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Synergistic interaction between caloric restriction and amphetamine in food-unrelated approach behavior of rats

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Abstract

Rationale—Approach behavior is regulated by the brain integrating information about environment and body state. Psychoactive drugs interact with this process.

Objectives—We examined the extent to which caloric (i.e., food) restriction, amphetamine and lithium interact in potentiating locomotor activity and responding reinforced by visual stimulus (VS), a reward unrelated to energy homeostasis.

Methods—Rats either had ad-libitum access to food or received daily rations that maintained 85-90% of their original body weights. Leverpressing turned on a cue light for 1 sec and turned off house light for 5 sec. Amphetamine and lithium were administered through intraperitoneal injections and diet, respectively.

Results—Food-restriction or amphetamine (1 mg/kg) alone had little effect on VS-reinforced responding; however, the combination of the two conditions markedly potentiated VS-reinforced responding (4-fold). Food restriction lasting 7 days or longer was needed to augment amphetamine's effect on VS-reinforced responding. Amphetamine (0.3 – 3 mg/kg) potentiated locomotor activity similarly between food-restricted and ad-libitum groups. Repeated injections of amphetamine sensitized locomotor activity, but not VS-reinforced responding. In addition, while chronic lithium treatments (0.2% lithium carbonate chow) reduced VS-reinforced responding, chronic lithium further augmented amphetamine-potentiated VS-reinforced responding.

Conclusions—Food-restriction interacts with psychoactive drugs to potentiate goal-directed responding unrelated to food-seeking in a much more powerful manner than previously thought. The novel finding that lithium can augment a psychostimulant effect of amphetamine suggests caution when combining lithium and psychostimulant drugs in clinical settings.

Keywords

dietary restriction; incentive motivation; manic-depressive illness; visual sensation; drug abuse; amphetamine; lithium

INTRODUCTION

The brain integrates information about body state and environment to regulate emotional and motivational states (Bindra, 1969; Panksepp, 1998; Damasio, 2010). Caloric or food

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restriction, which profoundly alters body state, is widely practiced for health and religious reasons (Trepanowski and Bloomer, 2010; NDP Group, 2013). However, little is known about the extent to which food restriction interacts with psychoactive drugs. Psychostimulant drugs such as amphetamine (AMPH) are widely used and abused because of their properties to decrease appetite and fatigue, heighten attention, elevate mood, and produce euphoria (Van Kammen and Murphy, 1975; Brauer and de Wit, 1996; National Task Force on the Prevention and Treatment of Obesity, 1996; Pliszka, 2007; Breitbart and Alici, 2010). Chronic use can lead to psychiatric issues including manic and psychotic symptoms (American Psychiatric Association, 2000; Ross, 2006), while preclinical studies have used AMPH to model psychiatric symptoms such as mania (O'Donnell and Gould, 2007). It is also used as a tool for investigating motivational mechanisms (Wyvell and Berridge, 2000; Shin et al., 2010). Available studies suggest that food restriction can further augment AMPH-potentiated responding reinforced by lateral hypothalamic stimulation (LHS) (so called intracranial self-stimulation) in rats (Cabeza de Vaca and Carr, 1998; Carr, 2002).

Accumulating evidence supports that food restriction and AMPH can potentiate approach behaviors that may not necessarily lead to nourishment (Ikemoto and Panksepp, 1999; Ikemoto, 2010). However, it is not clear how food restriction and AMPH interact in approach behavior unrelated to energy homeostasis. We used a behavioral procedure in which responding is reinforced by a brief presentation of light (or visual stimulus, VS) (Stewart and Hurwitz, 1958; Kish, 1966; Shin et al., 2010; Vollrath-Smith et al., 2012). Two important advantages of using VS over other common reinforcers such as food or LHS are the following. First, VS does not influence energy homeostasis. Foods are fundamental reinforcers because of their roles in energy homeostasis, and LHS, which does not contribute to energy homeostasis, induces feeding in the presence of food (Hoebel and Teitelbaum, 1962; Margules and Olds, 1962). Thus, the use of these popular reinforcers makes it difficult to study approach motivational processes independent of energy-homeostatic processes. In addition, VS reliably reinforces responding over weeks and yet is only moderately reinforcing (Stewart and Hurwitz, 1958; Kish, 1966), leaving room for detecting synergistic (supra-additive) interaction with other factors. Previous studies suggest that VS-reinforced responding is potentiated by AMPH (Fox, 1962; Kierman, 1965; Berlyne et al 1966; Shin et al., 2010) and by food restriction although food-restriction effect on this behavior is not consistent among studies (Forgays, 1959; Segal, 1959; Goodrick, 1965). Interaction between food restriction and AMPH in responding reinforced by VS has not been investigated. Moreover, we investigated whether food restriction needs to be chronic for response potentiation and whether repeated injections of AMPH sensitize responding. Although VS is distinct from LHS (or brain stimulation reward), similar processes may be responsible for augmenting approach behavior reinforced by these stimuli. LHS-reinforced responding is potentiated by chronic food restriction, but not acute food restriction or repeated injections of AMPH (Carr and Wolinsky, 1993; Wise and Munn, 1993; Carr, 2002; Cabeza de Vaca et al., 2004).

The second aim is to examine how the combination of food restriction and AMPH (food restriction/AMPH) alters behavior of rats chronically treated with the mood stabilizer lithium. Food restriction/AMPH-potentiated approach responding can be used as a model of mania, whose symptoms include heightened motivation and reward seeking (Hasler et al., 2006). Lithium, the gold standard of bipolar disorder treatment (Jefferson, 1990; Bowden et al., 1994), appears to reduce the euphoric effects of amphetamine (Van Kammen and Murphy, 1975; van Kammen et al., 1985). While high rates of substance abuse exist within the psychiatric population including bipolar disorder patients (Regier et al., 1990; Cassidy et al., 2001; McElroy et al., 2001), there is no documented information about how food restriction/AMPH affects behavior under the influence of lithium. We report here that food

restriction synergistically interacts with AMPH to potentiate VS-reinforced responding. Also, chronic lithium further augments food restriction/AMPH-potentiated VS-reinforced responding.

METHODS AND MATERIALS

Animals

One hundred twenty-four male Wistar rats (Harlan, Dublin, VA) were kept in a humidity- and temperature-controlled room on a reverse 12-h light-dark cycle (lights on at 8:00 PM). They were individually housed when experimental manipulations started as described below. Animals were assigned into groups in such a way that groups did not differ with respect to body weight, and if the information on leverpressing was available before any experimental manipulation started, this was also considered for group assignment. All procedures were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse Intramural Research Program and were in accordance with the *Guide for the care and use of laboratory animals* (National Research Council, 2011).

Drugs

D-Amphetamine sulfate and SCH 23390 (Sigma, St. Louis, MO) were dissolved in 0.9% saline. Rats were acclimated to the injection procedures by receiving saline injections (2-4 times) prior to testing, while experimental and control groups were treated equally. Lithium carbonate (Li_2CO_3) was administered through chow (0.2%) (Bio-Serv, Frenchtown, NJ).

Food restriction procedure

Food restriction was initiated by removing chow from home cages for 24 hrs. Thereafter, rats assigned for food restriction groups received limited amounts of standard rodent chow (6-15 g) once a day. Their body weights were gradually reduced over 2 weeks and maintained at 85-90% of their original weights (320-460 g at the onset of food restriction) throughout experiments. When behavioral testing was being conducted, rats received food just after each test session.

Behavioral apparatus and procedure

Operant conditioning chambers and the procedure of VS-reinforced responding were previously described (Shin et al., 2010; Vollrath-Smith et al., 2012; Webb et al., 2012). All experimentally naïve rats were habituated to their testing chambers by placing them once or twice (depending on the experiment) in the chambers with lights off and the levers retracted for 60 min prior to the first test session. All behavioral testing lasted 60 min per session. Active leverpressing illuminated the cue light above the lever for 1 sec and turned off the house light for 5 sec, during which leverpressing was counted, but produced no programmed consequence. Inactive leverpressing had no programmed consequence throughout the session. The left-right locations of the active and inactive levers were counterbalanced among rats; the assignment of active and inactive functions between the levers remained the same for each rat throughout the experiment. In addition, VS upon active leverpressing was presented on either a progressive ratio (PR) or a variable ratio (VR) 2 schedule, to facilitate differential responding between the two levers. In the PR schedule, the number of active leverpresses required to produce a presentation of VS increased by 1 every 10 VS presentations throughout the experiment. The PR schedule was used for all the experiments described below except one, which is described below and used a VR2 schedule. The VR2 schedule presented VS following 1, 2 or 3 responses on the active lever, and the required number changed randomly from trial to trial. In addition to leverpressing, locomotor activity was assessed with 4 pairs of infrared detector cells separated by 6 cm and detecting

“crosses”. A cross was counted only if the rat body part interrupted a different pair of cells from the last; that is, consecutive interruptions of the same pair were not counted. We used 6 different sets of rats for the present study, and some were used for multiple experiments. Table 1 summarizes how the 6 sets of rats were used for the experiments described below.

Experiment 1a: Effects of food restriction on VS-reinforced responding with a PR schedule

Experimentally naïve rats (set 1; $n = 24$) were tested for VS-reinforced responding on the PR schedule for the 14 consecutive days. Immediately after the session 2 test, eleven rats were placed on the food-restriction procedure, while 13 rats were kept on *ad libitum* (AL) diet.

Experiment 1b: Effects of food restriction on VS-reinforced responding with a VR2 schedule

Another set of naïve rats (set 2; $n = 16$) were tested for VS-reinforced responding on the VR2 schedule for 14 consecutive days. After the testing on day 2, a half of the rats ($n = 8$) were placed on the food-restriction condition, while the other half remained on the AL condition.

Experiment 1c: Effects of SCH 23390 on VS-reinforced responding

Then, the set 2 rats were tested for VS-reinforced responding on the PR schedule for 4 sessions before tested for effects of the D₁ receptor antagonist SCH 23390 (SCH) on VS-reinforced responding. They received IP injections of saline, 12.5 or 25 µg/kg SCH immediately before each of three sessions, separated by a day. A half of the rats received injections in this order, while the other in a reversed order. These doses were selected based on our previous study (Vollrath-Smith et al. 2012).

Experiment 1d: Effects of food restriction and AMPH on VS-reinforced responding

Dose-response effects of AMPH on VS-reinforced responding were determined in the 16 rats (set 2) after the SCH experiment described above. The rats were tested for VS-reinforced leverpressing immediately after IP injections of saline, 0.3, 1 and 3 mg/kg AMPH, in this order, over 4 sessions, separated by 24 hrs. These doses and the procedure were used before in our previous study (Shin et al. 2010).

Experiment 2a: Effects of food restriction without repeated behavioral testing

We examined whether a 2-week period of food restriction potentiates VS-reinforced responding. A half of experimentally naïve rats (set 3) were first food-restricted for 2 weeks, and then food restriction ($n = 16$) and AL ($n = 16$) rats were tested for VS-reinforced responding over 4 sessions, separated by 48 hrs.

Experiment 2b: Effects of repeated behavioral testing and AMPH injections

To examine effects of repeated testing and effects of repeated injections of AMPH on VS-reinforced responding, a half of each feeding condition received 8 injections of AMPH (1 mg/kg, IP), and the other half received saline injections over 8 sessions, separated by 48 hrs. Injections were separated by 48 hrs in this experiment, to minimize weight reduction in AL rats because of repeated injections of AMPH and its lasting appetite suppressant effect due to its long half-life (10-12 hrs).

Experiment 2c: Effects of different doses of AMPH

After 3 weeks of an injection-free period, effects of different doses of AMPH on VS-reinforced responding were examined. Immediately before behavioral tests, both AMPH-

and saline-experienced rats received IP injections of saline, 0.1, 0.3, 1, and 3 mg/kg AMPH in this order over 5 sessions, separated by 48 hrs.

Experiment 3a: Effects of food-restriction length on VS-reinforced responding

Effects of food-restriction length were determined in experimentally naïve rats tested for VS-reinforced responding with a within-subjects design. After 4 daily sessions of VS-reinforced responding, a half of the rats ($n = 10$) were food-restricted (day 0), while the other half ($n = 10$) remained on AL diet. The rats were tested for VS-reinforced responding daily. Before behavioral tests, both food restriction and AL rats received injections of AMPH (1 mg/kg, IP) on days 1, 3, 7, and 14, and no injection on other days.

Experiment 3b: Effects of SCH on food restriction/AMPH-potentiated VS-reinforced responding

Following the above experiment, the food-restricted rats were used to examine effects of SCH on AMPH-potentiated VS-reinforced responding. The rats received subcutaneous injections of 0, 12.5 and 25 μ g/kg SCH solutions, followed by AMPH (1 mg/kg, IP) injections over 3 sessions, separated by 48 hrs. The order to testing SCH was counterbalanced among the rats. The subcutaneous injection method appears to work at lower doses with SCH than the IP method, an effect that we learned after conducting experiment 1c.

Experiment 4a: Effects of lithium on VS-reinforced responding in food-restricted rats

Experimentally-naïve rats were food-restricted. A half ($n = 11$) received 0.2% lithium carbonate chow, while the other half ($n = 11$) received control chow containing the same nutrients without lithium carbonate. To help to correct hyponatremia caused by lithium intake, a saline solution (10 mM) in addition to tap water were made available in the home cages of all rats, irrespective of treatments (Thomsen and Olesen, 1974). Both groups had 85-87% of their baseline body weights by day 14. After 3 weeks of food restriction and lithium treatment, both groups of rats were tested for VS-reinforced responding for 8 sessions, separated by 1-2 days.

Experiment 4b: Interaction of lithium and AMPH on VS-reinforced responding in food-restricted rats

Then, effects of AMPH on VS-reinforced responding were examined in these rats. A set of more finely-spaced doses of AMPH were examined in this experiment than that of experiment 1 or 2, because we found, as reported below, that the 1 mg/kg dose had an increasing effect while the next dose, 3 mg/kg, had an opposite effect on VS-reinforced responding. Starting on the 35th day from the commencement of food restriction, the rats were tested for behavioral responses immediately after IP injections of saline, 0.5, 1, 2, and 4 mg/kg AMPH in this order over 5 sessions, separated by 48 hrs.

Experiment 5: Serum and brain lithium measurements

Naïve rats (set 6, $n = 10$) were food-restricted and maintained on either lithium- or control-diet as described above. After 42 days on the diet and 24 hrs following the last food rations, rats were anesthetized by isoflurane, and 1.5 mL of blood was drawn via a cardiac puncture. Then, rats were decapitated and their brains were excised and immediately frozen. Both brains and serum samples were stored in a -80° C freezer until the following assay procedure. Entire brains were homogenized with a polytron homogenizer (Kinematica AG, Model PT-MR 2100; Littau, Switzerland) in three volumes of 0.5 N trichloroacetic acid, followed by centrifugation (Gould et al., 2007). Lithium levels of both serum (mmol/l) and brain (mmol/kg, wet weight) samples were measured with a flame photometer (Cole-Palmer

Model 2655-00, Chicago, IL, USA). Serum and brain supernatant from control rats ($n = 5$) were used to prepare reference solutions. Lithium carbonate was dissolved in serum and brain supernatant to create samples containing known concentrations of lithium carbonate. These were used to create a “concentration curve” that was used to calculate lithium concentration in the samples from lithium rats ($n = 5$).

Statistical Analyses

All data on leverpressing and crossing are square-root transformed, to minimize heterogeneous variances for parametric statistical tests (McDonald, 2009). Square-root transformed data were analyzed with the ANOVA module of Statistica (version 6.1, StatSoft, Inc., Tulsa, OK). However, non-transformed data are presented in Figures 1-4, because square-root transformed data are not intuitively informative (McDonald, 2009). When Mauchly Sphericity Test detected violation of the sphericity assumption for repeated factors, the degrees of freedom for respective tests were adjusted with the Greenhouse-Geisser method. When a significant effect was found for a factor with more than two levels, the Tukey's Honestly Significant Difference post-hoc test was performed. To determine differential responding between active and inactive levers, they were analyzed together in the same ANOVAs; however, the data shown in Fig. 2c were followed by separate ANOVAs for active and inactive levers to help interpret statistical results concerning effects of other factors on each lever. This was justified by significant interactions involving the lever factor. Finally, all results of ANOVAs and Tukey's tests are not reported below unless they are relevant for interpreting data with respect to the questions that we laid out broadly in the introduction section and specifically in the result section for simplicity. As it is commonly practiced, statistics of non-significant results are not reported unless they are important for the question being asked or close ($p < 0.075$) to the significance level, $p < 0.05$.

RESULTS

Experiment 1a and b: food restriction modestly potentiates VS-reinforced responding over repeated sessions

We examined effects of food restriction in rats tested on a PR schedule ($n = 24$). One of the AL rats in the PR group was eliminated from all analyses, since it did not respond on the levers (only 2 leverpresses during the entire 14 daily sessions). Before implementing the food-restriction procedure (i.e., sessions 1-2), we confirmed that food-restriction groups did not differ from AL groups on any of the measures: active and inactive leverpressing, crossing or body weights (sessions 1 and 2 in Fig. 1a). During the first 2 session, rats responded on the active lever more than the inactive lever [lever: $F(1,21) = 26.18$, $p < 0.0001$], and leverpress rates decreased between sessions 1 and 2 [session: $F(1,21) = 5.83$, $p < 0.05$]. Weights increased over the two days [$F(1,21) = 33.73$, $p < 0.0001$]. Following these sessions, the rats in the food restriction groups received limited daily rations, the weights of the food-restricted rats gradually decreased to 89% of those of session 2 over 12 days, while the AL rats gradually gained weights to 113% (PR) [Tukey's test following a feeding $\times$ session interaction: $F(11,231) = 64.84$, $p < 0.0001$]. Feeding condition played an important role in leverpressing. Active, but not inactive, leverpressing of food-restricted rats was significantly potentiated more than the active or inactive leverpressing of AL rats [lever $\times$ feeding: $F(1,21) = 4.71$, $p < 0.05$, followed by Tukey's test]. Although the means shown in Figure 1a suggest a gradual increase in active leverpressing in food-restricted, but not AL, rats, the effect was not detected by a feeding $\times$ lever $\times$ session interaction. While lever discrimination was robust throughout the experiment [lever: $F(1,21) = 61.21$, $p < 0.0001$], the rats further increased active, but not inactive, leverpressing between sessions 3 and 14 [lever $\times$ session: $F(11,231) = 3.91$, $p < 0.005$]. Based on the significant feeding condition $\times$

lever interaction and the significant lever $\times$ session interaction, food restriction appears to have moderately potentiated VS-reinforced responding, and the effect emerged over repeated testing.

To confirm that food-restriction effects detected with the PR schedule are not schedule-specific, we examined effects of food restriction with another behavioral schedule, a VR2, which we thought to induce similar levels of leverpressing as the PR. Again, we confirmed that food-restriction groups did not differ from AL groups on any of the measures during sessions 1-2 (Fig. 1b). Rats responded on the active lever more than the inactive lever [lever: $F(1,14) = 28.80, p < 0.0001$] and gained weight [$F(1,14) = 13.63, p < 0.005$]. The weights of the food-restricted rats gradually decreased by 12% of those of session 2 over the next 12 days, while the AL rats gradually gained weights by 8% [feeding $\times$ session: $F(7,98) = 243.22, p < 0.0001$]. The rats on the VR2 schedule also responded on active lever more than inactive lever [lever: $F(1,14) = 44.43, p < 0.0001$]. Mean leverpresses of VR2 rats shown in Figure 1b also suggests a gradual increase in active leverpressing in food-restricted rats. However, effects of food restriction were not statistically significant [feeding $\times$ lever $\times$ session: *ns*; feeding $\times$ session: $F(11,154) = 2.41, p = 0.060$; feeding $\times$ lever: *ns*; feeding: *ns*].

Food restriction had no significant effect on locomotor activity [feeding: $F(1,21) = 0.83, p = 0.37$ and $F(1,14) = 0.29, p = 0.60$ for PR and VR2 schedules, respectively]. However, AL rats tended to have greater locomotor activity in early sessions and decrease it thereafter, while food-restricted rats tended to maintain it at a similar level between sessions 2 and 14 in PR rats [feeding $\times$ session: $F(11,231) = 1.74, p = 0.065$ and $F(7,98) = 1.38, p = 0.22$ for PR and VR2 schedules, respectively].

Experiment 1c: SCH similarly decreases VS-reinforced responding and locomotor activity

Previous studies showed that injections of dopamine receptor antagonists including SCH readily decrease spontaneous locomotor activity or exploration (Hoffman and Beninger, 1985; Bardo et al., 1993; Bevins et al., 2002). We examined whether SCH similarly decreases VS-reinforced responding and locomotor activity. IP injections of 25 $\mu\text{g/kg}$, but not 12.5 $\mu\text{g/kg}$, SCH significantly decreased crossing (Fig. 1c) [$F(2,28) = 18.91, p < 0.0001$]. Similarly, the 25 $\mu\text{g/kg}$, but not 12.5 $\mu\text{g/kg}$, dose significantly decreased leverpressing [dose: $F(2,28) = 12.96, p < 0.0005$]. Food restriction did not significantly interact with SCH for either leverpressing or crossing [feeding: *ns*; feeding $\times$ dose: *ns*; feeding $\times$ dose $\times$ lever: *ns*].

Experiment 1d: AMPH potentiates VS-reinforced responding in food-restricted rats

Our previous study found that systemic injections of AMPH moderately potentiate VS-reinforced responding (Shin et al. 2010). Here we examined whether food restriction further augments AMPH-potentiated VS-reinforced responding. While food restriction or AMPH alone potentiated leverpressing [feeding: $F(1,14) = 8.19, p < 0.05$; dose: $F(3,42) = 16.61, p < 0.0001$], these factors differentially altered active and inactive leverpressing (Fig. 1d). The injections of the 0.3 and 1, but not 3, mg/kg doses significantly increased active leverpressing in the food-restricted group ($n = 8$), while only the 1 mg/kg dose significantly increased active leverpressing in AL group ($n = 8$) [feeding $\times$ lever $\times$ dose interaction: $F(3,42) = 8.35, p < 0.005$, followed by Tukey's test]. Active leverpressing of food-restricted rats with the 1 mg/kg dose was greater than that of AL rats with the same dose ($p < 0.05$; Fig. 1d). The 1 mg/kg dose slightly increased inactive leverpressing of food-restricted rats compared to its vehicle value. All doses of AMPH significantly potentiated locomotor activity [dose: $F(3,42) = 41.92, p < 0.0001$; feeding $\times$ dose: *ns*]; the 1 mg/kg dose was more effective than the 0.3 or 3 mg/kg dose in stimulating locomotor activity. Thus, while AMPH injections similarly increased locomotor activity between food-restricted and AL rats,

AMPH injections increased active leverpressing much more potently in food-restricted rats than AL rats. It should be noted that prior experience with SCH injections did not seem to have influenced these effects of AMPH, since we obtained similar AMPH's effects in subsequent experiments (2b, c), involving rats without SCH experience.

Experiment 2a: Food restriction alone is not sufficient to potentiate VS-reinforced responding

The data shown in Fig. 1a suggest that food restriction moderately potentiates VS-reinforced responding. However, it is unclear whether food restriction alone is sufficient in causing it because the food-restricted rats received repeated behavioral tests during which they gradually reduced weights. Thus, it may be repeated tests, instead of food restriction. Here we examined with larger numbers of rats (food restriction: $n = 16$; AL: $n = 16$) whether a mere 2-week period of food restriction without behavioral test is sufficient for such potentiation. Figure 2a shows the data. First of all, the weights of food-restricted rats were significantly different from those of AL rats starting in session 1 [feeding: $F(1,30) = 563.46$, $p < 0.0001$; feeding $\times$ session: $F(3,90) = 81.66$, $p < 0.0001$, followed by Tukey's test]. The rats clearly discriminated active lever from inactive lever [lever: $F(1,30) = 57.12$, $p < 0.0001$], and the discrimination improved over sessions with decreasing inactive leverpressing [lever $\times$ session: $F(3,90) = 9.38$, $p < 0.0001$]. food restriction did not alter overall leverpressing [feeding: $F(1,30) = 0.27$, $p = 0.61$]; moreover, a feeding $\times$ lever $\times$ session interaction was not present [feeding: $F(3,90) = 0.26$, $p = 0.81$]. However, the food-restricted rats tended to increase leverpressing, while the AL rats decreased it over sessions [feeding $\times$ session: $F(3,90) = 3.36$, $p < 0.05$]. Therefore, a mere 2-week period of food restriction is not sufficient in potentiating VS-reinforced responding, although food restriction can affect VS-reinforced leverpressing over repeated behavioral sessions. The 2-week food restriction only had a tendency to decrease crossing [feeding: $F(1,30) = 3.77$, $p = 0.062$]. The next experiment examined whether additional repeated testing along with repeated injections of AMPH increase VS-reinforced responding.

Experiment 2b and c: Repeated injections of AMPH sensitize locomotor activity, but not VS-reinforced responding

Repeated injections of AMPH are known to sensitize locomotor activity (Robinson and Becker, 1986). However, previous studies reported that repeated injections of AMPH do not sensitize responding reinforced by LHS or intracranial self-stimulation (Wise and Munn, 1993; Cabeza de Vaca et al., 2004). We examined whether repeated injections of AMPH sensitize VS-reinforced responding. The 1 mg/kg dose was chosen because it was the most effective dose in potentiating VS-reinforced responding (Fig. 1d), and therefore, it gives enough room for detecting decrease in the behavior as a result of repeated amphetamine injections. In addition, injections were repeated 8 times to see whether increase in VS-reinforced responding in food-restricted rats (Fig. 1a) can be replicated here in saline-injected food-restricted rats.

The food-restricted ($n = 16$) and AL ($n = 16$) rats used in the above experiment were divided into AMPH and saline injection groups while maintaining the same feeding conditions. Consistent with the finding shown in Figure 1d, we observed a significant feeding $\times$ drug $\times$ lever interaction [$F(1,28) = 4.53$, $p < 0.05$ with a 2 feeding $\times$ 2 drug $\times$ 8 session $\times$ 2 lever ANOVA] (Fig. 2b). Active lever counts of food restriction/AMPH rats were greater than any other lever counts of the rats on food restriction, AL, AMPH or saline ($ps < 0.05$) except active lever counts of food restriction/saline rats ($p = 0.21$; Tukeys' test). This is explained by food restriction/AMPH rats tended to decrease active leverpressing ($p < 0.05$, the session 10 value compared to its vehicle value) and the food restriction/saline rats tended to increase active leverpressing ($p < 0.05$, the session 11 value compared to its vehicle value) while AL/

saline or AL/AMPH rats did not significantly change active leverpressing over the course of the sessions [feeding $\times$ drug $\times$ lever $\times$ session interaction: $F(7,196) = 3.40, p < 0.005$]. The latter result is consistent with the effect of food restriction on VS-reinforced responding shown in Fig. 1a. Inactive leverpress levels did not significantly change in all 4 groups (Tukey's test).

Following a 3-week injection-free period, we examined effects of different AMPH doses on leverpressing. A 2 feeding $\times$ 2 drug experience $\times$ 5 dose $\times$ 2 lever ANOVA indicates that none of the effects involving prior drug experience was statistically significant. We then conducted 2 feeding $\times$ 2 drug experience $\times$ 5 dose ANOVAs separately between active and inactive lever responses, to clearly determine effects of the factors on each lever. Again, prior AMPH-experience had no significant effect on active or inactive leverpressing. A feeding $\times$ dose interaction is significant for active lever [$F(4,112) = 8.99, p < 0.0005$]. The 0.3 and 1 mg/kg doses significantly increased active leverpressing in food-restricted rats, but not in AL rats, while the 3 mg/kg dose significantly decreased active leverpressing in both groups (Tukey's test). Although the mean of saline-experienced food-restricted rats at the 3mg/kg dose does not appear to be lower than its 0 mg/kg dose, the mean is distorted by one rat pressing 796 times while others pressing less than 20. A feeding $\times$ dose interaction is also significant for inactive lever [$F(4,112) = 5.86, p < 0.05$]. The 3 mg/kg dose significantly decreased inactive leverpressing in both food-restricted and AL rats, while other doses had no significant effect in either group. The interaction appears to be explained differential responding between the two groups at the 1 mg/kg dose: this dose tended to decrease inactive leverpressing in AL rats, while tending to increase it in food-restricted rats. The mean response of saline-experienced food-restricted rats at the 1 mg/kg dose is relatively high; however, because this is largely caused by one rat pressing over 400 times, it was not significantly greater than the 0 mg/kg dose.

Repeated AMPH injections increased crossing counts over sessions, while saline injections did not [session $\times$ drug: $F(7,196) = 3.60, p < 0.01$ with a 2 feeding $\times$ 2 drug experience $\times$ 5 dose ANOVA]. While AMPH group had greater crossing than saline group's in any of sessions 5-12, AMPH in sessions 6-12 had greater crossing than that in session 5 (Tukey's test). Although food-restricted rats receiving AMPH tended to have greater crossing counts than AL rats receiving AMPH, effects of food restriction are not significant [feeding: *ns*; feeding $\times$ session: *ns*; feeding $\times$ session $\times$ drug: *ns*]. However, when effects of different AMPH doses were examined 3 weeks later, food restriction significantly potentiated crossing [feeding: $F(1,28) = 12.34, p < 0.005$] and a significant feeding $\times$ dose interaction was found [$F(4,112) = 7.14, p < 0.001$]. Both food-restricted and AL groups increased crossing at the 0.3 and 1 mg/kg doses, and AL, but not food restricted, group showed significant decrease in crossing at the 3 mg/kg dose. Also, a significant drug experience $\times$ dose interaction was detected [$F(4,112) = 10.63, p < 0.0001$]. While both AMPH- and saline-experienced groups increased crossing at the 0.3 and 1 mg/kg doses when compared to respective saline values, AMPH-experienced group significantly increased crossing at the 0.1 mg/kg dose as well when compared to the saline value of saline-experienced group; moreover, the 3 mg/kg dose significantly decreased crossing in AMPH-, but not saline-, experienced group (Tukey's test). Therefore, these results suggest that prior AMPH-exposure shifted the dose response curve to the left and are consistent with the notion that prior AMPH-exposure sensitized rats with respect to locomotor activity.

It should be noted that repeated AMPH injections affected body-weight gain in AL rats. Although injections were separated by 48 hrs, the AL rats that received repeated injections of AMPH gained weights at a lower rate than the AL rats receiving saline injections [drug $\times$ session: $F(7,98) = 4.59, p < 0.05$]. Interestingly, after 3 weeks of no injection, the difference in weight between the AMPH- and saline-treated AL rats was significant [drug experience:

$F(1,14) = 6.17, p < 0.05$; drug experience $\times$ session: *ns*]. The same analyses do not apply to food-restricted rats, because experimenters controlled their weights.

Experiment 3a: Acute food restriction does not augment AMPH-potentiated VS-reinforced responding

Experiments 1d and 2b,c showed that food restriction readily augments AMPH-potentiated VS-reinforced responding. The food-restricted rats used in these experiments had been on the food-restriction condition for, at least, 2 weeks when they received the first AMPH injection. Thus, it is not clear whether chronic food restriction is necessary in inducing the potentiating effect. Previous studies suggest that the potentiating effect of food restriction on LHS-reinforced responding depends on continuous food restriction over several days or weeks (Carr and Wolinsky, 1993; Carr, 2002). Although LHS is distinct from VS, common mechanisms may be responsible for augmenting effects of food restriction on AMPH-potentiated responding reinforced by LHS and VS. If this is so, augmenting effect of food restriction is absent after a 24-hr food-deprivation, and food restriction's effects on AMPH-potentiated VS-reinforced responding grow larger and larger over the course of two weeks. To test this hypothesis, we compared AMPH-potentiated responding of AL rats with that of food-restricted rats on food-restriction days 0, 1, 3, 7, and 14.

When food-restricted rats received AMPH for the first time after a 24-hr food-deprivation (day 1), they did not display marked increase in VS-reinforced responding (Fig. 3a). Their active leverpressing did not significantly increase from that of no-injection day (day 0) or from that of AL rats. However, the food-restricted rats increased VS-reinforced responding more and more over the course of the 2 weeks as the food-restriction condition continued. Similar to our observations shown in Figures 1d and 2b, after 2 weeks of continuous food restriction, the food-restricted rats displayed marked increase in VS-reinforced responding compared to AL control rats. These observations are supported by a significant feeding $\times$ lever $\times$ day (0, 1, 3, 7 and 14) interaction [$F(4,72) = 6.04, p < 0.005$, followed by Tukey's test with a 2 feeding $\times$ 2 lever $\times$ 5 day ANOVA]. In contrast to active leverpressing, inactive leverpressing was not significantly altered by AMPH in either AL or food-restricted rats over the course of 14 days (Tukey's test). These results support the hypothesis stated above.

Crossing counts of days 1, 3, 7 and 14 were greater than that of day 0, and crossing counts of day 3, 7 and 14 were greater than that of day 1 (Fig. 4c) [length: $F(4,72) = 164.14, p < 0.0001$, followed by Tukey's test]. Moreover, the food-restricted group displayed more pronounced increase in crossing than the AL group [feeding $\times$ length: $F(4,72) = 3.49, p < 0.05$]. Food-restricted group's crossing counts of days 1, 3, 7 and 14 were greater than that of day 0, and those of day 3, 7 and 14 were greater than that of day 1, while AL group's crossing counts of days 1, 3, 7 and 14 were greater than that of day 0, but crossing counts of day 3, 7 and 14 were not greater than that of day 1 (Tukey's test). These data suggest that crossing was sensitized by repeated AMPH injections and continuing food restriction. The data on body weight are also shown in the figure. Over the 14-day period, the weights of food-restricted rats gradually decreased to 85% of the day 0, while those of AL rats gradually increased to 107% during the same period (Fig. 3) [feeding $\times$ length: $F(4,72) = 98.57, p < 0.0001$].

Experiment 3b: SCH similarly decreases food restriction/AMPH-potentiated VS-reinforced responding and locomotor activity

To determine the role of dopamine in food restriction/AMPH-potentiated VS-reinforced responding and locomotion, effects of SCH were examined. Both doses of SCH (12.5 and 25 $\mu\text{g/kg}$, SC) significantly decreased AMPH-potentiated active leverpressing, but not inactive leverpressing (Fig. 3b) [dose $\times$ lever: $F(2,18) = 11.31, p < 0.001$, followed by Tukey's test].

Similar to active leverpressing, both SCH doses also decreased locomotor activity [dose: $F(2,18) = 209.45, p < 0.0001$]. Thus, these results suggest that dopamine transmission via D1 receptors is similarly important between food restriction/AMPH-potentiated VS-reinforced responding and locomotor activity.

Experiment 4a and b: Lithium treatments reduce VS-reinforced responding, but augment it when combined with AMPH

Lithium has been the most effective treatment in the manic phase of bipolar disorder (Jefferson, 1990; Bowden et al., 1994). Symptoms of mood disorders include changes in motivation and reward seeking (Hasler et al., 2006); therefore, it is interesting to determine how lithium alters VS-reinforced responding under food restriction. Rats were fed with either lithium- or control-diet for more than 3 weeks before testing. In a separate group of rats ($n = 10$), we confirmed that this lithium diet regimen achieved a serum concentration of lithium equaling $0.61 \text{ mM} \pm 0.05$ (mean $\pm$ SEM). This level is comparable to the therapeutic levels of human bipolar patients, which ranges from 0.5-1.2 mM (Gould et al., 2007).

The lithium treatment decreased leverpressing (Fig. 4a) [lithium: $F(1, 20) = 5.51, p < 0.05$]: it selectively decreased active leverpressing, but not inactive leverpressing [lithium $\times$ lever: $F(1, 20) = 8.14, p < 0.01$, followed by Tukey's test]. When the rats were injected with AMPH, however, the lithium treatment further augmented VS-reinforced responding (Fig. 4b) [lithium $\times$ lever $\times$ dose: $F(4,80) = 4.69, p < 0.05$]. AMPH doses of 0.5, 1 and 2, but not 4, mg/kg increased active leverpressing in the lithium-diet group, while doses of 0.5 and 1, but not 2 or 4, mg/kg increased it in the control-diet group; inactive leverpressing was not significantly altered by AMPH doses in either group (Tukey's test). Thus, we found a novel effect of lithium on AMPH-potentiated behavior.

Consistent with previous studies, the lithium treatment decreased locomotor activity (Fig. 4a) [lithium: $F(1, 20) = 7.84, p < 0.05$]. Locomotor activity decreased over the repeated sessions [session: $F(7,140) = 10.11, p < 0.0001$, followed by Tukey's test; diet $\times$ session: $F(7,140) = 2.00, p = 0.11$]. The lithium treatment did not selectively alter AMPH-potentiated locomotor activity between the groups (Fig. 4b) [lithium $\times$ dose: *ns*; diet: *ns*]: all doses of AMPH (0.5-4 mg/kg) potentiated crossing [dose: $F(4,80) = 141.14, p < 0.0001$, followed by Tukey's test].

DISCUSSION

Rats learned to discriminate between active and inactive levers quickly from the very first session and maintained similar levels of VS-reinforced responding in many sessions over several weeks. Food restriction potentiated VS-reinforced responding. However, its effect was subtle and emerged only after repeated testing. That is, food restriction alone does not seem to be sufficient in increasing it. Because we provided the daily ration just after each behavioral testing, one possibility is that food restriction's potentiation effect on VS-reinforced responding reflects rats learning to expect food. This hypothesis remains to be evaluated.

The doses of SCH without AMPH injections similarly decreased locomotor activity and VS-reinforced responding in food-restricted and AL rats. Therefore, VS-reinforced responding depends on normal dopamine transmission in a similar manner as locomotor activity and perhaps exploratory behavior (Hoffman and Beninger, 1985; Bardo et al., 1993; Bevins et al., 2002).

Synergistic increase in VS-reinforced responding

Figure 5 summarizes the data shown in Figures 1-3 (sets 2-4; $n = 68$) and shows levels of VS-reinforced leverpressing in relation to that of unreinforced leverpressing as a function of food restriction and AMPH (first injection of the 1 mg/kg dose). Rats hardly responded on unreinforced or inactive lever (mean: 11.6 presses in 60 min; 100%). This is consistent with the notion that leverpressing is not an ethologically natural behavior for rats, and leverpressing needs to be reinforced for increase; therefore, increase in leverpressing should clearly reflect voluntary or purposeful processes. Food restriction or AMPH alone tended to decrease inactive leverpressing (a: 78% and b: 76%, respectively), while the combination of food restriction and AMPH tended to increase it (c: 142%). The unfilled bars indicate hypothetical values of leverpressing when the factors simply have additive effects with each other. VS-reinforced leverpressing is preferred over unreinforced leverpressing (d: 240%). The effect of food restriction alone on VS-reinforced leverpressing was subtle and hard to detect (cf. d and e), while the effect of AMPH injections alone was similarly small (cf. d and f). AMPH's potentiating effect on VS-reinforced responding in AL rats was detected in our previous study (Shin et al., 2010); however, it was not consistently detected in the present study. This may be largely explained by the robust effect of the food restriction/AMPH interaction masking the small effect of AMPH alone. It is especially striking how food restriction and AMPH synergistically interacted in potentiating VS-reinforced leverpressing compared to the hypothetical additive value (cf. g and h).

Although AMPH's effect on VS-reinforced responding largely depends on food restriction, food restriction only had small effects on locomotor activity, and its effects were not always detected, depending on the experiment. However, previous studies showed that chronic food restriction markedly augments AMPH-potentiated locomotor activity (Deroche et al. 1993; Marinkovic et al. 2007). The discrepancy between the present and the previous studies may be explained by a difference in procedure. The present study tested rats' locomotor activity multiple times in the same chamber, whereas those studies reported a marked potentiation tested it just once, thereby their test chambers were novel for rats. Therefore, the locomotor activity of the present study may reflect different processes than that of the previous studies, suggesting heterogeneous nature of locomotor activity. In any case, the present observation suggests that stimulant effects of AMPH have two components: food restriction-sensitive component detected by VS-reinforced responding and food restriction-insensitive component that can be detected by locomotor activity depending on the procedure. The finding that injections of SCH decreased both food restriction/AMPH potentiated VS-reinforced responding and locomotor activity in a similar manner suggests that both food restriction-sensitive and insensitive stimulant effects of AMPH depend on dopamine transmission.

Similarity between behavior reinforced by VS and other rewards

The present study seems to be the first to clearly show that food restriction interacts with psychoactive drugs in potentiating approach behavior more than an additive manner. This effect of food restriction is most likely not unique to responding reinforced by VS in the presence of AMPH. Inferences can be made from studies involving LHS and secondary reinforcers (or positive conditioned stimuli, CS^+), which are distal stimuli conditioned with primary reinforcers such as food, water or LHS. Although studies involving LHS have not shown supra-additive interactions between food restriction and psychoactive drugs, they did show that food restriction additively augments potentiating effect of AMPH and other rewarding drugs on LHS-reinforced responding (Cabeza de Vaca and Carr, 1998; Carr, 2002). There are more parallels between VS and LHS. Chronic food restriction lasting a week or two appeared to be needed to further augment AMPH-potentiated responding reinforced by VS. We observed that AMPH did not potentiate VS-reinforced responding in

24-hr food-deprived rats any more than in AL rats. However, after 1 or 2 weeks of continuous food restriction, the same rats did markedly increase VS-reinforced responding with AMPH, similar extent to which we observed in experiments 1 and 2. In addition, repeated injections of AMPH, which sensitized locomotor activity, did not sensitize VS-reinforced responding, similar to LHS (Wise and Munn, 1993; Cabeza de Vaca et al., 2004). Therefore, these parallels between VS and LHS suggest common mechanisms are responsible for potentiating approach responses reinforced by these stimuli.

The present results have an important implication in the use of visual stimuli as CS⁺. It is absolutely important to have a control visual stimulus, which is not paired with a primary reinforcer, to interpret effects of visual CS⁺ on behavior. Indeed, previous studies typically have provided “neutral” visual stimuli when they employed visual CS⁺. However, it may be important to keep in mind that “neutral” stimuli are not truly neutral, but CS⁻, which signals the absence of reward, i.e., a negative consequence.

Common mechanisms may also exist between VS and CS⁺. Discrete presentation of CS⁺ indicates motivational properties of primary reinforcers and, thus, reinforces approach behavior (so-called conditioned reinforcement). Previous studies have shown that responding reinforced by CS⁺ is potentiated by AMPH and other psychostimulant drugs, including methylphenidate (Robbins, 1978; Robbins and Koob, 1978; Beninger and Rinaldi, 1992) and nicotine (Olausson et al., 2004; Guy and Fletcher, 2013). Nicotine injections also potentiate responding reinforced by VS (Donny et al., 2003; Chaudhri et al., 2006). Although effects of food restriction on CS⁺-reinforced responding have not been examined for the most part, available data, involving CS⁺ associated with heroin administration, suggest that food restriction potentiates CS⁺-reinforced responding (D’Cunha et al. 2013). Based on this and the present study, we predict that food restriction will potentiate responding reinforced by CS⁺ associated with a variety of positive reinforcers, and this effect may be further augmented by injections of AMPH and other psychostimulant drugs.

Dopamine neurons projecting from the ventral tegmental area to the ventral striatum have been identified to play an important role in responding reinforced by VS, LHS, CS⁺ and drugs including AMPH and nicotine. Intracranial injections of AMPH directly into the ventral striatum not only reinforce responding (Ikemoto et al., 2005), but also potentiate responding reinforced by VS, LHS, or CS⁺ (Taylor and Robbins, 1984; Kelley and Delfs, 1991; Wyvell and Berridge, 2000; Shin et al., 2010; Duchesne and Boye, 2013). Also, nicotine reinforces responding via this dopamine projection system when injected into the ventral tegmental area (Ikemoto et al., 2006). The observations that low doses of SCH readily decrease food restriction/AMPH potentiated VS-reinforced responding (present study) and that SCH or sulpiride decreases VS-reinforced responding potentiated by intra-ventral striatal AMPH (Shin et al., 2010) are consistent with the notion that this dopamine system plays an important role in potentiating many types of approach behavior.

Implications on brain motivational processes

While food restriction or AMPH injections alone had relatively small effects on VS-reinforced responding, the combination of food restriction and AMPH markedly potentiated responding reinforced by VS. This finding suggests that rather than acting on the perceptual processes appraising the value of VS, AMPH acts on a system that integrates the information on the environment and body state to potentiate approach behavior. In other words, AMPH can be said to potentiate *the value of approach behavior* when appropriate conditions (i.e., food restriction and VS in this study) are met. This notion may also be extended to CS⁺ or LHS: AMPH increases the value of approach behavior involving CS⁺ or LHS rather than the value (or salience) of CS⁺ or LHS per se (Hill, 1970; Robbins, 1975; Taylor and Robbins, 1984; Wyvell and Berridge, 2000; Carr, 2002).

Although food restriction played a critical role in potentiating VS-reinforced responding involving AMPH, the VS neither satisfied energy needs nor was associated with food or water. This finding supports the notion that mechanisms of approach motivation are distinct from those of feeding. While VS-reinforced responding is an operant behavior, the vigor of operant responding, especially that potentiated by AMPH, is thought to be controlled by so-called Pavlovian incentive motivational system (PAMS), which appear to depend on dopaminergic mechanisms especially neurons projecting to the ventral striatum (Rescorla and Solomon, 1967; Bindra, 1968; Taylor and Robbins, 1984; Kelley and Delfs, 1991; Dickinson and Balleine, 1994; Dickinson et al., 2000; Wyvell and Berridge, 2000; Niv et al., 2007; Zhang et al., 2009; Shin et al., 2010; Flagel et al., 2011; Duchesne and Boye, 2013; Wassum et al., 2013). Thus, the PAMS appears to have the capacity to integrate homeostatic information for potentiation of behavior that does not necessarily lead to nourishment or other physiological needs. This notion is supported by various observations. For example, rats trained to approach and feed under a hungry state will seek out food regardless of being hungry or sated, if they have not experienced eating food under a sated state in the same context (Dickinson and Balleine, 1994). Similarly, even after the intake of a food has been associated with sickness, CS⁺ associated with the food can still reinforce responding (Parkinson et al., 2005; Gerdjikov et al., 2011). Moreover, while AMPH reduces food-intake, it potentiates responding previously reinforced by food if animals are tested without food (i.e., during an extinction phase) (Clark, 1966; Olds, 1970; Herling et al., 1979; Cohen, 1991). Therefore, although feeding motivational systems and PAMS typically work together hand in hand, they are probably distinct systems.

Effects of lithium on VS-reinforced responding

We observed that chronic lithium treatment reduced spontaneous locomotor activity, an effect that is consistent with previous studies (Staunton et al., 1982; O'Donnell and Gould, 2007). Lithium also decreased VS-reinforced responding. The observation that lithium did not reduce inactive leverpressing suggests that the lithium treatment did not cause general motor deficit. Although the lack of effect on inactive leverpressing could be explained by a floor effect, it is an accepted view that lithium treatments causing similar blood levels do not cause general motor deficit (O'Donnell and Gould, 2007; Young et al., 2011). However, previous studies are not consistent on how lithium treatments affect AMPH-potentiated locomotor activity. While some reported that lithium treatments reduced AMPH-potentiated locomotor activity, others and the present study found no detectable effect of lithium on AMPH-potentiated locomotor activity (O'Donnell and Gould, 2007; Young et al., 2011). Determining factors may include the animal strain, AMPH dose, and the dose, length, and route of lithium administration. The studies that employed chow to administer lithium tended to report the lack of lithium's effects on AMPH-potentiated locomotion (Ebstein et al., 1980; Fessler et al., 1982; Lerer et al., 1984). Lithium administered through diet typically results in weight loss compared to control-diet animals (Staunton et al., 1982). In our experience, it was not possible to maintain normal body-weight growth by giving lithium-containing (0.2%) chow to rats (unpublished observation), leading to not having a lithium-diet control group without food restriction in the present study. Thus, food restriction could be a contributing factor in producing inconsistent effects of lithium on this behavior.

While lithium did not reduce AMPH-potentiated locomotor activity in the present study, it further augmented AMPH-potentiated VS-reinforced responding. Our observation needs to be carefully interpreted, because we do not yet know how lithium would affect VS-reinforced responding, with or without AMPH, in rats of unaltered body weights. We can only make limited inference because, to the best of our knowledge, no prior research has systematically investigated effects of food restriction on behavior of lithium-treated animals

or effects of lithium on VS-reinforced responding. Based on synergistic interaction between food restriction, AMPH and VS reported above, food restriction probably played a critical role as well in the interaction between lithium and AMPH in VS-reinforced responding. This needs to be clarified by future research.

Clinical implications

Caloric restriction procedures similar to the present food restriction have been shown to have health benefits in rodents such as decreased morbidity and increased life span (Masoro, 2000). The synergistic interaction between food restriction and AMPH on purposeful behavior found in rats may have implications in humans exercising chronic dietary restriction. Dietary restriction is widely practiced in humans while AMPH is widely used and abused (Van Kammen and Murphy, 1975; Brauer and de Wit, 1996; National Task Force on the Prevention and Treatment of Obesity, 1996; Pliszka, 2007; Breitbart and Alici, 2010; Trepanowski JF, Bloomer RJ, 2010; NDP Group, 2013). In humans, chronic food restriction may potentiate responding to certain reinforcers and CS⁺ especially when administered with psychoactive drugs such as AMPH. Indeed, long-term caloric restriction (7 days or over) is associated with mood enhancement in humans (Michalsen, 2010). In addition, the present data on lithium raise the possibility that lithium treatment and its effects on motivation and approach behavior may be significantly altered when lithium-treated patients undergo caloric restriction and use psychostimulant drugs, leading to potentially dangerous behavior. Immediate actions may be warranted given the fact that combined medications of psychostimulant drugs with mood stabilizers are suggested for treatments of bipolar disorders that comorbid with attention-deficit/hyperactivity disorder (Bond et al. 2012).

In summary, food restriction appears to interact with psychoactive drugs in purposeful behavior much more potently than previously thought, and purposeful behavior need not be related to energy homeostasis. VS-reinforced responding, which is easily quantified and reliable over many repeated sessions, appears to be useful in examining effects of various manipulations. In this light, this procedure may be useful in elucidating mechanisms involved in psychiatric disorders including manic-depressive illness.

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Figure 1.

Effects of food restriction, SCH 23390 and AMPH on VS-reinforced responding. Data are means $\pm$ SEM. **a** Effects of food restriction (FR) on the PR schedule. **b** Effects of food restriction on the VR2 schedule. **c** Effects of SCH 23390. **d** Effects of AMPH. * $p < 0.05$, food-restriction value being significantly different from its session-1 value. ** $p < 0.01$, food-restriction value being significantly different from its 0-mg/kg value. + $p < 0.05$, AL value being significantly different from its session-1 value or 0-mg/kg value. # $p < 0.05$, dose value (AL and food restricted groups combined) being significantly different from its 0-mg/kg value. @ $p < 0.005$, food-restriction value being significantly different from corresponding AL value.

Figure 2.

Effects of food restriction, repeated testing and repeated AMPH. Data are means $\pm$ SEM. **a** Effects of two weeks of food restriction (FR) prior to testing. **b** Effects of repeated injections of AMPH. **c** Effects of different doses of AMPH. @ $p < 0.05$, food-restriction value being significantly different from corresponding AL value. * $p < 0.05$, food restriction-saline value at session 11 being significantly different from its session-5 value. * $p < 0.05$, food restriction-AMPH value at session 10 being significantly different from its session-5 value. # $p < 0.05$, dose value (AL and food-restricted groups combined) being significantly different from its saline value. #1 $p < 0.05$, dose value (AL and food-restricted groups combined) being significantly different from its saline value and from its session-5 value. * $p < 0.05$, food-restriction value (saline- and AMPH-experienced groups combined) being significantly different from its 0-mg/kg value. + $p < 0.05$, AL value (saline- and AMPH-experienced groups combined) being significantly different from its 0-mg/kg value.

Figure 3.

Acute food restriction (or a 24-hr food deprivation) does not augment AMPH-potentiated VS-reinforced responding. Data are means $\pm$ SEM. **a** Effects of acute food restriction (FR). Filled symbols in rectangular boxes indicate days when food-restricted and AL rats received AMPH injections. **b** Effects of SCH 23390 on food restriction/AMPH potentiated VS-reinforced responding and locomotor activity. * $p < 0.05$, food-restriction value being significantly different from its day-0 value. * $^1p < 0.05$, food-restriction value being significantly different from its day-1 value. + $p < 0.05$, AL value being significantly different from its day-0 value. + $^1p < 0.05$, AL value being significantly different from its day-1 value. # $p < 0.05$, dose value being significantly different from its 0-mg/kg value.

Figure 4.

Chronic lithium treatment decreases VS-reinforced responding, but augments it when food-restricted rats receive AMPH. Data are means $\pm$ SEM. **a** Effects of lithium diet. **b** Effects of different doses of AMPH. * $p < 0.001$, food-restricted control-diet value being significantly different from its 0-mg/kg value. *² $p < 0.001$, food-restricted lithium-diet value being significantly different from its 0-mg/kg value. # $p < 0.001$, dose value (lithium- and control-diet groups combined) being significantly different from its 0-mg/kg value.

Figure 5.

Synergistic interaction between food restriction, AMPH (1 mg/kg) and VS. The 100% baseline was derived from mean inactive lever counts of saline-injected AL rats. Gray and red bars indicate inactive and active leverpress levels, respectively. Unfilled bars indicate hypothetical additive levels, estimated by adding leverpress counts with respective factors subtracted by baseline level.

Table 1

Six sets of rats and experiments

Set	# of rat	Testing order	Experiment: Description
1	24	1	Exp. 1a: Effects of food restriction with progressive ratio (Fig. 1a)
2	16	1	Exp. 1b: Effects of food restriction with variable ratio 2 (Fig. 1b)
		2	Exp. 1c: Effects of SCH (Fig. 1c)
		3	Exp. 1d: Effects of AMPH (Fig. 1d)
3	32	1	Exp. 2a: Effects of food restriction (Fig. 2a)
		2	Exp. 2b: Effects of repeated AMPH injections (Fig. 2b)
		3	Exp. 2c: Effects of AMPH doses (Fig. 2c)
4	20	1	Exp. 3a: Effects of food-restriction length (Fig. 3a)
	10	2	Exp. 3b: Effects of SCH (Fig. 3b)
5	22	1	Exp. 4a: Effects of lithium (Fig. 4a)
		2	Exp. 4b: Effects of lithium (Fig. 4b)
6	10	1	Exp. 5: Lithium concentration analysis