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Interaction of chronic food-restriction and methylphenidate in sensation seeking of rats

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Abstract

Rationale—It is necessary to understand better how chronic food-restriction (CFR) and psychostimulant drugs interact in motivated behavior unrelated to food or energy homeostasis.

Objectives—We examined whether CFR augments methylphenidate (MPH)-potentiated responding reinforced by visual sensation (VS), and whether repeated MPH injections or prolonged CFR further augments such responses.

Methods—Before starting the following experiments, rats on a CFR diet received a limited daily ration in such a way that their body weights decreased to 85–90% of their original weights over 2 weeks. In experiment 1, rats on CFR and *ad-libitum* diet received four injections of varying MPH doses (0, 2.5, 5, and 10 mg/kg). In experiment 2, CFR and *ad-libitum* groups received repeated injections of MPH (2.5 mg/kg). In experiment 3, half of CFR rats received repeated injections of MPH (2.5 mg/kg), and the other half received saline, and following a 7-day abstinence, they all received the 2.5-mg/kg dose of MPH.

Results—CFR rats increased VS-reinforced responding more than *ad-libitum* rats when they received MPH. Repeated injections of MPH with prolonged CFR further increased VS-reinforced responding. We found a double dissociation where prolonged CFR (3 vs. 6 weeks) made VS-reinforced responding, but not locomotor activity, more responsive to MPH, whereas repeated MPH injections made locomotor activity, but not VS-reinforced responding, more responsive to MPH.

Conclusions—CFR markedly potentiates effects of MPH on VS-reinforced responding. The present study demonstrates that the longer CFR continues, the greater psychostimulant drugs augment behavioral interaction with salient stimuli.

Keywords

Visual stimulus; Ritalin; caloric restriction; approach motivation; instrumental reinforcement; behavioral sensitization; apathy

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INTRODUCTION

Caloric restriction or chronic food-restriction (CFR) increases motivation for drug self-administration and drug seeking (Carroll and Lac 1997; D'Cunha et al. 2013; Donny et al. 1998; Shalev 2012); psychostimulant-drug administration increases motivation to seek food, drugs, or conditioned stimuli (CSs) associated with food or drugs (Beninger and Ranaldi 1992; Hill 1970; Robbins 1975; Shalev et al. 2002). Therefore, it is not surprising to find that CFR interacts with psychostimulant drugs in potentiating motivated behaviors (Carr 2007; Keller et al. 2014). However, little investigation has been performed to elucidate the details of this interaction. Such investigation may have clinical implications for industrial societies where CFR and psychostimulant-drug use are prevalently prescribed by physicians and self-medicated.

The present study investigates interaction between CFR and psychostimulant drugs in motivated behavior induced by an unconventional reinforcer, flash of light or visual sensation (VS) (Gancarz et al. 2011; Keller et al. 2014; Kish 1966; Stewart 1960). Rats readily learn to make lever-press responses to obtain VS in an operant conditioning chamber equipped with two levers. Responding on the “active” lever is followed by VS, while responding on the “inactive” lever has no consequence. When introduced to the chamber for the first time, most rats display lever pressing within minutes and typically respond on the active lever twice as much as the inactive lever in a one-hour session, and this pattern of lever pressing is stable over many daily sessions (Keller et al. 2014). In this case, VS is mere physical perception without acquired motivational value, since it has not been paired with any conventional rewards.

Keller et al. (2014) found that while CFR or d-amphetamine administration alone has small effects on VS-reinforced responding, the combination of the two markedly increases it. In addition, because previous studies suggested that repeated injections of amphetamine potentiate motivation (Robinson and Berridge, 1993), Keller et al. (2014) examined effects of repeated injections of amphetamine on VS-reinforced responding and found that repeated injections of amphetamine do not sensitize it. The aim of the present study was threefold. First, we examined whether another psychostimulant drug methylphenidate (MPH; Ritalin) has similar effects as amphetamine on VS-reinforced responding. MPH is a popular psychostimulant drug prescribed for attention deficit hyperactive disorder (ADHD) and other clinical symptoms such as apathy. Second, we examined whether repeated injections of MPH sensitize VS-reinforced responding. Finally, we examined whether the capacity of CFR to interact with MPH in motivation depends on body-weight reduction.

METHODS AND MATERIALS

Animals

Male Wistar rats from Harlan (Dublin, VA) were individually housed in a humidity- and temperature-controlled room on a reverse 12-h light–dark cycle (lights on at 8:00 PM). Animals were randomly assigned into experimental groups. 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

Methylphenidate (National Institute on Drug Abuse, Bethesda, MD) was dissolved in 0.9 % saline and administered intraperitoneally at doses of 2.5, 5, and 10 mg/kg. Rats were acclimated to the injection procedures by receiving saline injections (one to four times) prior to testing. For all experiments, drugs were administered immediately before the start of each behavioral session.

Chronic food restriction

CFR rats were given one daily food ration of 8–15 g standard rodent chow throughout the duration of each experiment. Ad-libitum fed (AL) rats were given unlimited food, and their bodyweight rose gradually throughout the experiments, whereas the bodyweight of CFR rats was gradually reduced over two weeks and maintained between 85–90% of their original bodyweight throughout the duration of the experiment. Each experiment began exactly 3 weeks after the first day of CFR. During behavioral testing, rats were fed within an hour after completing their behavioral session.

Visual sensation-reinforced behavior

Operant conditioning chambers and the procedure of VS reinforced responding were previously described (Keller et al. 2014; Shin et al. 2010; Vollrath-Smith et al. 2012; Webb et al. 2012). Each chamber was housed in a box protected from outside noise and lights, and equipped with two levers mounted on a wall, a cue lightbulb covered by white lens above each lever, and a red house light mounted on opposite side of the lever-mounted wall. Each experimentally naive rat was habituated to the chambers for 60 min per day over two or three days prior to the beginning of each experiment. During testing, active lever-pressing illuminated the cue light above the lever for 1 sec and turned off the house light for 5 sec. During this 5-sec period, lever presses were recorded, but had no programmed consequence. Rats received VS reinforcement on a progressive ratio schedule, where the number of active lever presses required to produce a presentation of VS increased by 1 every 10 VS presentations throughout the session. Inactive lever pressing had no programmed consequence throughout each session. Each session lasted 60 min. 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 to lever pressing, locomotor activity was assessed with four 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.

Experiment 1: Effects of MPH doses and CFR on reinforced and non-reinforced responses

A half of experimentally-naïve rats ($n = 24$) were food-restricted as described above (CFR group) and maintained on this diet throughout the experiment. The other half ($n = 24$) had ad lib access to food (AL group). Each rat was habituated to the test chamber for three days. To

acclimate them to IP injections, they received one saline injection immediately before being placed in the chamber on the final habituation day. Over the next four sessions, each rat received IP injections of saline, 2.5, 5, and 10 mg/kg MPH in this order. Sessions were separated by 48 hours.

Experiment 2: Effects of repeated injections of MP with prolonged CFR

Immediately after experiment 1, 16 rats that were used in experiment 1 (8 CFR and 8 AL rats) received repeated injections of 2.5 mg/kg MPH (5 – 6 times separated by 48 hrs) followed by one week of abstinence from injections. Then, they again received saline, 2.5 and 5 mg/kg MPH in this order over three sessions, separated by 48 hours. At the time of the sensitization test, the CFR group was kept on CFR for 6 weeks.

Experiment 3: Which is more important for sensitized responses: repeated MPH injections or prolonged CFR?

Experimentally-naïve rats were food restricted as described above for three weeks before the start of this experiment, and were maintained on this diet for the rest of the experiment. After two habituation sessions with saline injections, one group (n = 12) received an injection of 2.5 mg/kg MPH just before each of the next 6 sessions, while the other (n = 12) received a saline injection before the next 6 sessions. Sessions were separated by 48 hrs. After a week of abstinence from injections and VS-reinforced responding, both groups received a dose of 2.5 mg/kg MPH injection before the last session, which occurred about 6 weeks after the start of CFR.

Statistical analyses and presentations

Because MPH injections produced skewed and widened distributions of lever-press counts (Fig. 1A–D), the data of experiments 1–3 were square-root transformed, to normalize distribution for parametric tests (McDonald 2014), including ANOVAs and student t-tests (Statistica, v. 6.1, StatSoft, Tulsa, OK). Means were calculated with back-transformed values of square-root values for presentation, to make it easier to compare and conceive effects (McDonald 2014). Lever preference ratios were derived with this formula: (active lever-presses + 1)/(inactive lever-presses + 1). When Mauchley sphericity test detected a violation of the sphericity assumption for repeated factors, the degrees of freedom for respective tests were adjusted with the Greenhouse–Geisser method. Post-hoc comparisons were performed with either Tukey's honestly significant difference tests or t-tests with the Bonferroni correction for multiple comparisons.

RESULTS

Experiment 1: Effects of MPH doses and CFR on reinforced and non-reinforced responses

Two rats (one from the CFR group and one from the AL group) were eliminated from the analyses below because they did not respond on active lever at all for the first two test sessions with saline and 2.5 mg/kg MPH. We examined the distribution of data plotted as a function of dose and CFR (Fig. 1A–D). While MPH injections increased locomotor activity of the rats uniformly by several fold (Fig. 1D), the same injections had a mixed effect on lever presses in the same rats: they had little or no effect in some rats, while markedly

increasing VS-reinforced lever presses in others (Fig. 1A). Two CFR rats with the 10 mg/kg dose responded 1,692 and 1,982 times; one of the CFR rats with the 5 mg/kg dose responded on the active lever 472 times; and one of the AL rats with the 10 mg/kg dose responded on the active lever 757 times in the one-hour session. Thus, MPH administration had an uneven effect on active lever-pressing. Similar effects were also seen for inactive lever-pressing and lever-preference ratio, although the ranges of variability were smaller (Fig. 1B, C).

To statistically evaluate the effects of CFR and MPH on lever pressing and locomotion, $2^{\text{group}} \times 4^{\text{dose}}$ mixed ANOVAs were performed in square-root transformed data. MPH significantly increased active lever-presses in a dose-related manner (Fig. 1A'; main dose effect: $F_{3,132} = 6.84$, $p < 0.01$), and the effect was larger for the CFR group than the AL group ($F_{1,44} = 9.65$, $p < 0.005$). However, despite of the pattern of mean responses between the two groups as a function of doses (Fig. 1A'), the group-by-dose interaction was not significant ($F_{3,132} = 2.11$, $p = 0.10$). This is understandable considering the observation that individual responses to VS and MPH injections varied enormously (Fig. 1A). Although MPH injections had small effects on inactive lever-pressing in both CFR and AL groups, MPH affected it more uniformly within each group (Fig. 1B'), yielding significant effects (a significant group-by-dose interaction: $F_{3,132} = 6.28$, $p < 0.001$; group effect: $F_{1,44} = 11.01$, $p < 0.005$; dose effect: $F_{3,132} = 7.68$, $p < 0.0001$). MPH doses produced an inverted u-curved effects on inactive lever-pressing for CFR group, while they decreased inactive lever-presses for the AL group.

Lever preference in both CFR and AL rats was similar. In the absence of MPH administration, the ratio was roughly 2 (Fig. 1C and C'). MPH administration increased lever preference in some rats, but not others (Fig. 1C). Overall, it produced a dose-related effect (Fig. 1C'; main dose effect: $F_{3,132} = 12.98$, $p < 0.0001$), and interestingly, the CFR condition had no detectable effect (main group effect: $F_{1,44} = 0.04$, $p = 0.85$; group x dose interaction: $F_{3,132} = 0.33$, $p = 0.80$). These results suggest that MPH injections (5 and 10 mg/kg), but not a 3-week CFR condition, increased motivational value or salience of VS in some rats.

MPH injections significantly increased crossing counts in a dose-dependent manner (Fig. 1D' $F_{3,132} = 230.16$, $p < 0.0001$) with a significant group x dose interaction ($F_{3,132} = 5.86$, $p < 0.005$) without main group effect ($F_{1,44} = 1.90$, $p = 0.18$). These results suggest that while the two did not differ with saline injections, the CFR group displayed slightly more locomotor activity with MPH than the AL group.

Overall, these results showed that MPH doses had different effects on lever presses, lever preference ratios, and crosses, suggesting that these effects are mediated by different mechanisms. To infer the extent to which these effects are regulated by the same mechanisms, we performed Spearman rank order correlational analyses. Active lever-presses were moderately correlated with inactive lever-presses. Although lever-presses were significantly correlated with crossing counts at lower doses, the levels of correlation were poor (Table 1). Crossing counts had no relationship at all with lever preference ratios. Therefore, the mechanisms by which MPH injections increased lever-preference ratios were

most likely distinct from the mechanisms by which MPH injections increased locomotor activity (Fig. 1C' and D').

Experiment 2: Effects of repeated injections of MP with prolonged CFR

We examined whether a manipulation consisting of repeated MPH injections with prolonged CFR sensitizes behavioral responses. Eight AL and eight CFR rats that were used in experiment 1 received repeated injections of 2.5 mg/kg MPH followed by a one-week abstinent period. Then, they again received saline, 2.5 and 5 mg/kg MPH in this order over three separate sessions. Responses of the CFR and AL groups were analyzed separately with $2^{\text{manipulation}} \times 3^{\text{dose}}$ repeated-measures ANOVAs. The manipulation had no detectable effects in AL rats (Fig. 2A–D) with the exception of conditioned locomotor activity with vehicle (Fig. 2D; manipulation $\times$ dose interaction: $F_{2,14} = 13.69$, $p < 0.001$). By contrast, after the manipulation, CFR rats displayed sensitized responses for active lever-pressing, lever preference, and crossing, but showed no sensitization of inactive lever-pressing (Fig. 2A'–D'). The CFR rats increased active lever-pressing (main manipulation effect: $F_{1,7} = 8.83$, $p < 0.05$) and yielded a significant manipulation $\times$ dose interaction ($F_{2,14} = 4.13$, $p < 0.05$). To determine the effect of each dose between before and after the manipulation, paired t-tests were conducted with the Bonferroni correction for multiple comparisons. The manipulation resulted in a significant increase in active pressing with saline ($p < 0.05$) and the 2.5 mg/kg dose ($p < 0.05$), but not with the 5 mg/kg dose. The manipulation also increased lever preference in CFR rats ($F_{1,7} = 7.68$, $p < 0.05$), while the manipulation $\times$ dose interaction was not significant. Similarly, the manipulation increased crossing ($F_{1,7} = 25.09$, $p < 0.005$) in CFR rats. Moreover, a significant manipulation $\times$ dose interaction ($F_{2,14} = 11.25$, $p < 0.005$) was found with significant differences in crossing counts at the 0 and 2.5 mg/kg doses. In summary, the manipulation significantly increased the effects of saline and MPH on VS-reinforced responding, lever-preference ratio, and locomotor activity in CFR rats. However, this experiment fails to point out which factor, repeated MPH injections or prolonged CFR, is more important in increasing these behavioral measures.

Experiment 3: Which is more important for sensitized responses: repeated MPH injections or prolonged CFR?

This final experiment sought to determine which factor (repeated MPH injections or prolonged CFR) is responsible for sensitized VS-reinforced responding, lever-preference ratio, and locomotor activity in CFR rats. Rats were chronically food restricted (3 weeks by session 1) and were assigned to one of two groups. One group received repeated injections (RptI) of MPH under a continuous CFR condition, while the other received saline injections and a single MPH injection (SngI) in the last session under a continuous CFR condition. The two groups did not differ with respect to active or inactive lever-pressing, lever-preference ratio, or locomotor activity prior to the start of the manipulations (sessions 1–2 of Fig. 3A–D; $2^{\text{group}} \times 2^{\text{session}}$ ANOVAs). By session 10, both groups were on the CFR condition for 6 weeks. We compared the two groups with a $2^{\text{group}} \times 2^{\text{session}}$ mixed ANOVA on active lever-presses and found a significant group $\times$ session interaction (Fig. 3A; $F_{1,22} = 5.07$, $p < 0.05$). Tukey's post hoc test revealed that active lever-presses between the two groups did not differ in session 3 when SngI and RptI rats received saline and the 2.5 mg/kg dose of MPH, respectively. This effect is consistent with what was found in experiment 1; the 2.5 mg/kg is

a sub-threshold dose for an acute condition. The RptI rats gradually increased lever pressing over repeated sessions, and their active lever-presses differed significantly between sessions 1 and 10, an effect that is consistent with what was found in experiment 2. Similarly, SngI rats markedly increased active lever-pressing in session 10 when they received 2.5 mg/kg MPH for the first time, compared to that of session 3 ($p < 0.01$). SngI's active lever-pressing in session 10 did not differ from that of RptI in session 10; however, SngI's active lever-pressing in session 10 was significantly greater than that of RptI in session 3 when the RptI rats received MPH for the first time. These results suggest that prolonged CFR, but not repeated injections of MPH, is responsible for the sensitized responding reinforced by VS. Inactive lever-pressing was not affected by RptI (main group effect: $F_{1,22} = 1.86$, $p = 0.19$ or group x session interaction: $F_{1,22} = 0.63$, $p = 0.44$), although both groups responded slightly more in session 10 ($F_{1,22} = 14.90$, $p < 0.001$; Fig. 3B).

Effects on lever preference were somewhat similar to those on active lever-pressing, but with an interesting difference: A significant group x session interaction was found on lever-preference ratios (Fig. 3C; $F_{1,22} = 9.62$, $p < 0.01$). Preference ratios did not differ between the two groups in session 3 when SngI and RptI rats received saline and the 2.5 mg/kg dose of MPH, respectively. Although the RptI rats tended to increase preference for active lever over repeated sessions, preference did not differ significantly between sessions 1 and 10. By contrast, SngI rats significantly increased preference for active lever in session 10 when they received 2.5 mg/kg MPH for the first time, compared to that of session 3 ($p < 0.01$). SngI's active lever-preference in session 10 was significantly greater than that of the RptI group in sessions 10 and session 3. These results suggest that continuous CFR, but not repeated injections of MPH, is responsible for the development of sensitized preference for VS lever induced by acute MPH. Moreover, repeated MPH injections appear to interfere with the development of sensitized interaction between CFR and MPH in VS lever preference.

Unlike active lever-pressing and lever-preference, crossing was sensitized by repeated MPH injections, and prolonged CFR did not have any detectable effect (Fig. 3D). A $2^{\text{group}} \times 2^{\text{session}}$ mixed ANOVA on crossing revealed significant effects for group ($F_{1,22} = 24.71$, $p < 0.0001$), session ($F_{1,22} = 146.80$, $p < 0.0001$), and group x session interaction ($F_{1,22} = 4.73$, $p < 0.05$). The crossing counts of the RptI group were significantly greater than those of the SngI group in session 3 when the RptI and SngI groups received 2.5 mg/kg MPH and saline, respectively. Thus, the dose that did not significantly increase active lever-presses (Fig. 3A) significantly increased crosses. The RptI rats gradually increased crossing over repeated sessions, and their crossing counts were greater in session 10 than those of session 3 ($p < 0.001$). The SngI group also increased crosses when they received MPH in session 10 for the first time compared to session 3 ($p < 0.001$). While the crosses of the SngI group in session 10 did not differ from those of the RptI group in session 3, the RptI group's crosses in session 10 was significantly greater than that of the SngI group in session 10 ($p < 0.05$), an effect that is consistent with MPH-induced sensitization. These results suggest that repeated injection of MPH, but not continuous CFR, sensitized locomotor activity. The significant increase detected after saline injections shown in Fig. 2D' must have indicated a conditioned effect of repeated MPH, but not prolonged CFR.

Mean original weights of the Sngl and RptI groups were 387 g (SEM, 11.6) and 381 g (SEM, 17.8), respectively. By the first test session, their weights were reduced to and maintained at 84% and 84%, respectively, and their weights were maintained throughout the experiment with 87% and 86% at the time of final test, respectively. Thus, while their weights did not undergo further reduction between sessions 3 and 10, the active lever-pressing and lever preference ratios of the single MPH group increased significantly between these sessions, suggesting weight loss does not account for the additional potentiation in VS-reinforced responding or lever-preference ratio.

DISCUSSION

MPH administration alone had small effects on VS-reinforced behavior, but when MPH was combined with CFR, rats displayed vigorous VS-reinforced responding. The most important finding of the present study is that the property of CFR to interact with MPH in augmenting VS-reinforced responding did not stop after 3 weeks of CFR. Rather, CFR continued to exert its potentiating effect for at least several more weeks, even if the rats' body weights were kept at a constant level. This was demonstrated in a double dissociation, where prolonged CFR made VS-reinforced responding, but not locomotor activity, more responsive to an acute MPH injection than before, whereas repeated MPH injections made locomotor activity, but not VS-reinforced responding, more responsive to acute MPH injection than before. This suggests that VS-reinforced responding is affected by prolonged CFR and regulated differently from locomotor activity. We believe that these findings have important implications in understanding motivation mechanisms, especially in relation to psychostimulant drugs and their interaction with CFR. Below we discuss implications and issues associated with the present findings.

Effects of MPH injections and CFR on motivation: theoretical consideration

Here we will discuss the effects of MPH injections and CFR on behavioral responses with two respects: behavioral direction controlled by VS and behavioral vigor indicated by lever pressing, using a working model described in the following key assumptions (Fig. 4): First, lever pressing indicates rats' willingness to interact with the environment, which is referred to as *approach motivation* (Ikemoto 2010). Second, inactive lever-pressing indicates approach motivation induced upon perceiving novelty or a familiar environment unexplored for some time, which is referred to as *general investigatory or general approach motivation* (Butler 1957; Harlow 1950; Montgomery 1954; Pavlov 1927). Third, rats can internally represent motivational value of VS, which we designate as VS*. VS can be associated with the context of the test chamber whose perception triggers VS*. Forth, active lever-pressing indicates approach motivation consisting of general motivation and acquired, specific motivation (or *incentive motivation*) induced by VS*; motivational value of the reinforcer will affect relative preference between active and inactive lever-pressing. Therefore, VS* can be estimated by dividing active lever-presses by inactive lever-presses.

AL rats increased active lever-pressing, but not inactive lever-pressing, when they received MPH; as a result, preference ratio for active lever over inactive lever increased as a function of MPH dose (Fig. 1A'–C'). These results suggest that MPH increased VS*, while having

little effect on investigatory motivation (note that the 10 mg/kg dose decreased inactive lever-pressing). Although the 3-week CFR (CFR_{3wk}) alone had no effect on active or inactive lever-pressing or lever-preference ratio, CFR_{3wk} interacted with MPH and increased both active and inactive lever-pressing more than MPH alone; however, MPH with CFR_{3wk} did not differ from MPH alone in lever preference ratio. Thus, these results suggest that CFR_{3wk} interacted with MPH injections in increasing general approach motivation, but not VS*.

In the third experiment, interaction of the 6-week CFR (CFR_{6wk}) was investigated with the low MPH dose (2.5 mg/kg). This was a sub-threshold dose in interacting with CFR_{3wk} and did not reliably augment VS-reinforced responding. The low MPH with CFR_{6wk} significantly increased active lever-pressing more than MPH with CFR_{3wk}, while keeping inactive lever-pressing level the same, resulting in increased lever-preference ratio (Fig. 3A–C), thereby increased VS*. In summary, CFR_{3wk} and CFR_{6wk} may exert distinct effects on VS-reinforced responding: CFR_{3wk} interacts with MPH and increases rats' willingness to interact with the environment given the opportunity, while CFR_{6wk} interacts with MPH (low dose) and increases salience of salient stimuli. It is of interest to investigate if other abused drugs such as amphetamine and opioids exert qualitatively different effects on motivated behavior as a function of CFR length.

Systemic injections of psychostimulant drugs may have had dual action exerting opposing effects on VS-reinforced responding. Psychostimulant drugs such as MPH and d-amphetamine stimulate the mesolimbic dopamine system to increase motivated behavior (Blackburn et al. 1992; Ikemoto and Panksepp 1999; Robinson and Berridge 1993). Indeed, Shin et al. (2010) showed that injections of d-amphetamine directly into the ventral striatum (the terminal region of the mesolimbic dopamine system), but not the dorsal striatum, vigorously augment VS-reinforced responding in AL rats. Increased dopamine transmission in the ventral striatum appears to play an important role in approach motivation, but little or no role in VS* because of two key observations: First, those injections increase both active and inactive lever-presses while having little effect on lever preference ratio; in addition, injections of dopamine receptor antagonists into the ventral striatum decrease both active and inactive lever-presses without affecting lever-preference ratio (Shin et al. 2010). Thus, these results also suggest that the mesolimbic dopamine system is not responsible for increasing VS*, but approach motivation, a notion that is consistent with inferences derived from a review of other studies (Ikemoto and Panksepp 1999). The finding that CFR_{3wk} interacts with MPH in increasing approach motivation is consistent with previous studies suggesting that CFR sensitizes the mesolimbic dopamine system for motivational effects of psychostimulant drugs (Carr 2016; Pothos et al. 1995; Stouffer et al. 2015).

Systemic injections of psychostimulant drugs may recruit inhibitory effects over approach motivation, i.e. both active and inactive lever-presses. While mere unilateral injections of amphetamine directly into the ventral striatum significantly increase VS-reinforced responding, systemic injections of amphetamine do not significantly increase it in the same AL rats (Shin et al. 2010). Moreover, systemic amphetamine injections markedly increase locomotor activity, while unilateral injections of ventral striatal amphetamine have a smaller effect on locomotion than systemic injections (Shin et al. 2010). Similarly, the present study

found that systemic injections of MPH weakly increased VS-reinforced responding, while markedly increasing locomotor activity. Given that the ventral striatum plays a key role in locomotor activity induced by psychostimulant drugs (Ikemoto 2002; Kelley et al. 1989), these observations suggest that systemic injections of psychostimulant drugs recruit inhibitory effects over approach motivation. Little is known about inhibitory actions of psychostimulant drugs over motivated behavior. Extracellular levels of 5-HT increased by amphetamine administration have been implicated in “calming” effects of the drug as assessed by locomotor activity (Gainetdinov et al. 1999). However, MPH doses tested here vigorously increased locomotor activity; in addition, MPH is known to have little or no effect in extracellular 5-HT levels (Kuczenski and Segal 1997). In summary, it is unknown what mechanisms may be recruited by systemic MPH injections to inhibit approach motivation, while having little or no inhibitory action in locomotor activity.

Effects of repeated MPH injections on VS-reinforced responding

Previous studies have shown that repeated injections of psychostimulant drugs are known to sensitize behavioral responses associated with CSs, i.e. incentive motivation (Robinson and Berridge 1993; Wyvell and Berridge 2001). However, repeated injections of MPH (present study) or d-amphetamine (Keller et al. 2014) did not sensitize VS-reinforced responding even though they sensitized locomotor activity in the same animals. In fact, repeated MPH injections prevented CFR from interacting with MPH in increasing lever preference ratio (Fig. 3C), thereby VS*. Does this finding imply that the incentive motivation that responds to CSs associated with primary reinforcers such as food is distinct from that of CS associated with VS? One possibility is that the difference is not in motivational process, but procedure. Previous studies that successfully demonstrated sensitized incentive motivation effects treated animals with psychostimulant drugs in the absence of primary reinforcers or CSs (Mead et al. 2003; Taylor and Horger 1999; Taylor and Jentsch 2001; Wyvell and Berridge 2001). The present study differs from those studies in the following respects: both the primary reinforcer and the CS were paired with MPH, and the CS was a contextual, but not discrete, stimulus; therefore, these differences may have disrupted development of sensitization. This notion is consistent with the phenomenon called conditioned taste aversion or avoidance (Hunt and Amit 1987; Parker 1995; Wise et al. 1976) where pairing of fluid intake with administration of psychostimulant drugs does not result in conditioned preference for the taste of the fluid, but avoidance. Similarly, rats that have received repeated injections of d-amphetamine leading to sensitized locomotor activity do not become sensitized with lateral hypothalamic self-stimulation when self-stimulation sessions are conducted repeatedly with the injections (Wise and Munn 1993); however, Cabeza De Vaca et al. (2004) do not observe sensitized self-stimulation even though the rats receive repeated amphetamine in their home cages, not during self-stimulation performance.

Individual difference

Some rats responded little for VS, and their responding changed little with MPH, CFR, or both (Fig. 1A). By contrast, every rat increased its locomotor activity as a function of MPH injections (Fig. 1D). The variable effect of MPH treatments on VS-reinforced responding may depend on genetic variation among rats.

It is interesting to determine whether the variation in VS-reinforced responding has anything in common with other traits of compulsive behaviors. *Sensation seeking* is “a trait describing the tendency to seek novel, varied, complex, and intense sensations and experiences and the willingness to take risks for the sake of such experience” (Zuckerman 1994), and this personality trait has been linked to drug abuse and addiction (Zuckerman 1994). Similarly, the behavioral trait of “sign-tracking” (Pavlovian responding to CSs paired with food while waiting for food delivery, as opposed to responding immediately to food-delivery location, a.k.a. “goal-tracking”) has also been linked to compulsive disorders including addiction (Flagel et al. 2009). Future studies are warranted to investigate whether these behavioral differences, and the genetic factors that underlie them, account for individual variation in VS-reinforced responding.

How does CFR interact with MPH in motivation?

Moderate CFR procedures similar to the one used here are known to promote health of rodents and prolong their lifespan rather than to cause detrimental physiological and psychological stress (McCay et al. 1935; Schwartz et al. 2000; Tucker 1979). Indeed, our CFR rats looked healthy without any health concerns. Other than the mesolimbic dopamine system discussed above, little is known about physiological mechanisms through which CFR alters motivational state.

When rats receive daily meals at the same time every day, they display anticipatory arousal prior to their daily meal, indicated by increased locomotor activity (Mistlberger 1994). The present CFR rats were fed within one hour after the completion of the test session. Therefore, anticipation of the daily meal may have affected the performance of VS-reinforced responding. Although we did not address this issue with an experiment, preliminary data suggest that this is unlikely. In a pilot experiment, we examined effects of a meal given 3-hrs or 1-hr before the test or immediately after the test in rats that had been used to getting meals just before regular testing time. The preliminary results suggested that the rats did not decrease VS-reinforced responding even when they had just been fed (Online Resource 1). Although this experiment did not directly address whether meal anticipation has any effect on the behavioral measures, the results reinforce the notion that the capacity of CFR to interact with MPH in motivation cannot be explained by acute mechanisms such as blood sugar level, but mechanisms regulated on the order of days and weeks.

In summary, we found that MPH administration or CFR on its own had relatively small effects on VS-reinforced responding, but the two strongly potentiate VS-reinforced responding when they are combined. Our analysis suggests that systemic injections of MPH most likely have an inhibitory action over approach motivation, but not VS* or locomotor activity, and CFR makes the mesolimbic dopamine system more sensitive to MPH to overcome its inhibitory action. In addition, the present study found that the property of CFR to increase MPH's effects on VS-reinforced responding does not stop when rats' body weight stabilizes at 85–90% of their original level, but continues to exert its potentiating effect for at least several more weeks if CFR continues. A theoretical analysis suggests that the treatment of MPH under a prolonged CFR condition makes individual rats more responsive to salient stimuli by increasing their willingness to interact with the environment

and by making the thought of salient stimuli more attractive. We believe that it is important for healthcare professionals to pay attention to such intriguing interactions between stimulant drugs and caloric restriction in voluntary behavior because of the prevalence of stimulant-drug use and caloric-restriction practice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Effects of methylphenidate doses on lever pressing, lever preference ratio, and locomotor activity. Panels A–D show responses of individual ad-libitum (AL) or chronic food-restriction (CFR) rats with means (green lines). Panels A'–D' were back-transformed values of means of the square-root data (McDonald 2014). ¹, ², and ³ $p < 0.05$, 0.005 , and 0.0001 , respectively; significant dose effects different from the 0-mg/kg value. * $p < 0.05$, significantly different from the 0-mg/kg value.

Figure 2.

Effects of repeated injections of MPH with prolonged CFR on lever presses, lever preference, and locomotor activity in AL (A–D) and CFR (A'–D') groups. * $p < 0.05$, significantly different from the same dose value before the manipulation, which consisted of 5–6 repeated injections of 2.5 mg/kg MPH with CFR followed by a one-week abstinent period, as described in the Methods (Experiment 2). ¹ and ² $p < 0.01$ and 0.005 , respectively; significant dose effects different from the 0 mg/kg value.

Figure 3.

Prolonged CFR with a single and repeated MPH injections differentially affect lever pressing and locomotor activity. The first saline injection of SngI rats is labeled as “1”; the first and last cocaine injections of RptI rats in sessions 3 and 10 are labeled as “2” and “3”, respectively. A significant difference between the value of 1, 2, or 3 and the value of the last MPH injection in session 10 is shown by placing the number by the symbol in session 10.

Figure 4.

A model for interpreting data generated with the VS-reinforced responding procedure. Note that it is shown here that VS^* is derived by active lever-presses divided by inactive lever-presses for the sake of conceptual simplicity; as described in the method section, VS^* is actually derived with this formula, $(a + 1)/(b + 1)$, to avoid cases where a or b is zero.

Table 1

R² values between lever pressing, crossing, and lever preference as a function of MPH doses

	Methylphenidate (mg/kg)			
	0	2.5	5	10
Active vs. Inactive lever-pressing	0.29 *	0.45 *	0.24 *	0.41 *
Active lever-pressing vs. Crossing	0.11 *	0.17 *	0.10 *	0.05
Inactive lever-pressing vs. Crossing	0.18 *	0.15 *	0.08	0.06
Lever preference ratio vs. Crossing	0.01	0.01	0	0.01

*
 $p < 0.05$