA in tests 4 through 8 ($t(11) = 2.2$; $t(11) = 2.1$; $t(11) = 2.4$; $t(11) = 2.6$; $t(11) = 3.1$; all $p < .05$). In a direct comparison of the two feeding groups, based on CPA difference scores via two-way mixed design ANOVA (diet \times day), the difference between diet groups in the final three tests was confirmed ($F(1, 22) = 4.25$, $p = .05$) with no effect of test day and no interaction between diet and test day. Results are shown in Fig. 5.

Fig. 3 Morphine CPP. Levels of pSer845-GluA1, pERK1, pERK2, and corresponding total proteins in nucleus accumbens core (*left*) and shell (*right*) of ad libitum-fed (*AL*) and food-restricted (*FR*) rats. Results (mean \pm SEM) are expressed in comparison with the normalized control, defined as the AL group. Representative immunoblots are included beneath each bar graph. * $p < .05$



Examination of time spent in the LiCl-paired side, irrespective of time spent elsewhere (Fig. 2, middle), suggests that diet groups diverged beginning with the second test following the return from a 3-week hiatus, though on this measure the difference between groups across the final three test sessions was not statistically significant.

Immediately following the final behavioral test, in which the ad libitum-fed group displayed a CPA and the food-restricted group did not, levels of pSer845-GluA1 in the NAc core and shell of food-restricted rats were higher than in ad libitum-fed rats (core: $t(22) = 3.06$, $p < .01$; shell: $t(22) = 4.35$, $p < .0001$). Levels of pERK2 were also higher in NAc core ($t(22) = 2.77$, $p < .02$) and shell ($t(22) = 2.18$, $p < .05$; Fig. 6) of food-restricted relative to ad libitum-fed rats. These differences were not seen in CPu. There were no differences between diet groups in levels of total GluA1 or pERK1, and no significant correlations between any biochemical measure in any brain region and CPA difference scores.

Naloxone-precipitated withdrawal CPA

Prior to conditioning (pre), the 17 rats in this experiment showed no unconditioned preference for either side of the place preference apparatus. In the first post-conditioning expression test, rats displayed a strong aversion to the naloxone-

paired side of the chamber ($t(16) = 7.66$, $p < .001$). Using these results, two groups were matched on CPA expression and side of chamber associated with naloxone. The resulting groups both displayed CPA (ad libitum fed: $t(7) = 4.74$, $p = .001$; food restricted: $t(8) = 5.86$, $p < 0.001$). This was followed by a 3-week hiatus during which the group assigned to food restriction attained and stabilized at target body weight. Upon the resumption of testing, extinction criterion was met by the ad libitum-fed group in sessions 15–17, as well as in the 18th session that preceded brain harvesting. Although food-restricted rats did not meet criterion for CPA in session 15, they resumed CPA expression in sessions 16 ($t(8) = 3.3$, $p = .005$), 17 ($t(8) = 2.69$, $p < .02$), as well as in the 18th session that preceded brain harvesting ($t(8) = 1.93$, $p < .05$). In a direct comparison of the two feeding groups, based on CPA difference scores via two-way mixed design ANOVA (diet \times day), the difference between diet groups in the final three tests was confirmed ($F(1, 15) = 4.38$, $p = .053$) with no effect of test day and no interaction between diet and test day. Results are displayed in Fig. 7.

Examination of time spent in the naloxone-paired side, irrespective of time spent elsewhere (Fig. 2, bottom), suggests that the food-restricted group maintained a relatively stable avoidance while the ad libitum-fed group tended toward increasing time in the naloxone-paired side after the

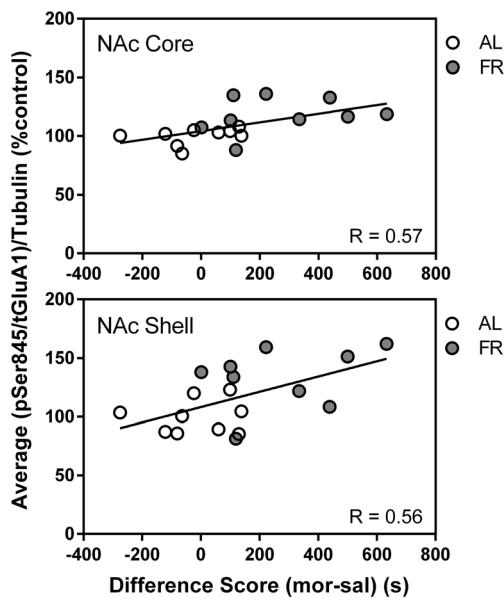


Fig. 4 Morphine CPP. Scatter plots: correlation between pSer845-GluA1 in NAc core (*top*) and shell (*bottom*) and morphine CPP difference score in the final test session among all subjects in the experiment

7th test session (final three test sessions: $F(1, 15) = 5.68$, $p < .05$).

Subjects in the naloxone CPA experiment spent an appreciable portion of their 1200 s test sessions in the center compartment of the three-compartment apparatus. To more closely evaluate the behavior of these subjects, times spent in each of the three locations are plotted in Fig. 8. It is clear that ad libitum-fed and food-restricted subjects spent equal time in the center with a similar subtle increase over the course of successive test sessions (slopes: ad libitum fed = +2.89; food restricted = +3.42). Importantly, over the course of testing, the food-restricted group maintained separation between time spent in the saline- and naloxone-paired sides (slopes: saline = -1.78; naloxone = -2.06) while the ad libitum-fed group displayed convergence (slopes: saline = -6.84; naloxone = +3.95), again suggesting CPA extinction in the ad libitum fed but not the food-restricted group.

Immediately following the final behavioral test, in which the food-restricted group displayed a robust CPA and the ad libitum-fed group did not, levels of pSer845-GluA1 in the NAc core and shell of food-restricted rats were higher than in ad libitum-fed rats (core: $t(15) = 2.7$, $p < .025$; shell: $t(15) = 2.29$, $p < .05$; Fig. 9). These differences were not seen in CPu. Diet groups did not differ in levels of total GluA1, pERK1, or pERK2 in any brain region. However, among all subjects, regression analyses indicated a negative correlation between difference scores (time spent in the saline-paired compartment – time spent in the naloxone-paired compartment) and pERK1 ($r = -.54$; $t(15) = 2.49$, $p = .025$ and

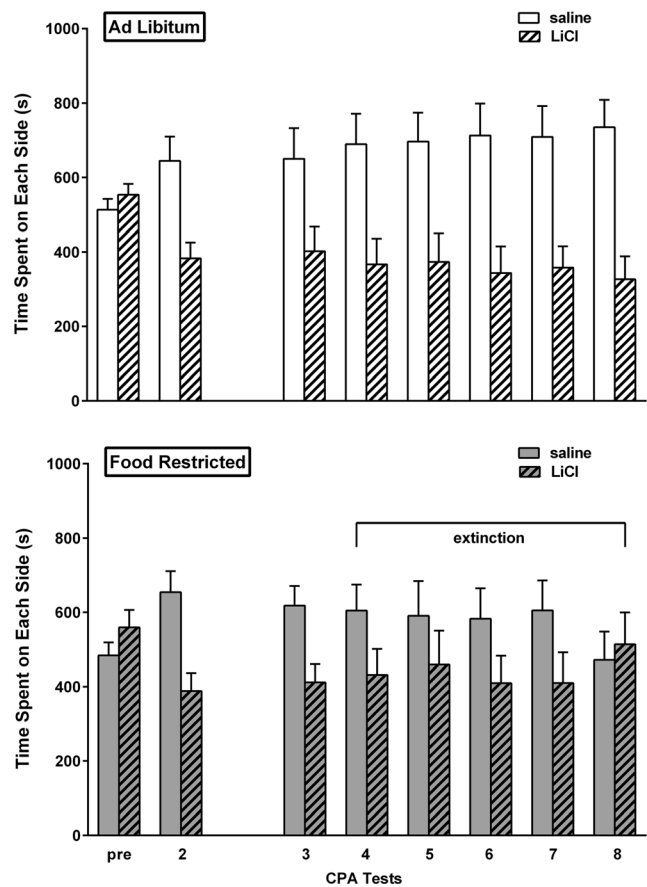


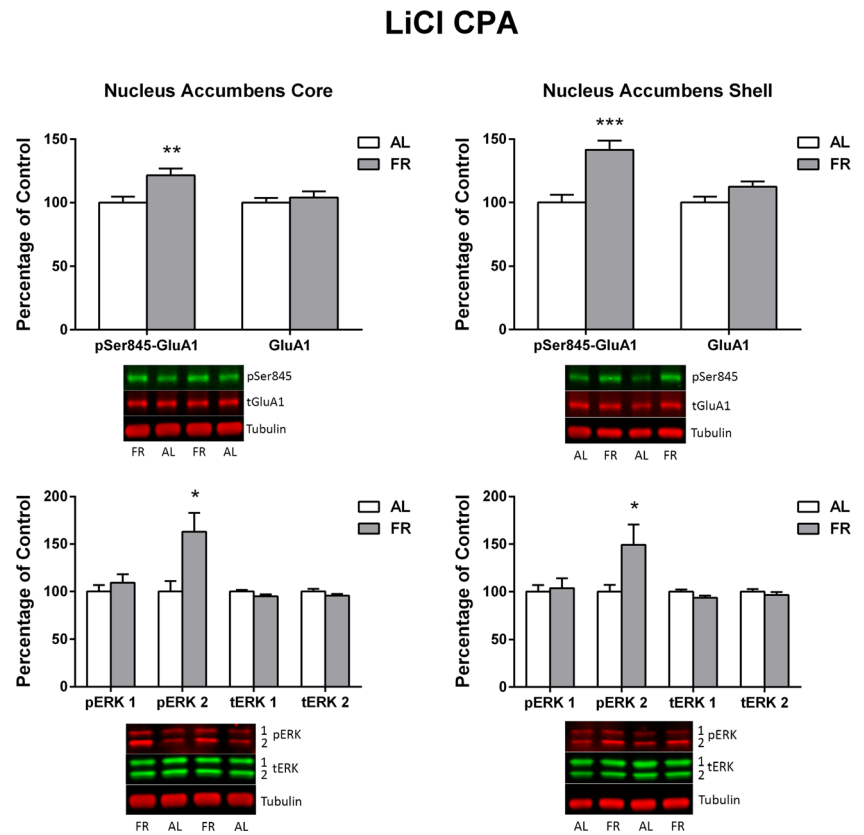
Fig. 5 LiCl CPA. Mean (\pm SEM) time spent (s) on the LiCl- and saline-paired sides of the CPA apparatus during the pre-conditioning session (*pre*), the first post-conditioning test session (*1*), and all tests after the 3-week hiatus (*3–8*) in ad libitum-fed ($n = 12$; *top*) and food-restricted ($n = 12$; *bottom*) rats. Testing continued until the food-restricted group satisfied extinction criterion (sessions 4–8 as indicated by *horizontal bracket*)

pERK2 ($r = -.47$; $t(15) = 2.07$, $p = .056$) in NAc core (Fig. 10). There were no significant correlations between pSer845-GluA1 or total GluA1 and behavior.

Dependence of increased pSer845-GluA1 on pairing of context with drug injection

A common finding among the three experiments is elevated pSer845-GluA1 in the NAc of food-restricted rats whose brains were harvested immediately after behavioral testing. In order to determine whether these elevations depend on drug-context conditioning, a control experiment was conducted. Eighteen subjects were “conditioned” in eight sessions with saline injected prior to each session. Nine subjects each were then assigned to ad libitum and food-restricted diet treatments. Three weeks later, daily “expression” testing was conducted and brains were harvested immediately after the seventh test. Levels of pSer845-GluA1 did not differ between diet groups (Fig. 11).

Fig. 6 LiCl CPA. Levels of pSer845-GluA1, pERK1, pERK2, and corresponding total proteins in nucleus accumbens core (left) and shell (right) of ad libitum-fed (AL) and food-restricted (FR) rats killed immediately after the final LiCl CPA test. Results (mean \pm SEM) are expressed in comparison with the normalized control, defined as the AL group. Representative immunoblots are included beneath each bar graph. $**p < .01$; $*p < .05$



Discussion

The first aim of the present study was to determine whether the enhancing effect of food restriction on CPP persistence applies to morphine as it does to cocaine. Thus, in experiment 1, food restriction was implemented after conditioning and confirmation of a morphine CPP. When testing resumed 3 weeks later, CPP was found to extinguish in subjects which were ad libitum fed throughout the study but continued to be expressed by subjects switched to food restriction. This result matches the result previously obtained with cocaine (Zheng et al. 2012). Interestingly, the divergence between diet groups in both studies reflected not only decreasing time spent on the drug-paired side by ad libitum-fed subjects but increasing, rather than stable, time spent by the food-restricted subjects. This observation adds weight to the possibility that food restriction enhances the incentive effect of the drug-paired context with repeated exposure, at least for a time, similar to the sensitization that might be seen if the drug itself were being repeatedly administered.

Immediately following the final morphine CPP test, levels of pSer845-GluA1 were higher in NAc core and shell of food-restricted relative to ad libitum-fed rats and the magnitude of CPP correlated with levels of pSer845-GluA1. The increase in pSer845-GluA1 appears to be triggered by exposure to the context previously paired with drug administration, rather than the basal state of

subjects, because food-restricted subjects that underwent “mock” conditioning and testing showed no increase. GluA1 is phosphorylated at Ser845 downstream of D1 dopamine receptor signaling (Snyder et al. 2000) and the upregulated response of food-restricted rats to context exposure matches their upregulated response to D1 agonist administration (Carr et al. 2010). Considering that phosphorylation at Ser845 increases AMPA currents, and trafficking and stabilization of GluA1 in the synaptic membrane (Barry and Ziff 2002; He et al. 2009; Kessels and Malinow 2009; Choquet 2010), the relationship between levels of this phosphorylated AMPA receptor subunit and CPP expression may reflect increased excitation of D1 receptor-expressing medium spiny neurons, known to increase approach and enhance CPP (Lobo et al. 2010).

The second aim of this study was to determine whether the enhancing effect of food restriction extends to CPA. Thus, in experiment 2, LiCl, which induces visceral illness, taste aversion (Nachman and Ashe 1973), and CPA (Parker and McDonald 2000) was used to condition a CPA in ad libitum-fed rats. Following confirmation of CPA expression, subjects were assigned to ad libitum and food-restricted diet groups, and CPA testing resumed 3 weeks later. Interestingly, the effect of food restriction was, if anything, opposite as that observed in the CPP studies. After the switch to food restriction, subjects displayed a weak CPA that extinguished rapidly, while subjects that remained ad libitum fed continued to

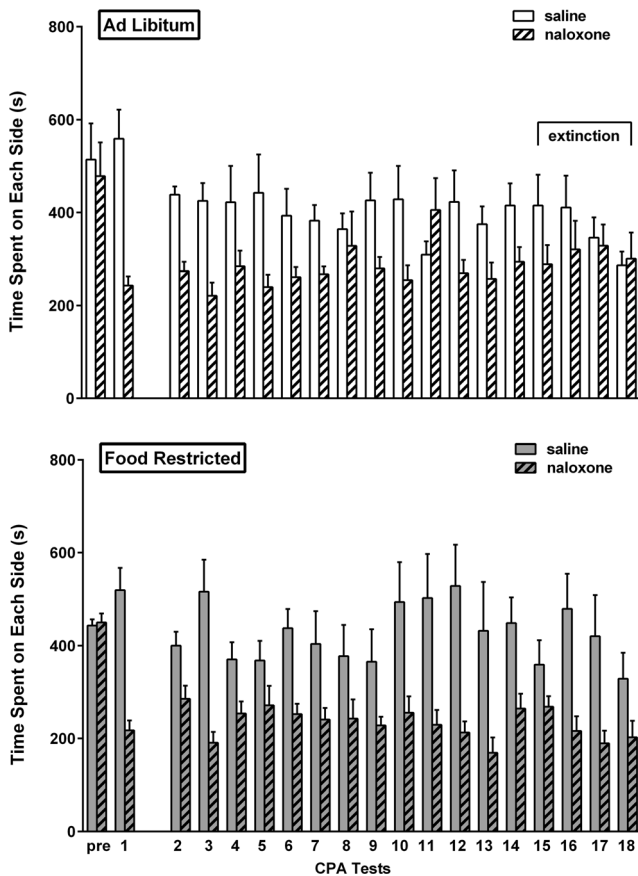


Fig. 7 Naloxone CPA. Mean (\pm SEM) time spent (s) on the naloxone- and saline-paired sides of the CPA apparatus during the pre-conditioning session (*pre*), the first post-conditioning test session (*1*), and all tests after the 3-week hiatus (*2–18*) in ad libitum-fed ($n = 8$; *top*) and food-restricted ($n = 9$; *bottom*) rats. Testing continued until the ad libitum-fed group satisfied extinction criterion (sessions 15–18 as indicated by *horizontal bracket*)

display a CPA. Despite this, food-restricted subjects again displayed elevated levels of pSer845-GluA1 in NAc core and shell. In addition, pERK2 levels were markedly higher in NAc core and shell of food-restricted relative to ad libitum-fed rats. Both of these biochemical findings are curious given that ERK phosphorylation in NAc core is necessary for expression of a cocaine CPP (Miller and Marshall 2005b) as well as reconsolidation of both cocaine and morphine CPP (Miller and Marshall 2005b; Valjent et al. 2006). Moreover, pERK plays a role in maintaining NAc synaptic incorporation of GluA1-containing AMPARs (Ferrario et al. 2011), including during the reconsolidation of a morphine CPP (Lv et al. 2015). The current methods do not enable determination of whether increased pERK2 and pSer845-GluA1 occur in a common population of medium spiny neurons. However, if co-expression is assumed, there is the seeming paradox that biochemical findings in food-restricted rats should favor increased expression of a conditioned response. Having said that, NAc involvement in CPA expression is not as well

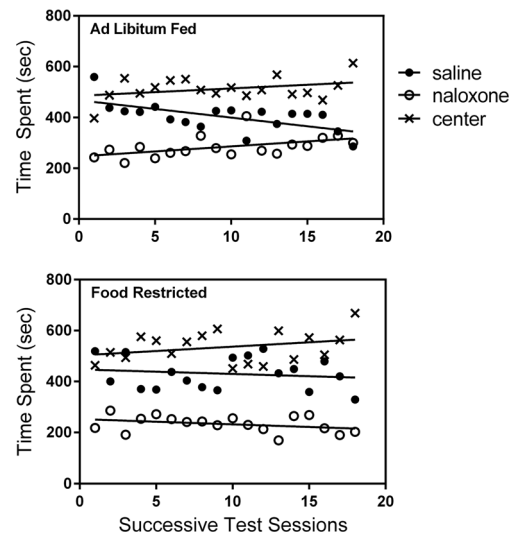
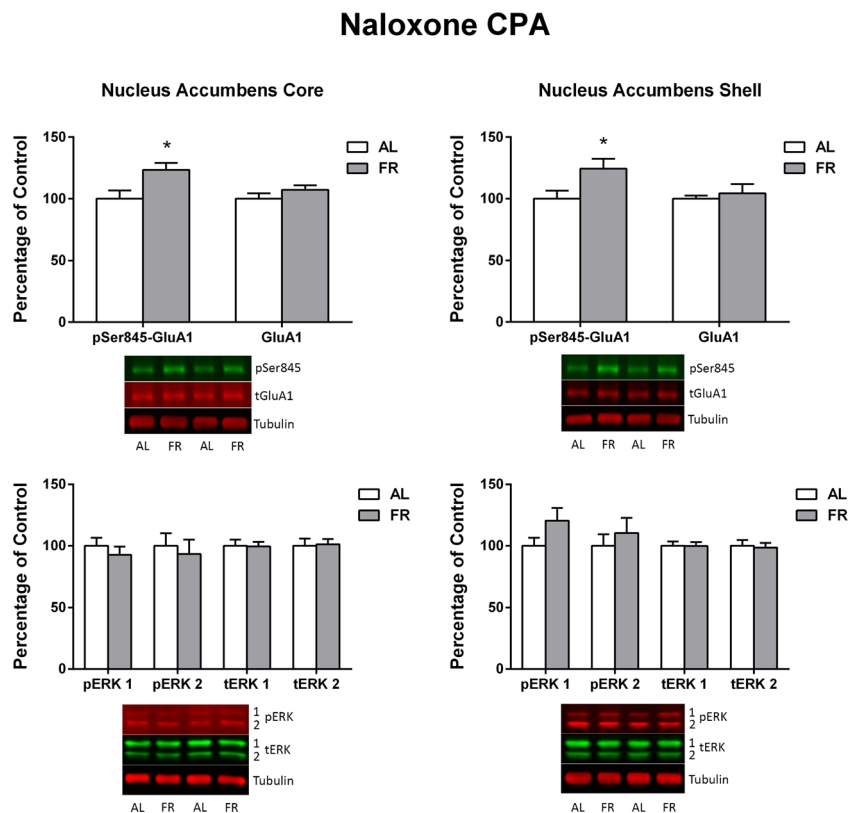


Fig. 8 Naloxone CPA. Scatter plots: average time spent, in seconds, in the saline-paired side, naloxone-paired side, and center compartment of the place conditioning apparatus over the course of 18 successive test sessions by ad libitum fed (*top*) and food-restricted (*bottom*) rats

investigated as CPP, and fundamental differences have been reported. For example, NMDA receptors in NAc mediate reconsolidation of morphine CPP but not naloxone CPA (Wu et al. 2012), and unlike cocaine CPP (Miller and Marshall 2005b), blockade of ERK phosphorylation does not decrease expression of LiCl CPA (Longoni et al. 2011). In fact, contrary to the positive role of ERK phosphorylation in CPP expression, ERK phosphorylation in multiple forebrain regions has been associated with CPA extinction (Wang et al. 2012; Wang et al. 2015).

While the role of pSer845-GluA1, pERK2, and any mechanistic relationship between the two in LiCl CPA cannot be resolved based on the current data, it is of interest that food restriction exerted opposite behavioral effects on cocaine/morphine CPP and LiCl CPA. This raises the possibility that food restriction facilitates reward and diminishes aversion, particularly in relation to food, proxies for food (i.e., drugs of abuse), and impediments to feeding. Under conditions of food scarcity, a more enduring behavioral response to a reward-associated context coupled with increased “risk taking,” as in returning to an environment associated with visceral illness, may have adaptive value by increasing the probability of food acquisition. Parsimony might then point to increased pSer845-GluA1 as a “switch” that alters the balance between reward-directed and avoidant behavior. This idea receives support from findings that blockade of GluA1-containing/GluA2-lacking AMPA receptors in NAc reverses the enhanced rewarding effect of d-amphetamine in food-restricted rats (Peng et al. 2014) and exacerbates the pain and depression associated with spared nerve injury (Goffer et al. 2013). A caveat to this proposed scheme is the finding that viral vector mediated overexpression of GluA1 in NAc

Fig. 9 Naloxone CPA. Levels of pSer845-GluA1, pERK1, pERK2, and corresponding total proteins in nucleus accumbens core (left) and shell (right) of ad libitum-fed (AL) and food-restricted (FR) rats killed immediately after the final naloxone CPA test. Results (mean ± SEM) are expressed in comparison with the normalized control, defined as the AL group. Representative immunoblots are included beneath each bar graph. * $p < .05$



shell decreases rather than increases brain stimulation reward (Todtenkopf et al. 2006). Reconciliation of these seeming contradictory findings will likely require identification of affected neuronal phenotype(s) in NAc (Lobo et al. 2010; Kravitz et al. 2012) and characterization of changes in AMPA receptor subunit abundance in the post-synaptic density.

In experiment 3, food restriction increased persistence of the naloxone CPA and was accompanied by increased pSer845-GluA1 in NAc core and shell, plus an overall negative correlation between CPA and pERK1/pERK2 levels in NAc core. These results militate against a scheme in which food restriction generally shifts the balance between reward and aversion. Differential effects of food restriction on LiCl and naloxone CPA could be explained by the substantially different neuroanatomical patterns of neuronal activation induced by these two aversive stimuli (Gracy et al. 2001; Ferreira et al. 2006) or, to the extent that both are regulated by NAc, the balance between phosphorylation of GluA1 and ERK. If increased pSer845-GluA1 across experiments is to be assigned a consistent function, it may relate to enhanced recognition of stimulus salience or responsiveness to the context with positive valence but, in the case of CPA, is subject to override by ERK phosphorylation that mediates extinction learning. Thus, in LiCl CPA, the robust increase in pERK2 prevailed and CPA extinguished, while in naloxone CPA, increased pSer845-GluA1 prevailed but in inverse relation to the

level of pERK. Understanding the relationship between changes in pSer845-GluA1 and pERK as they relate to CPP vs. CPA and their modulation by food restriction may depend

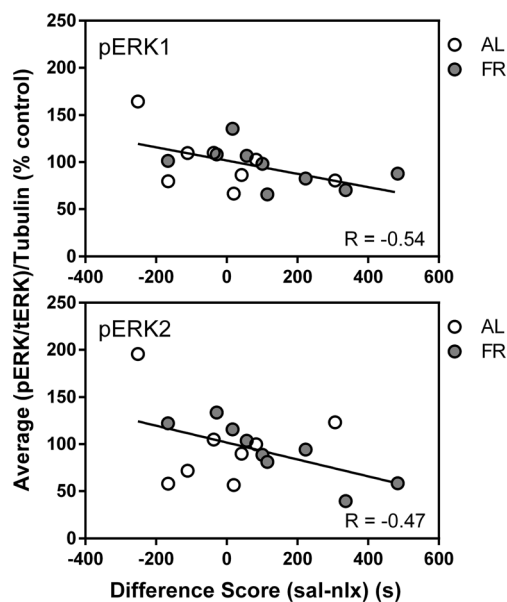


Fig. 10 Naloxone CPA. Scatter plots: correlation between pERK1 (top) and pERK2 (bottom) in nucleus accumbens core and naloxone CPA difference score in the final test session among all subjects in the experiment

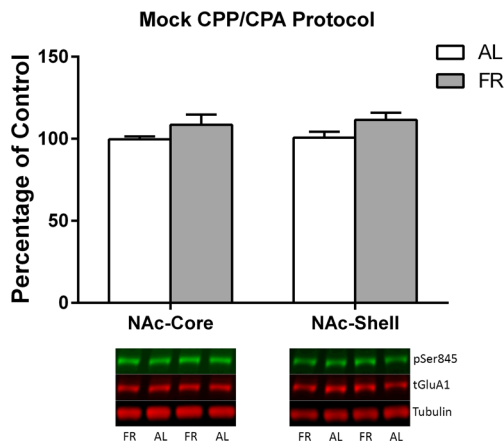


Fig. 11 Mock CPP/CPA protocol. Levels of pSer845-GluA1 in nucleus accumbens core (*left*) and shell (*right*) of ad libitum-fed (AL; $n = 9$) and food-restricted (FR; $n = 9$) rats killed immediately after the final “expression” test in a mock conditioning protocol. Results (mean \pm SEM) are expressed in comparison with the normalized control, defined as the AL group. Representative immunoblots are included beneath each pair of bars

not only on localization of these molecular changes within cells and NAc subregions, but also between subregions. For example, in contrast to the key involvement of NAc core in CPP expression (Miller and Marshall 2005a, b; Théberge et al. 2010), a glutamatergic projection from paraventricular thalamus to NAc shell mediates expression of a naloxone CPA (Li et al. 2011).

From a behavioral standpoint, what is clear based on present results and prior results with cocaine, is that food restriction does not enhance expression of all forms of place conditioning but, so far, has increased the persistence of place conditioning induced by administration and withdrawal from drugs of abuse. Given the importance of cues and contexts as triggers of craving and relapse, these findings add further support to the contention that food restriction and key CNS concomitants not only increase vulnerability to initial use by enhancing drug reward magnitude, but may also increase vulnerability to relapse triggered by exposure to environmental cues and contexts.

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