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Food restriction increases acquisition, persistence and drug prime-induced expression of a cocaine-conditioned place preference in rats

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ABSTRACT

Cocaine conditioned place preference (CPP) is more persistent in food-restricted than *ad libitum* fed rats. This study assessed whether food restriction acts during conditioning and/or expression to increase persistence. In Experiment 1, rats were food-restricted during conditioning with a 7.0 mg/kg (i.p.) dose of cocaine. After the first CPP test, half of the rats were switched to *ad libitum* feeding for three weeks, half remained on food restriction, and this was followed by CPP testing. Rats tested under the *ad libitum* feeding condition displayed extinction by the fifth test. Their CPP did not reinstate in response to overnight food deprivation or a cocaine prime. Rats maintained on food restriction displayed a persistent CPP. In Experiment 2, rats were *ad libitum* fed during conditioning with the 7.0 mg/kg dose. In the first test only a trend toward CPP was displayed. Rats maintained under the *ad libitum* feeding condition did not display a CPP during subsequent testing and did not respond to a cocaine prime. Rats tested under food-restriction also did not display a CPP, but expressed a CPP following a cocaine prime. In Experiment 3, rats were *ad libitum* fed during conditioning with a 12.0 mg/kg dose. After the first test, half of the rats were switched to food restriction for three weeks. Rats that were maintained under the *ad libitum* condition displayed extinction by the fourth test. Their CPP was not reinstated by a cocaine prime. Rats tested under food-restriction displayed a persistent CPP. These results indicate that food restriction lowers the threshold dose for cocaine CPP and interacts with a previously acquired CPP to increase its persistence. In so far as CPP models Pavlovian conditioning that contributes to addiction, these results suggest the importance of diet and the physiology of energy balance as modulatory factors.

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Preclinical addiction research has been productively guided by the hypothesis that drugs of abuse usurp the neurocircuitry that mediates appetitive motivation and reward (Kelley and Berridge, 2002; Cardinal and Everitt, 2004; Di Chiara, 2005; Volkow and Wise, 2005). The common involvement of mesoaccumbens dopamine neurons in adaptive and drug-induced incentive motivation and synaptic plasticity have been supported by animal models and human neuroimaging (Hyman et al., 2006; Kalivas and O'Brien, 2008; Robinson and Berridge, 2008; Volkow et al., 2008). Support for the regulation of drug effects by mechanisms of energy balance and body weight regulation are provided by demonstrations that metabolic hormones (e.g., Marinelli et al., 1996; DiLeone, 2009; Daws et al., 2011) and feeding-related peptides (e.g., Tessari et al., 2007; Chung et al, 2009; Cason et al., 2010) alter behavioral and/or neurophysiological responses to abused drugs. While the influence of these numerous signaling systems on drug abuse vulnerability and addiction remain to be fully worked out, there are clear-cut behavioral observations that appear to reflect their influence. The most thoroughly characterized phenomenon is the increased self-administration of abused drugs by food-deprived and -restricted subjects (Carroll et al., 1979; Carroll and Meisch, 1984). Complementing these results are more recent reports that rats with free access to high fat diet display impaired acquisition of cocaine self-administration (Wellman et al., 2007) and amphetamine-conditioned place preference (Davis et al., 2008).

Using a learning-free measure that capitalizes on the positive interaction between drugs of abuse and rewarding electrical brain stimulation, it has been shown that food restriction increases the reward magnitude of numerous drugs of abuse and dopamine receptor agonists (Cabeza de Vaca and Carr, 1998; Carr et al., 2000). Related biochemical studies have provided insight into neuroadaptations underlying these effects (Carr, 2007; Carr et al., 2010). An increase in the acute rewarding effect of abused drugs may increase vulnerability to initial use and may explain the low dose threshold and enhanced acquisition of self-administration in food-restricted subjects (Carroll and Meisch, 1984). However, one of the most challenging problems in treatment of addictive disorders is the craving and relapse induced in detoxified

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addicts by contextual stimuli (environments and cues) that are associated with past drug effects (Childress et al., 1988; O'Brien et al., 1992). This phenomenon, which relies on Pavlovian conditioning, may be modeled in rodents using the conditioned place preference (CPP) paradigm (Carr et al., 1989; Mueller and Stewart, 2000). There have been several previous studies of food restriction effects on CPP, although they have focused exclusively on acquisition of the place preference. For example, food-restricted rats acquired an amphetamine CPP at lower conditioning doses than those required in *ad libitum* fed subjects (Stuber et al., 2002). In a cocaine study, both *ad libitum* fed and food-restricted rats acquired a CPP at the conditioning doses used but the magnitude of preference was greater in the food-restricted rats (Bell et al., 1997). In both of these studies the first post-conditioning CPP test was the terminal test conducted. Recently, this laboratory observed that when *ad libitum* fed and food-restricted rats were conditioned with a dose of cocaine that initially induced a similar CPP in the two feeding groups, continuation of testing revealed that CPP extinguished after several test sessions in *ad libitum* fed rats but persisted in food-restricted rats (Liu et al., 2011). This raises the question of whether food restriction only interacts with cocaine during conditioning to induce a more persistent CPP or whether it also acts during the expression phase to increase persistence.

Consequently, in the present cocaine CPP study, effects of confining food restriction to the conditioning phase versus the expression phase were evaluated. Each cohort of rats was conditioned under the same feeding condition (*ad libitum* or restricted). After the first expression test, half the rats were switched to the opposite feeding condition for approximately three weeks prior to resumption of testing. Results indicate that if rats are conditioned with cocaine when in the food-restricted state but switched to the *ad libitum* fed state for testing, their CPP extinguishes more rapidly than rats that remain in the food-restricted state. In addition, if rats are conditioned with cocaine in the *ad libitum* fed state but switched to the food-restricted state for testing, their CPP is more persistent than rats that remain in the *ad libitum* fed state. Finally, if *ad libitum* fed rats are conditioned with a dose of cocaine that is too low to induce a CPP but are then switched to food restriction and administered a priming dose of cocaine before testing, they display a CPP.

1. Method

1.1. Subjects and food restriction

All subjects were male Sprague–Dawley rats (Taconic Farms, Germantown, NY) weighing 350–400 g at the start of the experiment. Rats were individually housed in plastic cages in a central facility and maintained on a 12 hour light:dark cycle (lights on at 0700 h). Rats had free access to water and standard lab pellets (Purina Laboratory Rodent Diet #5001) except when restricted feeding conditions applied (see below). 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 Animal Care (NIH Publication no. 85-23).

In Experiment 1, all rats were initially placed on a food restriction regimen, which involved daily feeding of a single 10 g meal, delivered at 1700 h, until body weights decreased by 20% (approximately two weeks). Food allotments were then adjusted on a daily basis to clamp body weight at the new value. During this period, daily meals ranged from 10 to 18 g; the daily intake of *ad libitum* fed rats was in the 25–30 g range. After the first CPP test, half the rats were returned to *ad libitum* feeding, while the other half continued to be maintained on food restriction. Behavioral testing resumed after three weeks, by which time the body weights of rats returned to *ad libitum* feeding had rebounded to at least pre-restriction levels.

In Experiments 2 and 3, all rats were initially conditioned under the *ad libitum* feeding condition. After the first CPP test, half the rats were switched to food restriction as described above. Testing resumed after three weeks, by which time the food-restricted rats had met the 20% body weight loss criterion and had been maintained at that value for one week prior to (and for the remainder of) testing.

Table 1 depicts the sequence and duration of experimental manipulations as well as the mean (±SEM) body weights of subjects at key time-points for each experiment.

1.2. Place preference apparatus

Behavioral conditioning and testing were conducted in a three-compartment apparatus. Each Lucite test chamber (61×30.5×30.5 cm) consisted of two large side compartments (25.4×30.5×30.5 cm) separated by a small center area (10.2×30.5×30.5 cm). One of the large compartments had black walls with horizontal white stripes and a white grid floor composed of parallel stainless steel rods (0.2 cm diameter mounted 1.0 cm apart), whereas the other had white walls and a black wire mesh floor (1.3×1.3 cm^2). The small center compartment had white walls and a smooth ceramic floor. Removable partitions matching the compartment walls were used to isolate rats within specific compartments during conditioning. During CPP test sessions, the partitions were removed to allow rats free access to the entire apparatus. Automated data collection was accomplished through 24 infrared photo-beam detectors along the length of the test chamber; the number and location of beam interruptions were scanned at 100 times per second. Information about beam status was stored and later transformed into a complete record of activity during a session (VersaMax system, Accuscan, Columbus, OH). The dependent measure was time spent in each compartment. During pre-conditioning test sessions (one per experiment) rats displayed no unconditioned preference for one side of the apparatus over the other (see below).

1.3. Procedure

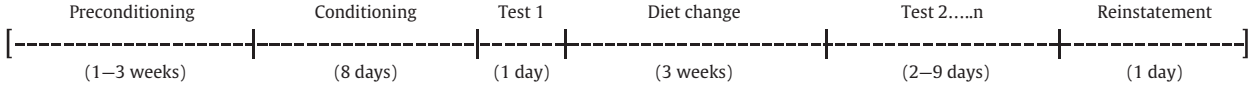
1.3.1. General conditioning and testing procedures

Prior to experiments, rats were habituated to transport and handling on at least five occasions. Rats, remaining in their home cages, were wheeled along an interior corridor from the animal facility to laboratory, weighed, and maintained in a quiet holding area within the laboratory for several hours before being returned to the animal facility. The first day of each experiment was a pre-conditioning test session in which each rat was placed in the center compartment of the CPP apparatus with partitions removed and allowed to move freely 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 cocaine in one of the two compartments.

Each rat underwent eight conditioning sessions, each of 20 min duration, over eight consecutive days. On alternate days, rats were injected with cocaine HCl immediately before being confined to the cocaine-paired compartment. On intervening days, rats received saline-vehicle injections before being confined to the opposite compartment.

The first CPP test was conducted two days after the eighth conditioning session. During this test, no injection was administered before placing each rat in the center compartment with partitions in place for 15 s. Partitions were subsequently removed and rats were allowed to move freely in the apparatus for 20 min. After the first CPP test, rats were divided into two groups matched for CPP test performance with an effort to also match for compartment associated with cocaine. A new dietary condition was then implemented for one group. Three weeks later, CPP testing resumed. CPP tests were conducted on consecutive days until the CPP extinguished in at least one diet group. Extinction was defined, as previously (Liu et al., 2011), as three consecutive CPP test sessions in which time spent on the cocaine-paired side

Table 1
Mean (±SEM) body weights (g) at selected time points during each experiment and a line diagram representing the sequence and duration of each experimental manipulation.



	Pre-conditioning body weights				Post-conditioning body weights			
Cocaine dose	Initial weight	Initial diet	Pre-exposure	Conditioning sessions	CPP-1	Diet change	CPP-2	CPP-final
Exp 1 7 mg/kg	397.3 ±6.9	*FR*	312.9 ±4.3		302.1 ±4.3	*AL*	437.2 ±5.4	449.4 ±6.4
	397.2 ±6.9	*FR*	313.8 ±5.0		302.8 ±4.3	No change	310.1 ±4.5	304.5 ±5.3
Exp 2 7 mg/kg	398.5 ±3.1	*AL*	426.8 ±4.4		449.1 ±5.1	*FR*	346.4 ±4.3	345.8 ±4.4
	395.2 ±5.3	*AL*	425.8 ±3.4		448.3 ±4.1	No change	495.9 ±5.4	491.3 ±5.4
Exp 3 12 mg/kg	350.8 ±7.8	*AL*	385.6 ±5.3		425.3 ±5.7	*FR*	335.2 ±5.0	338.0 ±5.1
	354.8 ±3.5	*AL*	383.3 ±3.9		419.2 ±4.9	No change	488.7 ±7.0	490.8 ±7.9

Note: *FR* and *AL* refer to restricted and *ad libitum* feeding, respectively. CPP-final is the last CPP test conducted for the two groups together.

did not differ from time spent on the saline-paired side, as determined by separate one-tailed *t*-tests for correlated samples.

1.3.2. Experiment 1

Twenty food-restricted rats were conditioned with 7.0 mg/kg cocaine. Following the first post-conditioning CPP test, subjects were divided into two groups of ten matched for CPP test performance. *Ad libitum* access to food was restored to one group and all subjects remained in home cages in the animal facility for the next three weeks where they were periodically weighed. CPP testing then resumed with each group tested once per day. The *ad libitum* fed group satisfied the extinction criterion after the sixth test. Prior to the seventh test they were food deprived for 24 h as a 'priming' treatment. Three days later this group was tested an eighth time preceded by a priming injection of cocaine (7.0 mg/kg). The food-restricted group was tested on all days when the *ad libitum* fed group was tested, but the food-restricted group did not receive the 24 h food deprivation or cocaine prime treatments.

1.3.3. Experiment 2

Twenty-four *ad libitum* fed rats were conditioned with 7.0 mg/kg cocaine. Following the first post-conditioning CPP test, subjects were divided into two groups of twelve matched for CPP test performance. Food restriction was initiated in one group and all subjects remained in home cages in the animal facility for the next three weeks where they were periodically weighed. CPP testing then resumed with each group tested once per day. Both groups satisfied the extinction criterion after the third test. Prior to the fourth test session, subjects in both groups were injected with a priming dose of cocaine (7.0 mg/kg). Due to expression of a CPP in the food-restricted group in this test, they were tested once more, without a cocaine prime, two days later.

1.3.4. Experiment 3

Twenty-four *ad libitum* fed rats were conditioned with 12.0 mg/kg cocaine. Following the first post-conditioning CPP test, subjects were divided into two groups of twelve matched for CPP test performance. Food restriction was initiated in one group and all subjects remained in home cages in the animal facility for the next three weeks where they were periodically weighed. CPP testing then resumed with each group tested once per day. The *ad libitum* fed group satisfied the extinction criterion after the fifth test. Prior to the sixth test they received a priming injection of cocaine (12.0 mg/kg). The food-restricted groups were also tested on this day, without cocaine prime, and were tested on three subsequent days to confirm continued CPP expression.

1.4. Drugs

Cocaine HCl (NIDA; Research Triangle Institute) was dissolved in sterile 0.9% saline and administered intraperitoneally (i.p.) at doses of 7.0 mg/kg (Experiments 1 and 2) and 12.0 mg/kg (Experiment 3) in a volume of 1.0 ml/kg.

1.5. Data analysis

In each experiment, time spent (in seconds) in the two compartments during the pre-conditioning test was compared by *t*-test for correlated samples to confirm absence of an unconditioned preference for one of the two conditioning compartments. Results obtained in the first post-conditioning CPP test were also analyzed by *t*-test as time spent on the cocaine-paired side versus the saline-paired side. By design, results of each CPP test following the three-week dietary manipulation were separately evaluated for each diet group until at least one group met the extinction criterion (see above). Although criteria for establishing extinction versus persistence were defined *a priori* and determined the duration of testing, *post hoc* comparison of the two diet groups across the three test days that constituted evidence of CPP extinction in one diet group (always the *ad libitum* fed group), was carried out by 2-way mixed factors ANOVA performed on difference scores (i.e., time spent on the cocaine-paired side minus time spent on the saline-paired side), with test day as the within subjects factor and dietary condition as the between subjects factor. CPP expression in any subsequent test session (i.e., prime-induced reinstatement of an extinguished CPP or assessment of continuing expression in the food-restricted group) was assessed by *t*-test for correlated samples.

Finally, inspection of results suggested an unanticipated effect, namely sensitization of CPP in subjects tested under food restriction. Difference scores over CPP test days were therefore evaluated for the presence of a linear trend by performing single degree of freedom polynomial contrasts following a one-way repeated measures ANOVA.

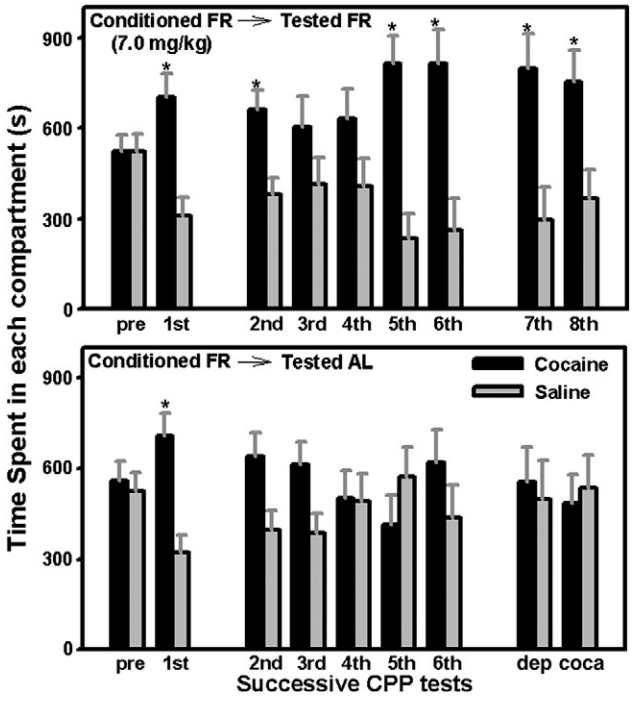


Fig. 1. In Experiment 1, rats ($n=20$) were all food-restricted (FR) during conditioning with a 7.0 mg/kg (i.p.) dose of cocaine. After the first CPP test, half of the rats were switched to *ad libitum* feeding (AL) (bottom panel), half remained on FR (top panel), and three weeks later this was followed by resumption of daily testing until at least one group met extinction criterion. Mean (±SEM) time spent (s) on the cocaine- and saline-paired sides of the CPP apparatus during the pre-conditioning session (pre) and subsequent post-conditioning test sessions (1st–6th) are displayed for both groups. For the AL group, the final two test sessions were preceded by 24-h food deprivation (7th session, dep) and administration of a priming injection of cocaine (7.0 mg/kg, i.p; 8th session, coca). *Refer to results of separate *t*-tests for correlated samples (at least, $p<.05$).

2. Results

2.1. Experiment 1

Fig. 1 displays results obtained in the group conditioned with 7.0 mg/kg cocaine when food-restricted, with half of the subjects remaining food-restricted for the duration of testing (top) and half switched to *ad libitum* feeding after the first CPP test and for the three weeks preceding and then continuing through the resumption of CPP testing (bottom). Prior to conditioning (Pre), rats did not display preference for either side of the conditioning chamber ($t(19)=0.2$, $p=0.8$). During the first CPP test, conducted 48 h after the final conditioning session (1st), a strong preference was expressed for the cocaine-paired side relative to the saline-paired side ($t(19)=4.32$, $p<.001$). This result was followed by establishment of two diet groups and a three week hiatus. Following the resumption of testing, a marginally significant cocaine-side preference was seen in the *ad libitum* fed group on the 2nd ($t(9)=1.77$, $p=.055$) and 3rd ($t(9)=1.68$, $p=.063$) test days. With continued testing, clear-cut fulfillment of the extinction criterion was established in this group, as defined by absence of a cocaine-side preference on the 4th ($t(9)=0.07$, $p=.47$), 5th ($t(9)=0.83$, $p=.21$), and 6th ($t(9)=0.85$, $p=.20$) test days. Although the food-restricted group did not display a significant CPP on the 4th test day ($t(9)=1.22$, $p=.12$), CPP expression resumed on the 5th ($t(9)=3.48$, $p=.003$) and 6th ($t(9)=2.56$, $p=.015$) test days. In a direct comparison of the two feeding groups from test day 4 through 6, 2-way mixed factors ANOVA revealed a significant main effect of diet ($F(1,18)=5.32$, $p=.033$) and no effect of test day ($F2,36)=1.97$, $p=.15$) or interaction between factors ($F2,36)=2.12$, $p=.13$), indicating that CPP during this period was greater in food-restricted than in *ad libitum* fed rats. Further, CPP was not reinstated in the *ad libitum* fed group by either 24 h food deprivation (dep; $t(9)=0.25$, $p=.40$) or a priming dose of cocaine (coca; $t(9)=0.25$, $p=.40$). On these latter two test occasions, the food-restricted rats, which did not receive priming treatments, displayed a CPP ($t(9)=2.29$, $p=.024$; $t(9)=1.92$, $p=.044$).

In addition to CPP being more persistent in the food-restricted relative to the *ad libitum* fed group, inspection of Fig. 1 (top) suggested that the magnitude of CPP in food-restricted animals tended to increase across test days. This was confirmed by the presence of a significantly increasing linear trend in the difference scores ($F(1,9)=5.94$, $p=.038$).

2.2. Experiment 2

Fig. 2 displays results obtained in the group conditioned with 7.0 mg/kg cocaine when *ad libitum* fed, with half of the subjects remaining *ad libitum* fed for the duration of testing (top) and half switched to food-restriction after the first CPP test and for the three weeks preceding and then continuing through the resumption of CPP testing (bottom). Prior to conditioning (Pre), rats did not display preference for either side of the conditioning chamber ($t(23)=1.1$, $p=.24$). During the first CPP test conducted 48 h after the final conditioning session (1st), rats displayed only a trend toward preference of the cocaine-paired side relative to the saline-paired side ($t(23)=1.43$, $p<.10$). This result was followed by establishment of two diet groups and a three week hiatus. When testing resumed, CPP continued to be absent in the *ad libitum* fed group on the 2nd ($t(11)=1.43$, $p=.08$) and 3rd($t(11)=0.32$, $p=.37$) test days, and was similarly absent in the group switched to food restriction on the 2nd ($t(11)=0.5$, $p=.29$) and 3rd($t(11)=0.03$, $p=.48$) test days. However, in response to a priming dose of cocaine (coca), the food-restricted

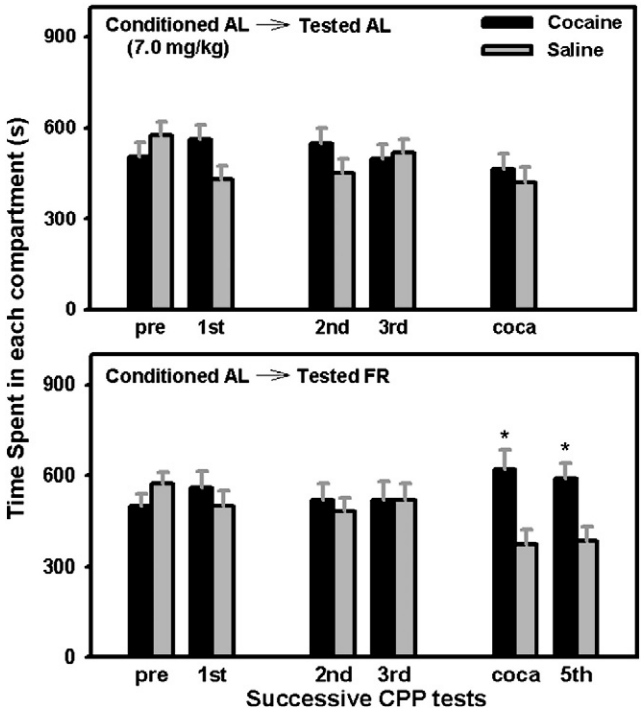


Fig. 2. In Experiment 2, rats ($n=24$) were all *ad libitum* fed (AL) during conditioning with a 7.0 mg/kg dose of cocaine. After the first CPP test, half of the rats were switched to food restriction (FR) for three weeks, followed by resumption of daily testing (bottom panel), while half remained AL throughout (top panel). Mean (±SEM) time spent (s) on the cocaine- and saline-paired sides of the CPP apparatus during the pre-conditioning session (pre) and subsequent post-conditioning test sessions (1st–3rd) are displayed. The fourth test session was preceded by administration of a priming injection of cocaine (7.0 mg/kg, i.p., coca) but the fifth was drug-free. *Time spent on the cocaine-paired side was significantly greater than time spent on the saline-paired side (at least, $p<.005$).

group displayed a CPP ($t(11)=3.08$, $p=.0029$) and the *ad libitum* fed group did not ($t(11)=0.64$, $p=.26$). Interestingly, when the food-restricted group was re-tested two days later (5th), without a cocaine prime, they retained the CPP ($t(11)=2.98$, $p=.0034$).

2.3. Experiment 3

Because the cocaine dose used in Experiment 2 failed to induce a significant CPP in *ad libitum* fed rats, the experiment was repeated using a higher, 12.0 mg/kg, dose of cocaine. Fig. 3 displays results obtained in rats that remained *ad libitum* fed for the duration of testing (top) and rats that were switched to food-restriction after the first CPP test and for the three weeks preceding and then continuing through the resumption of CPP testing (bottom).

Prior to conditioning (Pre), rats did not display preference for either side of the conditioning chamber ($t(23)=0.07$, $p=.94$). During the first CPP test conducted 48 h after the final conditioning session (1st), rats displayed a strong preference for the cocaine-paired side ($t(23)=8.41$, $p<.0001$). This result was followed by establishment of two diet groups and a three week hiatus. Following the resumption of testing, CPP extinguished in the *ad libitum* fed group, as defined by absence of a cocaine-side preference on the 3rd($t(11)=0.36$, $p=.36$), 4th ($t(11=1.65$, $p=.064$) and 5th ($t(11)=1.55$, $p=.074$) test days. The food-restricted group continued to display a significant CPP on the 3rd($t(11)=5.05$, $p<.001$), 4th ($t(11)=2.96$, $p=.007$) and 5th ($t(11)=2.91$, $p=.007$) test days. In a direct comparison of the two feeding groups from test day 3 through 5, 2-way mixed factors ANOVA revealed no significant effects. Thus, the difference between diet groups during this period was not as robust as in Experiment 1 yet satisfied the *a priori* criterion of three consecutive test days without significant CPP in one diet group (*ad libitum* fed) with

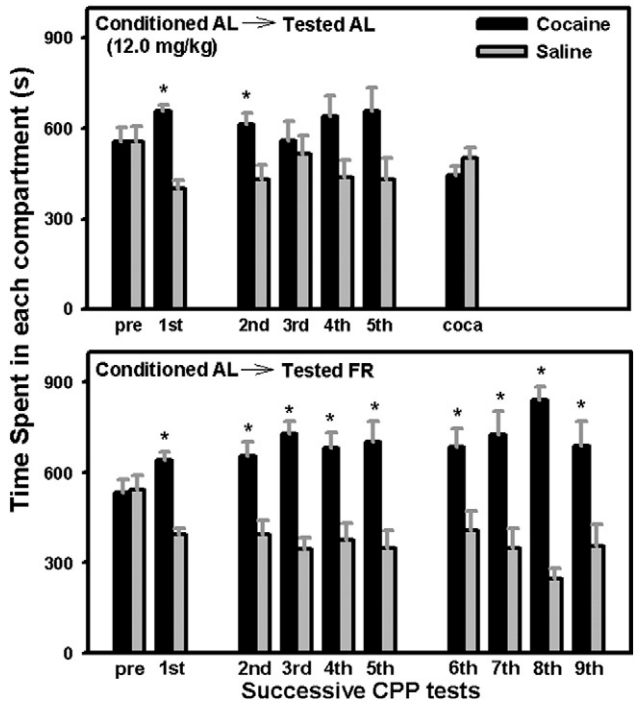


Fig. 3. In Experiment 3, rats ($n=24$) were all *ad libitum fed* (AL) during conditioning with a 12.0 mg/kg dose of cocaine. After the first CPP test, half of the rats were switched to food restriction (FR) for three weeks, followed by resumption of daily testing (bottom panel), while half remained AL throughout (top panel). Testing continued until one group met extinction criterion. Mean (±SEM) time spent (s) on the cocaine- and saline-paired sides of the CPP apparatus during the pre-conditioning session (pre) and subsequent post-conditioning test sessions (1st–5th) are displayed. For the AL group, the sixth test session was preceded by administration of a priming injection of cocaine (12.0 mg/kg, i.p, coca). *Refer to results of separate *t*-tests for correlated samples (at least, $p<.05$).

persistent CPP expression in the other (food-restricted). Further, *ad libitum* fed rats did not display CPP in response to a priming dose of cocaine (coca; $t(11)=1.17$, $p=0.17$), but on the same test occasion the food-restricted rats, which did not receive a priming treatment, displayed a CPP ($t(11)=2.18$, $p=.026$) and continued to do so in the next four test sessions (only two are displayed in Fig. 3), after which testing was discontinued.

As in Experiment 1, the magnitude of CPP in food-restricted animals appeared to increase across test days. This was confirmed by the presence of a significantly increasing linear trend in the difference scores ($F(1,11)=6.18$, $p=.03$).

3. Discussion

In the present study a 7.0 mg/kg dose of cocaine induced a strong CPP in food-restricted rats but produced only a trend toward CPP in *ad libitum* fed rats. In our prior study, this 7.0 mg/kg dose of cocaine induced a CPP in both diet groups, although the CPP in *ad libitum* fed rats extinguished rapidly over subsequent test sessions and the CPP in food-restricted rats did not (Liu et al., 2011). It is likely that 7.0 mg/kg is the approximate threshold dose distinguishing feeding groups in our paradigm. Further, the difference in conditioning efficacy agrees with the finding of a lower dose threshold for d-amphetamine CPP in food-restricted relative to *ad libitum* fed rats (Stuber et al., 2002). In addition to the differential efficacy of 7.0 mg/kg cocaine in *ad libitum* fed and food-restricted rats, the other primary findings of the current study include the observation that if rats are conditioned with cocaine when in the food-restricted state but are switched to the *ad libitum* fed state for testing, their CPP extinguishes more rapidly than rats that remain in the food-restricted state. In addition, if rats are conditioned with cocaine in the *ad libitum* fed state but are switched to the food-restricted state for testing, their CPP is more persistent than rats that remain in the *ad libitum* fed state. Finally, if *ad libitum* fed rats are conditioned with a dose of cocaine that is too low to induce a CPP but are then switched to food restriction and administered a priming dose of cocaine before testing, they display a CPP.

Food restriction increases behavioral responsiveness to cocaine and other drugs of abuse in self-administration, electrical brain stimulation reward, and locomotor activity assays (Carroll and Meisch, 1984; Deroche et al., 1995; Cabeza de Vaca and Carr, 1998). The present results indicate that food restriction also increases responsiveness to an environmental context previously paired with cocaine. Increased magnitude or persistence of a CPP conditioned and tested under food restriction in previous studies (Bell et al., 1997; Stuber et al., 2002; Liu et al., 2011) might have been due exclusively to increased drug reward and/or enhanced associative learning during the conditioning phase. However, the current demonstrations that restoration of *ad libitum* feeding after cocaine conditioning in food-restricted rats hastens extinction of the CPP, while implementation of food-restriction after cocaine conditioning in *ad libitum* fed rats increases persistence of the CPP support a specific effect of food restriction on responsiveness to the drug-paired environment. Post-conditioning facilitatory effects of food restriction were also indicated by observation that *ad libitum* fed rats conditioned with a low dose of cocaine did not display a CPP until they were switched to food restriction and injected with a priming dose of cocaine. A possible explanation of the present results is that enhanced responsiveness of food-restricted animals to the cocaine-paired environment reflects an adaptive response to underfeeding, in which cocaine has become a proxy for food and is thereby subject to incentive sensitization (Robinson and Berridge, 2008). There are, nevertheless, alternative explanations of the persistent CPP that cannot be ruled out at this time. For example, food restriction may enhance recall of the cocaine-context association (Hashimoto and Watanabe, 2005; Deng et al., 2009) or impair extinction learning or its expression (Koot et al., 2009). However, in both Experiments 1 and 3 food-restricted subjects were not only resistant to extinction of

the CPP but displayed trends indicative of CPP sensitization. This observation suggests that food restriction may enhance the incentive effects of the drug-paired environment to the extent that CPP is initially strengthened and perpetuated as it might be by cocaine itself. Presumably, this effect dissipates with repeated drug-free testing and is followed by extinction. The duration of testing of food-restricted subjects in the present study was linked to the performance of *ad libitum* fed subjects; consequently, the full time-course of CPP expression and eventual extinction were not assessed.

Prior findings that food restriction and body weight loss enhance rewarding and psychomotor stimulant effects of abused drugs are considered to be of potential clinical significance based on the high comorbidity of disordered eating and substance abuse (Root et al., 2010), the relationship between dieting and substance abuse in adolescents (Pisetsky et al., 2008; Seo and Jiang, 2009), and the deliberate use of cocaine, nicotine and other psychostimulants with anorexic properties as diet aids (Klesges et al., 1997; Cochrane et al., 1998). However, the modulatory effect of food restriction on acute drug effects may primarily increase vulnerability to initial use. The impact of food restriction on later stages and other components of drug abuse or addiction have received little attention. Given the importance of Pavlovian conditioning in the triggering of drug craving and relapse in detoxified addicts (Childress et al., 1988; O'Brien et al., 1992), the present results may have implications for management of abstinence. The possibility that food scarcity or physiological concomitants of underfeeding promote drug seeking was previously indicated by the finding that one day of total food deprivation triggered relapse to heroin seeking in rats (Shalev et al., 2001; Maric et al., 2011). However, the functional and physiological mechanisms underlying the effect of acute food deprivation and chronic food restriction are probably different. For example, leptin treatment blocked the reinstating effect of one day food deprivation (Shalev et al., 2001) but had no effect on the potentiation of d-amphetamine reward by chronic food restriction (Hao et al., 2006). Moreover, chronic food restriction produces a variety of reward-related striatal neuroadaptations that would be expected to develop over days or weeks rather than hours. These include decreased maximum velocity of dopamine uptake (Zhen et al., 2006), downregulation of basal preprodynorphin gene expression (Haberny and Carr, 2005), upregulation of D-1 dopamine receptor stimulation-induced MAP kinase and CREB phosphorylation (Haberny et al., 2004), and accumulation of the stable transcription factor ΔfosB (Stamp et al., 2008; Vialou et al., 2011; Peng et al., 2011).

The changes in brain and behavior observed in food-restricted subjects could be dependent on sustained alterations in levels of peripheral metabolic or stress hormones that modulate brain reward circuitry. For example, the food restriction regimen employed in the present study produces a 5–10-fold increase in plasma corticosterone, a 75% increase in activated ghrelin, a 50% decrease in insulin-like growth factor-1, and an 80% decrease in insulin (Liu and Carr, in progress). All of these peripheral signals interact with the brain dopamine system (e.g., Deroche et al., 1995; Abizaid et al., 2006; Bondy et al., 1992; Figlewicz et al., 2003). Corticosterone, in particular, has been implicated in several of the enhanced behavioral responses of food-restricted subjects to drugs of abuse (Deroche et al., 1995; Campbell and Carroll, 2001; however, see Carr, 2002).

Previous studies of brain regional mechanisms involved in CPP expression and persistence (e.g., Kaddis et al., 1995; Miller and Marshall, 2005a,b; Meyers et al., 2006; Rademacher et al., 2006; Shen et al., 2006; Bahi et al., 2008; Tropea et al., 2008), coupled with studies of brain regional effects of food restriction (e.g., Duan et al., 2001; Haberny et al., 2004; Carr et al., 2010; Liu et al., 2011) indicate multiple points of convergence and will enable the framing of mechanistic hypotheses to guide future studies. Testing hypotheses relating to involvement of specific brain regional mechanisms, and metabolic hormone responses to food restriction that regulate those mechanisms, could lead to a better understanding of some factors that increase relapse risk and suggest novel pharmacological and behavioral approaches to relapse prevention.

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