

Continuous, but not Intermittent, Antipsychotic Drug Delivery Intensifies the Pursuit of Reward Cues

Anne-Marie Bédard¹, Jérôme Maheux², Daniel Lévesque² and Anne-Noël Samaha^{*1,3}

¹Department of Pharmacology, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada; ²Faculty of Pharmacy, Université de Montréal, Montreal, QC, Canada; ³CNS Research Group, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada

Chronic exposure to antipsychotic medications can persistently change brain dopamine systems. Most studies on the functional significance of these neural changes have focused on motor behavior and few have addressed how long-term antipsychotic treatment might influence dopamine-mediated reward function. We asked, therefore, whether a clinically relevant antipsychotic treatment regimen would alter the incentive motivational properties of a reward cue. We assessed the ability of a Pavlovian-conditioned stimulus to function as a conditioned reward, as well as to elicit approach behavior in rats treated with haloperidol, either continuously (achieved via subcutaneous osmotic minipump) or intermittently (achieved via daily subcutaneous injections). Continuous, but not intermittent, treatment enhanced the ability of amphetamine to potentiate the conditioned reinforcing effects of a cue associated with water. This effect was not related to differences in the ability to attribute predictive value to a conditioned stimulus (as measured by conditioned approach behavior), but was potentially linked to the development of behavioral supersensitivity to amphetamine and to augmented amphetamine-induced immediate early-gene expression (*c-fos* and *Nur77*) in dorsal striatopallidal and striatonigral cells. By enhancing the ability of reward cues to control behavior and by intensifying dopamine-mediated striatopallidal and striatonigral cell activity, standard (ie, continuous) antipsychotic treatment regimens might exacerbate drug-seeking and drug-taking behavior in schizophrenia. Achieving regular but transiently high antipsychotic levels in the brain (as modeled in the intermittent condition) might be a viable option to prevent these changes. This possibility should be explored in the clinic.

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INTRODUCTION

Nearly one in two individuals with schizophrenia symptoms have a history of drug abuse or addiction (Kavanagh *et al*, 2002; Regier *et al*, 1990). Two principal hypotheses have been suggested to explain this. First, the vulnerability to psychosis and drug abuse might share overlapping neural substrates and could be likely to co-occur in the same individual (Chambers *et al*, 2001). However, the phenotypic heterogeneity in schizophrenia makes it unlikely that a single neurobiological deficit underlies the illness (Keshavan *et al*, 2008). Second, drugs might be abused to cope with the illness (Khantzian, 1985) and antipsychotic medication side effects (Schneier and Siris, 1987). However, this idea lacks strong empirical support (Gregg *et al*, 2007;

Krystal *et al*, 2006), and drug use aggravates rather than alleviates schizophrenia symptoms and medication side effects (Pencer and Addington, 2003; Potvin *et al*, 2006). A third, complementary, but often overlooked hypothesis is that chronic antipsychotic treatment might alter reward function (Kosten *et al*, 1996; LeDuc and Mittleman, 1995). This possibility is supported by pre-clinical evidence. Thus, although acute exposure to antipsychotics/D2 antagonists generally reduces the incentive motivational properties of primary and conditioned rewards (Beninger *et al*, 1989; Le Moal and Simon, 1991), chronic antipsychotic treatment can enhance reward function. For example, chronic antipsychotic treatment augments operant responding for intravenous injections of cocaine (Howell and Byrd, 1992; Roberts and Vickers, 1987), facilitates the acquisition of heroin self-administration (Stinus *et al*, 1989), enhances cocaine-induced psychomotor sensitization (Fukushiro *et al*, 2008; LeDuc and Mittleman, 1993), and facilitates conditioned place preference to cocaine (Fukushiro *et al*, 2007; Kosten *et al*, 1996) and heroin (Stinus *et al*, 1989).

How might antipsychotic treatment alter reward function? One possibility is by inducing dopamine receptor

*Correspondence: Dr A-N Samaha, Department of Pharmacology, Faculty of Medicine, Université de Montréal, C P 6128, Succursale Centre-ville, Montreal, QC, Canada H3C 3J7. Tel: +1 514 343 6111; Fax: +1 514 343 2291; E-mail: anna.samaha@umontreal.ca
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supersensitivity within dopamine pathways. Although all antipsychotics occupy dopamine D2/3 receptors and reduce dopamine neurotransmission acutely, their chronic use can induce dopamine receptor supersensitivity. This is linked to increases in the number and sensitivity of striatal D2 receptors (Ginovart *et al*, 2009; Samaha *et al*, 2007, 2008) and an enhanced psychomotor response to dopaminergic drugs (Pudiak and Bozarth, 1997; Rebec *et al*, 1982; Samaha *et al*, 2007; Smith and Davis, 1975). In addition to mediating psychomotor behavior, dopamine functions as a 'teaching signal' to enable the association of neutral environmental cues with rewards (Schultz, 1998), and an 'incentive signal' to mediate the motivational value of reward cues (Berridge and Robinson, 1998). This is of significance in addiction because drug-paired cues generate motivational states that can precipitate or invigorate drug-seeking behavior (Arroyo *et al*, 1999; Panlilio *et al*, 1996), and elicit craving and relapse (de Wit and Stewart, 1981; O'Brien *et al*, 1998; Shaham *et al*, 2003).

In considering the ability of chronic antipsychotic medication to induce dopamine supersensitivity, the importance of the dopamine system in the processing of reward cues, and the role such cues have in drug-seeking and -taking behavior, we were led to ask the following question: Does chronic antipsychotic treatment, using clinically relevant doses, alter responsiveness to reward cues? We addressed this question by evaluating in rats the effects of chronic haloperidol treatment on the operant pursuit of a reward-associated cue. We compared the effects of continuous haloperidol administration (achieved via subcutaneous (SC) osmotic minipump) *vs* intermittent administration (achieved via daily SC injection). This is because continuous treatment induces behavioral dopamine supersensitivity, but intermittent treatment does not (Ericson *et al*, 1996; Samaha *et al*, 2008). Our findings show that following an acute amphetamine injection, rats with a history of continuous—but not intermittent—antipsychotic treatment pursued the reward cue more vigorously than antipsychotic-naïve animals. This was not related to changes in the predictive value of reward-predicting stimuli, but was potentially linked to the development of behavioral supersensitivity to dopaminergic stimulation, and to increased amphetamine-induced mRNA for the immediate early-genes *c-fos* and *Nur77* in striato-pallidal and striatonigral neurons of the caudate-putamen.

SUBJECTS AND METHODS

Subjects

Male Sprague–Dawley rats (Charles-River, Montréal, Canada; 200–225 g) were housed two/cage in a vivarium (12-h reverse light/dark cycle. Lights off at 0800 h). Testing was conducted during the dark phase of the animals' circadian cycle. Procedures complied with the Université de Montréal's animal care committee.

Drugs

Haloperidol (HAL; Sabex, Boucherville, Canada) was dissolved in 0.5% glacial acetic acid/water solution (pH 5) for administration via SC minipump (Alzet model 2ML2,

19-day drug delivery; Durect, Cupertino, CA, USA) because HAL maintains striatal D2 receptor occupancy over time in this vehicle (Kapur *et al*, 2003; Samaha *et al*, 2007). For administration through SC injection, HAL was dissolved in 20 mmol/l phosphate-buffered saline (PBS) because a glacial acetic acid/water solution is very acidic for instant delivery to a single site and could irritate the animals, which were injected daily for 17 days (see below). Importantly, using either PBS (Samaha *et al*, 2008) or a glacial acetic acid/water solution (Li *et al*, 2007) does not alter HAL's behavioral effects. *D*-amphetamine sulfate (AMPH; Sigma-Aldrich, Dorset, UK) was dissolved in 0.9% saline. All injections were given SC route in a volume of 1 ml/kg.

Antipsychotic Treatment

We wished to compare the effects of continuous *vs* intermittent HAL using clinically meaningful and equivalent doses. Multiple lines of evidence have suggested that doses of antipsychotics that are clinically efficacious occupy a target range of striatal D2 receptors. Although the relationship between the degree of striatal D2 receptor occupancy and the quality of the therapeutic response is complex (Pilowsky *et al*, 1992; Wolkin *et al*, 1989), clinical and brain imaging studies have generally supported the idea that for typical antipsychotics, therapeutic efficacy and a reduced risk of motor side effects can be seen with 65–75% D2 receptor occupancy ((Farde *et al*, 1992; Kapur *et al*, 2000), the therapeutic window is reported to lie lower—at 40–60%—for many atypical antipsychotics (Abi-Dargham and Laruelle, 2005)). Pre-clinical studies with typical antipsychotics also show antipsychotic-like efficacy at doses that provide 65–80% occupancy and an increased incidence of extra-pyramidal side effects at >80% occupancy (Natesan *et al*, 2006; Wadenberg *et al*, 2000).

In rats, HAL administration through a minipump produces continuously high levels of striatal D2 occupancy (Kapur *et al*, 2003; Samaha *et al*, 2007), whereas SC injections produce transiently high occupancy, which is diminished at 24 h post injection (Kapur *et al*, 2003). HAL administration through a minipump in rats, models the kinetics of standard antipsychotic treatment in humans because D2 occupancy in human striatum can remain high for several days following a dose (Farde *et al*, 1989; Tauscher *et al*, 2002). To meaningfully compare the continuous (minipump) *vs* intermittent (SC injection) conditions, we chose doses that held achieved dose/peak D2 occupancy constant under the two conditions. In rats, 0.5 mg/kg/day HAL via minipump achieves clinically relevant levels of D2 occupancy (73%; unpublished observations. See also (Kapur *et al*, 2003; Samaha *et al*, 2007)). However, when given through SC injection, 0.5 mg/kg leads to >80% D2 occupancy (Wadenberg *et al*, 2001) and is cataleptogenic (Natesan *et al*, 2006), and 0.05 mg/kg is sufficient to produce clinical levels of occupancy without catalepsy (74%, 2 h post injection (Kapur *et al*, 2003)). Thus, we studied three groups: CONT-HAL, which received 0.5 mg/kg/day HAL through a minipump, INT-HAL, which received 0.05 mg/kg/day HAL through daily SC injection, and VEH, which received PBS through daily SC injection.

Under 1.5% isoflurane, CONT-HAL rats were implanted with minipumps as in (Samaha *et al*, 2008), and INT-HAL

and VEH animals received an incision, closed with clips. The next day and for 17 consecutive days, the INT-HAL group was injected with HAL and all remaining animals received PBS. On the 18th day, minipumps were removed from CONT-HAL rats. INT-HAL and VEH animals were sham operated.

Experiment 1: Operant Responding for Conditioned Reward and AMPH-induced Locomotion

Here we assessed HAL-induced effects on operant responding for conditioned reward. We also assessed AMPH-induced locomotion to determine whether responding for conditioned reward was related to the development of dopamine supersensitivity.

Pavlovian conditioning and operant responding for conditioned reward. Procedures are illustrated in Figure 1a. In standard operant chambers (Med Associates, St Albans, VT, USA), water-restricted rats (2 h/day) were trained to associate the delivery of 0.1 ml tap water (the unconditioned stimulus; UCS) into a receptacle with a light/tone stimulus (the conditioned stimulus; CS) as in (Fletcher, 1995). Animals were then assigned to the VEH, INT-HAL, and CONT-HAL groups. Following antipsychotic treatment cessation, rats received a reminder conditioning session followed by two lever-training sessions in which they could press an 'active' lever to obtain CS presentations (now a conditioned reward) according to a random-ratio 2 schedule, and an 'inactive' lever which produced no consequences. No water was delivered. Sessions ended following 10 active presses or after 40 min. Two conditioned reward tests were then given under the same conditions, but without limiting the number of active lever presses. Immediately before testing, rats were injected with saline (first test) or 0.5 mg/kg AMPH (second test).

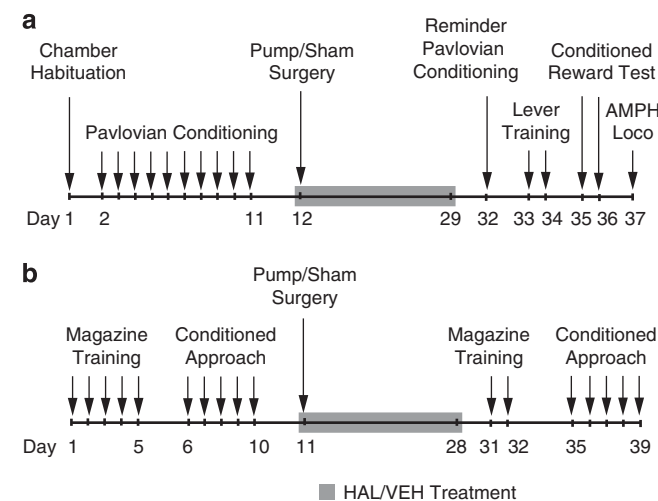


Figure 1 Timeline of behavioral training, testing, and antipsychotic treatment for Experiment 1 (a), in which operant responding for a conditioned reward was assessed following haloperidol (HAL) treatment cessation, and Experiment 2 (b), in which conditioned approach behavior to localizable reward-predicting stimuli was assessed before and starting on the 7th day following HAL treatment cessation. AMPH, amphetamine; Loco, locomotion; VEH, vehicle.

AMPH-induced locomotion. One day following conditioned reward testing, AMPH (1.5 mg/kg)-induced locomotion was measured in Plexiglas cages as was measured in (Samaha *et al*, 2007).

Experiment 2: Conditioned Approach Behavior to a Reward-predicting Cue

A reward cue more effectively reinforces an operant response in animals with a propensity to approach localizable conditioned stimuli (Robinson and Flagel, 2009). Thus, we determined whether HAL-induced changes in operant responding for conditioned reward could involve changes in approach behavior to reward-predicting stimuli. In the presence of a reward cue, some rats preferentially approach/engage with the cue ('sign trackers'), whereas others preferentially approach the location of reward delivery ('goal trackers'; (Flagel *et al*, 2007)). We determined which rats are goal trackers and which are sign trackers before assessing HAL-induced changes in sign-tracking vs goal-tracking conditioned responses.

Conditioned approach behavior. Procedures are illustrated in Figure 1b. Food-restricted rats were given once-daily magazine-training sessions (~25-min) for 5 days, where 50 banana-flavored food pellets (VWR, Ville Mont-Royal, Canada) were delivered into a recessed magazine on a variable interval 90-s schedule. All rats ate most pellets by the 5th day. We then assessed conditioned approach behavior in daily, 35–40 min autoshaping sessions for 5 days (25 trials/session), as in (Flagel *et al*, 2007). On each trial, the left lever was extended into the chamber for 8 s (the CS). This was followed by the delivery of one banana-flavored pellet (the UCS). We recorded lever contacts and magazine entries during the CS. As in (Flagel *et al*, 2007), CS-UCS pairings led to conditioned approach behaviors consisting of lever contacts and magazine entries. Rats in the top 33%, based on average lever contacts during autoshaping, were categorized as sign trackers and rats in the bottom 33% were categorized as goal trackers (Flagel *et al*, 2007). Sign and goal trackers were distributed equally in the CONT-HAL, INT-HAL, and VEH groups. Following antipsychotic treatment cessation, rats were given two reminder magazine-training sessions. Conditioned approach behavior was then re-assessed daily for 5 days. On days 4 and 5, rats were injected with saline or 0.5 mg/kg AMPH, 5 min before testing.

Experiment 3: *C-fos* and *Nur77* mRNA

We used *in situ* hybridization techniques to examine the effects of HAL on AMPH-induced neuronal activation by quantifying *c-fos* and *Nur77* mRNA in cortical and striatal regions.

Procedures. At 9 and 10 days following HAL treatment cessation, rats from each group were injected with 1.5 mg/kg AMPH or saline in the home cage and decapitated 1 h later. Brains were extracted, frozen in isopentane on dry ice, and stored (−80°C). Simultaneous radioactive ($[^{35}\text{S}]\text{UTP}$ -labeled *Nur77* or *c-fos* riboprobe) and nonradioactive (digoxigenin-labeled preproenkephalin riboprobe)

hybridizations were performed on cryostat-cut coronal brain sections (12- μ m), as in (Beaudry *et al*, 2000; Tremblay *et al*, 1999). An experimenter, blind to condition, translated optical gray densities from autoradiographs into μ Ci/g of tissue using a 14 C standard curve (ARC-146A, American Radiolabeled Chemicals, St Louis, MI, USA) and ImageJ software (NIH, Bethesda, MD, USA). Background values obtained from the corpus callosum of each section were subtracted from analysis. mRNA levels were measured in caudate-putamen quadrants at +1.6, +1.2, +0.8, +0.4, and 0.0 mm relative to Bregma, the nucleus accumbens (+2.0, +1.2, and +0.8 mm), the prelimbic and infralimbic cortices (+2.6 mm), and the orbitofrontal cortex (+3.0 mm) according to (Paxinos and Watson, 1986).

The greatest group differences in AMPH-induced gene expression were observed in the dorsal caudate-putamen (+0.8 mm). Approximately 95% of caudate-putamen cells are medium-sized spiny neurons forming the striatopallidal and striatonigral pathways. Striatopallidal cells preferentially express preproenkephalin mRNA (ENK+) and project to the lateral globus pallidus, whereas striatonigral cells do not preferentially express preproenkephalin mRNA (ENK-), and project to the medial globus pallidus and substantia nigra (Gerfen, 1992). To identify the cellular circuits in which HAL treatment could influence the neurobehavioral response to AMPH, we examined AMPH-induced *c-fos* and Nur77 mRNA in ENK+ and ENK- cells. This was achieved by manually counting seven cell types in three fields (0.26 mm²/field) of the dorsolateral (DL) caudate-putamen: cells containing mRNA for *c-fos*, Nur77 or ENK, both *c-fos* or Nur77 and ENK, and *c-fos* or Nur77 without ENK. Quantification was carried out at $\times 400$ using an optical microscope (Carl Zeiss, Montréal, Canada).

Statistics

Lever presses in Experiment 1 were analyzed with three-way ANOVA. When interaction effects were significant, simple effects were analyzed using the Bonferroni test. Within-group AMPH vs saline effects on lever pressing were analyzed using paired *t*-tests. Conditioned approach behaviors and AMPH-induced locomotion were analyzed using two-way ANOVA. AMPH effects on conditioned approach behaviors were analyzed using one-way ANOVA followed by Tukey's Multiple Comparison Test (TMCT). mRNA levels and counted cells were analyzed using one-way ANOVA followed by TMCT.

RESULTS

Experiment 1: Operant Responding for Conditioned Reward and AMPH-Induced Locomotion

Following antipsychotic treatment cessation, rats were given a 'reminder' Pavlovian conditioning session. During this session, all groups nose-poked more into the magazine containing the water receptacle during the 5-s CS period than in the 5-s period before the onset on the CS (PCS), and this behavior did not differ between groups (data not shown; one-way ANOVA on average CS/PCS ratio; $F(2, 39) = 1.1$, $p = 0.35$). This indicates that rats in all groups retained the CS-UCS contingency. As shown in Figure 2, all

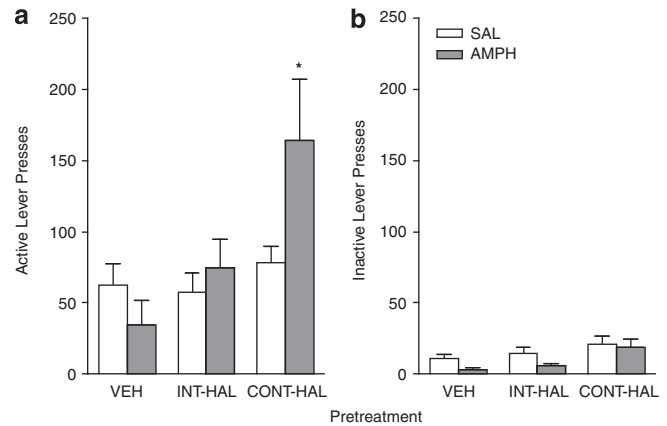


Figure 2 Continuous (CONT-HAL), but not chronic and intermittent (INT-HAL) haloperidol (HAL), treatment enhances operant responding for a conditioned reward following an injection of amphetamine (0.5 mg/kg). n 's = 14 per condition. Presses on the active lever are shown in (a) and presses on the inactive lever are shown in (b). CONT-HAL, group receiving 0.5 mg/kg/day haloperidol through minipump; INT-HAL, group receiving 0.05 mg/kg/day haloperidol through daily subcutaneous injection. * $p < 0.05$ compared with vehicle (VEH).

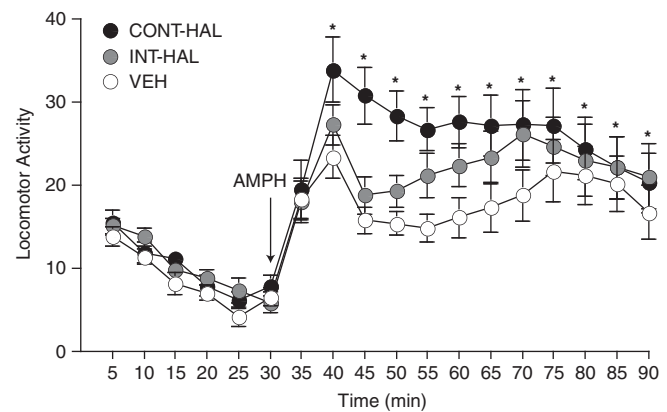


Figure 3 Continuous (CONT-HAL), but not chronic and intermittent (INT-HAL) haloperidol (HAL), treatment enhances the locomotor response to amphetamine (1.5 mg/kg). n 's = 14 per condition. CONT-HAL, group receiving 0.5 mg/kg/day haloperidol through minipump; INT-HAL, group receiving 0.05 mg/kg/day haloperidol through daily subcutaneous injection. * $p < 0.05$ compared with vehicle (VEH). AMPH, amphetamine.

groups pressed more on the active (A) vs inactive (B) lever ($F(1, 39) = 41.18$, $p < 0.0001$). This indicates that all groups discriminated between the two levers and spontaneously acquired a new operant response, reinforced solely by the conditioned reward. There was a significant Group-Injection \times Lever interaction ($F(2, 39) = 4.39$, $p = 0.019$). *Post hoc* investigation of this interaction revealed that following amphetamine, active lever presses were greater in the CONT-HAL group relative to the VEH group ($p = 0.009$). The INT-HAL group was not different from the VEH group ($p = 1.00$). Following amphetamine, inactive lever presses were also greater in the CONT-HAL group relative to both the INT-HAL and VEH groups (p 's < 0.05). However, AMPH did not alter inactive lever presses in the CONT-HAL rats compared with saline ($t = 0.33$, $p = 0.75$). There were no group differences in either active or inactive lever presses following an injection of saline (all p 's > 0.05).

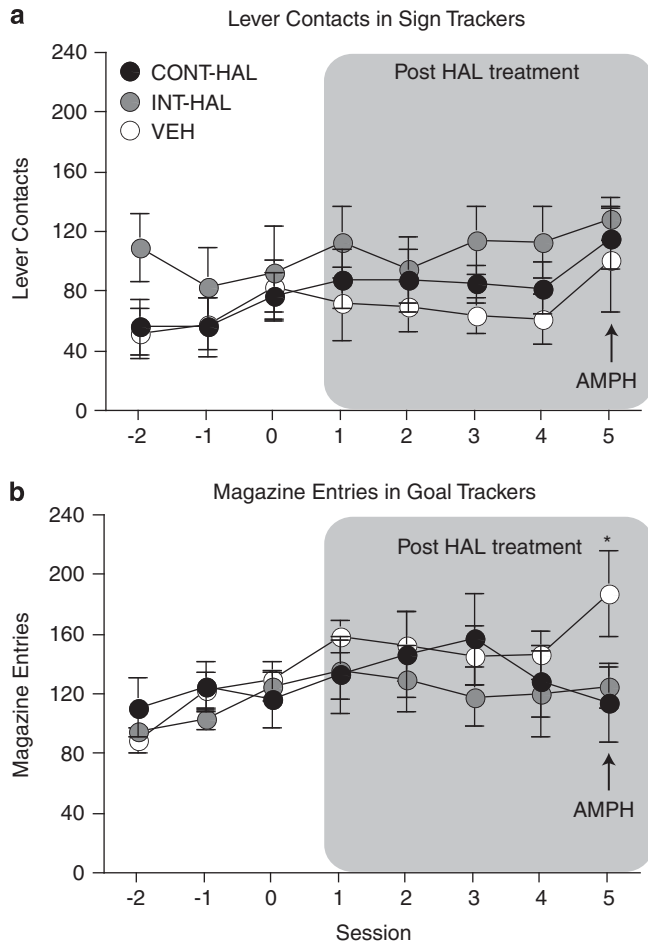


Figure 4 Neither continuous (CONT-HAL) nor chronic and intermittent (INT-HAL) haloperidol (HAL) treatment altered conditioned stimulus-directed (a) or goal-directed (b) conditioned approach responses. n 's = 6–7 per condition. CONT-HAL, group receiving 0.5 mg/kg/day haloperidol through minipump; INT-HAL, group receiving 0.05 mg/kg/day haloperidol through daily subcutaneous injection. * $p < 0.05$ compared with CONT-HAL and INT-HAL. AMPH, amphetamine.

AMPH-induced locomotion was greater in CONT-HAL compared with VEH (Figure 3; main effect of Group between 40–90 min; $F(1, 26) = 5.56, p = 0.03$). INT-HAL rats were no different from VEH rats ($p > 0.05$).

Experiment 2: Conditioned Approach Behavior to a Reward-Predicting Cue

Figure 4 shows conditioned approach behavior in sign trackers (a) and goal trackers (b) before antipsychotic treatment (sessions –2, –1, and 0) and following antipsychotic treatment cessation (sessions 1–5). Regardless of the mode of treatment (continuous vs intermittent) and of the nature of the conditioned response (sign tracking vs goal tracking), previous antipsychotic exposure did not alter approach behavior to a conditioned stimulus (sessions 1 to 4, main effect of Group; (a), $p = 0.31$; (b), $p = 0.73$). Acute AMPH did not influence sign-tracking behavior in any group ((A), session 5, $p > 0.05$), but it increased goal-tracking behavior in VEH compared with CONT-HAL and INT-HAL rats ((B); session 5, $F(2, 16) = 5.84, \text{all } p$'s < 0.05).

Supplementary Figure 1 shows lever contacts in goal trackers (a) and magazine entries in sign trackers (b), both before antipsychotic treatment (sessions –2, –1, and 0) and following antipsychotic treatment cessation (sessions 1–5). Antipsychotic treatment had no effect on either the number of lever contacts in goal trackers ((A) sessions 1–4, $p > 0.05$) or the number of magazine entries in sign trackers ((b) sessions 1–4, $p > 0.05$). Furthermore, acute AMPH did not influence these behaviors ((a) and (b), session 5, all p 's > 0.05).

Experiment 3: *C-fos* and *Nur77* mRNA

There were no group differences in AMPH-induced *Nur77* or *c-fos* mRNA expression in any cortical region analyzed (data not shown, all p 's > 0.05). Supplementary Table S1 shows saline- vs AMPH-induced *c-fos* mRNA in the nucleus accumbens core and shell averaged from +2.0 to 0.8 mm relative to Bregma. There were no group differences in saline-induced *c-fos* mRNA in either the nucleus accumbens core or shell (data not shown, all p 's > 0.05). Thus, saline-treated rats were pooled to form a single SAL group for each subdivision. All AMPH-treated groups had greater *c-fos* mRNA expression than SAL (all p 's < 0.001), and there was no effect of antipsychotic treatment. Similar results were seen with *Nur77* (data not shown). Supplementary Table S2 shows saline- vs AMPH-induced *Nur77* mRNA expression in the quadrants of the caudate-putamen (dorsomedial (DM), DL, ventromedial (VM), and ventrolateral (VL)), averaged from +1.6 to 0 mm relative to Bregma. AMPH-induced *Nur77* mRNA was greater in CONT-HAL relative to the other AMPH-treated groups in all quadrants but the VM (DM, $F(2; 96) = 8.96$, DL, $F(2; 96) = 7.7$, VL, $F(2, 96) = 5.33$; all p 's < 0.05). In addition, saline-induced *Nur77* expression was decreased in INT-HAL relative to the other saline-treated groups in all quadrants (all p 's < 0.05). Similar results were seen with *c-fos* mRNA (Supplementary Table S3).

The greatest group differences in AMPH-induced gene expression in the caudate-putamen were seen at level +0.8 mm relative to Bregma. These results are shown in Figures 5–7. There were no group differences in saline-induced *Nur77* or *c-fos* mRNA in any quadrant of the caudate-putamen (data not shown, all p 's > 0.05). Thus, saline-treated rats were pooled to form a single SAL group for *Nur77* (Figure 5) and for *c-fos* (Figure 6) mRNAs. As shown in Figure 5, all AMPH-injected groups had greater *Nur77* mRNA expression than SAL in the DM and in the VM caudate-putamen (DM, $F(3, 47) = 22.54$, VM, $F(3, 47) = 29.05$; all p 's < 0.05). In addition, there were group differences in amphetamine-induced *Nur77* mRNA. In the DL and in VL caudate-putamen, AMPH enhanced *Nur77* mRNA expression above SAL only in CONT-HAL rats (DL, $F(3, 47) = 6.76$, VL, $F(3, 47) = 4.98$; all p 's < 0.05). In addition, in the DM quadrant, AMPH-induced *Nur77* mRNA levels were greater in CONT-HAL rats than in VEH or in INT-HAL rats (all p 's < 0.01). In the VM and VL quadrants, AMPH-induced *Nur77* mRNA levels were also greater in CONT-HAL than in INT-HAL rats (all p 's < 0.05). No other comparisons were significant.

As shown in Figure 6, all AMPH-treated groups had greater *c-fos* mRNA levels relative to SAL in all quadrants of the caudate-putamen (DM, $F(3, 47) = 31.7$, DL, $F(3, 47) = 20.2$, VM, $F(3, 47) = 38.2$, and VL, $F(3, 47) = 18.3$; all

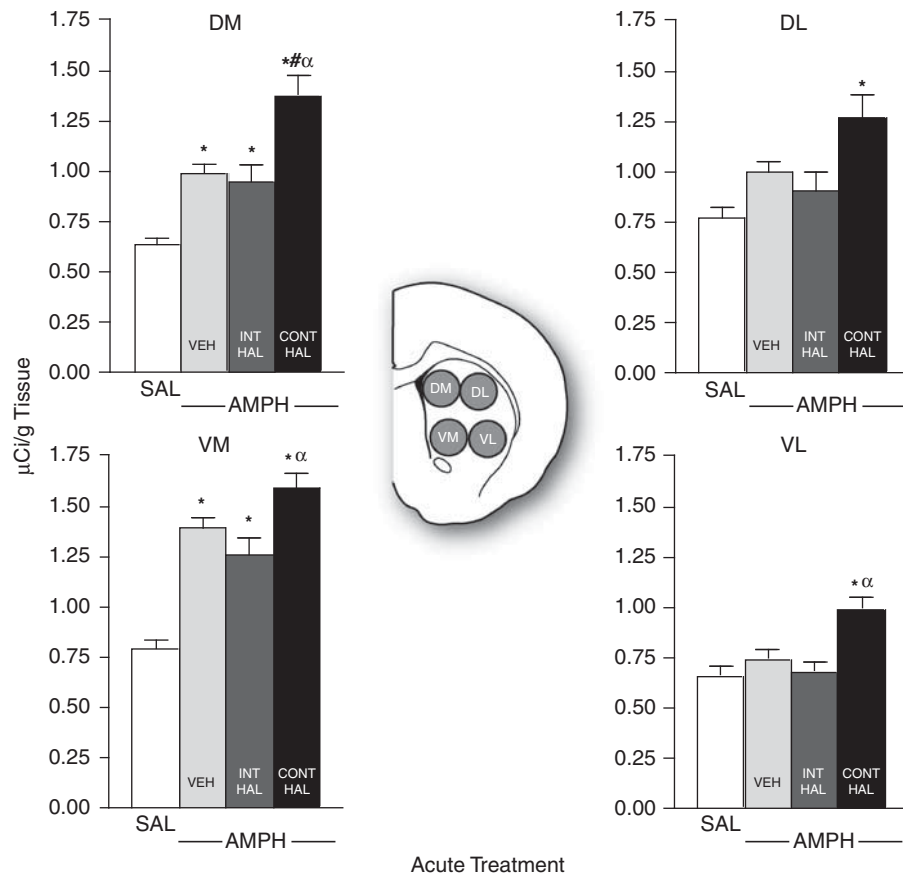


Figure 5 Continuous (CONT-HAL), but not chronic and intermittent (INT-HAL), haloperidol (HAL) treatment potentiates amphetamine-induced Nur77 mRNA expression in the caudate-putamen. n 's = 24 in the SAL condition, and 9 per condition in remaining groups. CONT-HAL, group receiving 0.5 mg/kg/day haloperidol through minipump; INT-HAL, group receiving 0.05 mg/kg/day haloperidol through daily subcutaneous injection; SAL, combined control group receiving saline. * p < 0.05 compared with SAL, # p < 0.05 compared with VEH, α p < 0.05 compared with INT-HAL. AMPH, amphetamine; DL, dorsolateral; DM, dorsomedial; VL, ventrolateral; VM, ventromedial.

p 's < 0.05). In addition, AMPH-induced *c-fos* mRNA expression was greater in CONT-HAL rats relative to VEH control rats in the DM, DL, and in VL quadrants (all p 's < 0.05). In the VL quadrant, *c-fos* mRNA expression was also greater in CONT-HAL than in INT-HAL rats (p < 0.01). No other comparisons were significant.

Figure 7 shows AMPH-induced *c-fos* and Nur77 mRNA expression in ENK+ and ENK− cells. There were no group differences in the number of any cell type following saline. Thus, all saline-injected rats were pooled to form a single SAL control group for each cell type (panels a–f). The effects of AMPH on *c-fos* and Nur77 mRNA expression depended upon previous antipsychotic treatment. AMPH increased the number of each cell type relative to SAL only in CONT-HAL rats, and the number of each cell type following AMPH was greater in CONT-HAL rats than in VEH or in INT-HAL rats ((a), $F(3, 40) = 13.71$; (b), $F(3, 40) = 7.86$; (c), $F(3, 38) = 13.70$; (d), $F(3, 41) = 6.81$; (e), $F(3, 41) = 8.53$; with the exception of (f), in which CONT-HAL > VEH only, $F(3, 41) = 5.52$; all p 's < 0.0004).

DISCUSSION

We show here that a history of continuous exposure to haloperidol, potentiates instrumental responding for a reward cue (a light-tone stimulus paired with water)

following an amphetamine challenge, and that this effect is not seen following a history of chronic, but intermittent antipsychotic exposure. This effect of continuous antipsychotic treatment was observed using a clinically relevant dose and mode of administration, was behaviorally specific (responding on an inactive lever was unaffected), and was persistent—lasting for at least 1 week following the cessation of antipsychotic administration. Consistent with previous work (Mead *et al*, 2004; Robbins, 1978; Robbins *et al*, 1983), the dose of amphetamine used here (0.5 mg/kg) had no effect on operant responding for conditioned reward in vehicle-treated control animals. However, this dose potentiated responding for a conditioned reward in rats with a history of continuous exposure to antipsychotic medication. The ability of previous continuous antipsychotic treatment to enhance amphetamine-induced potentiation of conditioned reward was potentially linked to the development of behavioral supersensitivity to dopaminergic stimulation, because continuous (but not intermittent) treatment also induced an exaggerated psychomotor response to amphetamine. Importantly, the two modes of antipsychotic treatment led to different outcomes in spite of the fact that achieved dose, peak levels of striatal D2 receptor occupancy, duration, and route of administration were identical under the two conditions. We have shown previously that the ability of continuous, but not intermittent,

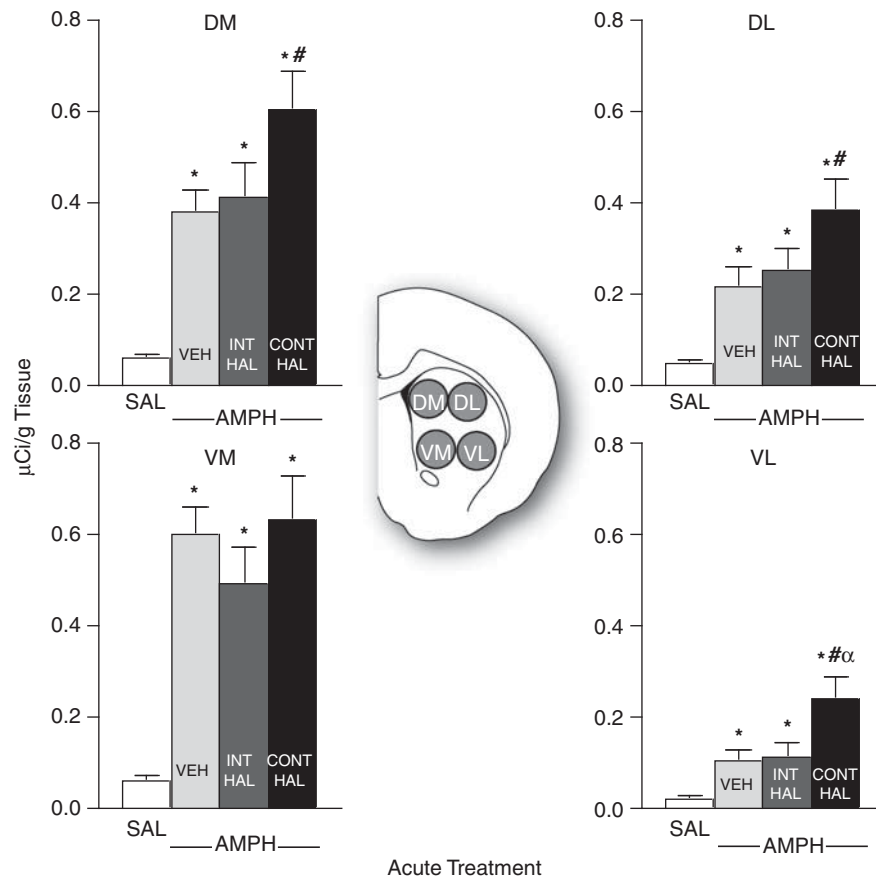


Figure 6 Continuous (CONT-HAL), but not chronic and intermittent (INT-HAL), haloperidol (HAL) treatment potentiates amphetamine-induced *c-fos* mRNA expression in the caudate-putamen. n 's = 24 in the SAL condition, and 9 per condition in remaining groups. CONT-HAL, group receiving 0.5 mg/kg/day haloperidol via minipump; INT-HAL, group receiving 0.05 mg/kg/day haloperidol via daily subcutaneous injection; SAL, combined control group receiving saline. * $p < 0.05$ compared with SAL, # $p < 0.05$ compared with VEH, $^{\alpha}p < 0.05$ compared with INT-HAL. AMPH, amphetamine; DL, dorsolateral; DM, dorsomedial; VL, ventrolateral; VM, ventromedial.

antipsychotic treatment to induce a state of behavioral supersensitivity to dopamine is associated with decreased efficacy in animal models of antipsychotic-like effects (Samaha *et al*, 2007, 2008). The current findings extend this previous work by showing that antipsychotic-induced behavioral supersensitivity to dopamine could also lead to changes in reward function.

A reward cue is a more effective conditioned reinforcer in animals that have a greater propensity to attribute predictive value to and approach localizable conditioned stimuli (Robinson and Flagel, 2009). As such, we speculated that continuous antipsychotic treatment could augment amphetamine-induced operant responding for a reward cue by increasing the propensity to attribute predictive value to and approach reward cues. However, using Pavlovian autoshaping procedures, we found that neither continuous nor intermittent antipsychotic treatment altered basal, or amphetamine-induced approach of a conditioned stimulus (sign tracking; Flagel *et al*, 2007, 2008). Thus, a history of continuous (but not intermittent) antipsychotic treatment enhances the ability of reward cues to reinforce the learning of new actions following an acute amphetamine challenge (Experiment 1), but it does not necessarily alter the ability of such cues to attract (Experiment 2). This is not the first time that a dissociation between the incentive vs predictive

properties of reward cues has been reported (Robinson and Flagel, 2009). These two properties of reward cues have overlapping but also potentially distinct neural underpinnings. For example, although the ability of stimuli that have been paired with a reward to acquire predictive properties and elicit approach (ie, elicit sign-tracking behavior) depends on the nucleus accumbens, lesions of nucleus accumbens sub-regions do not completely abolish operant responding for such stimuli (Parkinson *et al*, 1999). Thus, it is possible that a history of continuous exposure to antipsychotic medication preferentially alters the neural substrates that mediate the incentive properties of reward cues vs the substrates that mediate their predictive properties.

It has previously been shown that chronic antipsychotic treatment disrupts the acquisition of sign-tracking behavior (Danna and Elmer, 2010). Our findings extend this work by showing that previous antipsychotic treatment does not affect the expression of previously learned sign-tracking behavior. HAL treatment did however have a modest effect on the expression of another form of conditioned approach behavior; approach of the site of reward delivery (goal tracking). Following acute amphetamine, goal-tracking behavior was modestly but significantly increased in control rats relative to both antipsychotic-treated groups. This

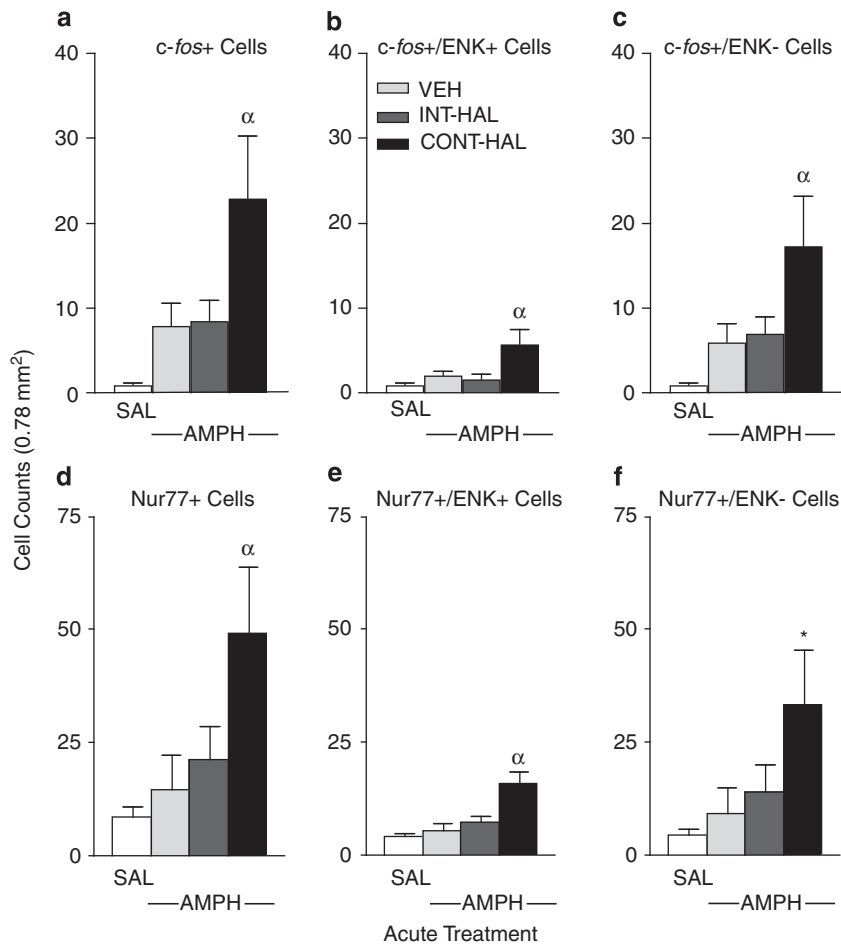


Figure 7 Continuous (CONT-HAL) but not chronic and intermittent (INT-HAL) HAL treatment potentiates amphetamine-induced *c-fos* and Nur77 mRNA expression in both preproenkephalin-containing (ENK+; (b) and (e), respectively) and preproenkephalin-lacking (ENK-; (c) and (f), respectively) cells of the caudate-putamen. *n*'s = 23–24 in the SAL condition, and 6–9 per condition in remaining groups. CONT-HAL, group receiving 0.5 mg/kg/day haloperidol via minipump; INT-HAL, group receiving 0.05 mg/kg/day haloperidol via daily subcutaneous injection; SAL, combined control group receiving saline. *Indicates $p < 0.05$ compared to SAL and VEH, α indicates $p < 0.05$ compared to SAL, VEH and INT-HAL. AMPH, amphetamine; ENK, preproenkephalin.

effect of amphetamine is consistent with previous work (Holden and Peoples, 2010). One possible explanation for the lack of effect of amphetamine in antipsychotic-treated rats is that a history of chronic antipsychotic exposure might alter the brain substrates underlying the expression of goal-tracking behavior. These substrates could include altered levels of tyrosine hydroxylase, dopamine transporter, and D2 mRNA in mesolimbic structures (Flagel *et al*, 2007).

To investigate the neural mechanisms by which continuous antipsychotic treatment might enhance amphetamine-induced potentiation of conditioned reward, we assessed amphetamine-induced gene regulation in brain circuits that mediate incentive motivation for reward cues. We found that a history of continuous, but not intermittent, antipsychotic treatment intensified amphetamine-induced *c-fos* and Nur77 mRNA expression in striatopallidal and striatonigral cells of the caudate-putamen. *C-fos* and Nur77 are immediate early genes that rapidly respond to changes in cell activity. Both genes transform acute cellular events into lasting changes in cell function by regulating the transcription of late-onset genes (Levesque and Rouillard, 2007; Robertson *et al*, 1991). Thus, our findings indicate that continuous exposure to antipsychotic medication

induces lasting changes in both the behavioral and neurobiological impact of amphetamine. It is not known whether there is a causal relationship between the ability of amphetamine to engage caudate-putamen neurons and its ability to potentiate conditioned reward. However, several lines of evidence support the idea that the actions of amphetamine within the caudate-putamen mediate, at least in part, the drug's effects on the operant pursuit of conditioned reward. First, intra-caudate infusions of amphetamine are sufficient to enhance operant responding for conditioned reward (Taylor and Robbins, 1984). Second, dopamine neurotransmission within the caudate-putamen is thought to provide an incentive motivational signal that directs attention and behavior towards reward-predicting cues (Palmiter, 2008). On the basis of these observations, we speculate that in individuals with a history of continuous antipsychotic treatment, dopaminergic stimulation (achieved here by an amphetamine challenge), could lead to altered signaling in the caudate-putamen—possibly through changes in D2 receptor number and function (Ginovart *et al*, 2009; Samaha *et al*, 2007, 2008)—and that this in turn could lead to aberrant assignment of incentive motivational value to reward cues.

It is of particular note that continuous haloperidol treatment augmented amphetamine-induced gene expression in striatopallidal cells. Amphetamine is often reported to induce immediate early gene expression in striatonigral cells, with little if any expression in striatopallidal cells—particularly when amphetamine is given in the animal's home cage as was done here (Berretta *et al*, 1992; Cenci *et al*, 1992; Johansson *et al*, 1994; Ruskin and Marshall, 1994). However, more recent work shows that when administered under conditions that promote sensitization to the behavioral effects of the drug (eg, in a novel environment), amphetamine does induce *c-fos* expression in striatopallidal cells (Badiani *et al*, 1999; Uslaner *et al*, 2001).

Does continuous HAL exposure enhance the effects of amphetamine on conditioned reward, on locomotion, and on gene regulation through pre- or post-synaptic processes? This question is an important avenue for future research. Pre-synaptic mechanisms could involve changes in dopamine release or reuptake, or both. Post-synaptic mechanisms could involve modifications in post-synaptic receptor number and/or sensitivity that could in turn lead to changes in the post-synaptic, intra-cellular response to amphetamine. Although this issue remains to be resolved, there are several reasons to suspect preferential involvement of post- rather than pre-synaptic mechanisms. First, a history of chronic antipsychotic treatment enhances the behavioral and neurobiological effects of amphetamine without a pre-synaptic increase in amphetamine-induced dopamine availability in the striatum (Compton and Johnson, 1989; Ichikawa and Meltzer, 1992; See *et al*, 1992). Second, chronic HAL treatment increases both the number and sensitivity of striatal D2/3 receptors (Ginovart *et al*, 2009; Samaha *et al*, 2007, 2008). Though these studies did not determine on which side of the synapse these D2/3 receptor changes were located, the ability of pre-synaptic autoreceptors to regulate dopamine overflow is unchanged following chronic HAL treatment (administered through the oral route; Chesi *et al*, 1995). Third, chronic HAL exposure augments the locomotor response to intra-accumbens or intra-caudate-putamen infusions of dopamine (Halperin *et al*, 1983). Finally, our current gene findings demonstrate that a history of continuous HAL treatment alters the post-synaptic, intra-cellular signaling cascades engaged by amphetamine. Taken together, these observations suggest that post-synaptic neuroadaptations likely underlie the ability of continuous antipsychotic treatment to enhance the behavioral and neurobiological response to amphetamine.

Why does a history of continuous, but not intermittent, antipsychotic treatment enhance the ability of amphetamine to potentiate the incentive motivational properties of a reward cue? We hypothesize that by continuously occupying D2 receptors, continuous antipsychotic treatment involves unrelenting perturbation of normal dopamine neurotransmission. This elicits compensatory neuroadaptations, including increased striatal D2 receptor number and function (Ginovart *et al*, 2009; Samaha *et al*, 2007, 2008), and altered amphetamine-induced gene regulation in striatopallidal and striatonigral circuits (present study). Our findings suggest that a functional consequence of these neuroadaptations could be a dopamine supersensitive state and hypersensitivity to reward following dopaminergic stimulation (achieved here through an acute amphetamine

injection). In contrast, by occupying D2 receptors for only a few hours in a 24-h period, intermittent treatment might prevent supersensitivity-related neuroadaptations and altered reward processing. Regardless of the precise underlying mechanisms, the present findings suggest that some form of regular, but intermittent, treatment strategy, which would include fixed drug-free periods, should be entertained in some schizophrenia patients vulnerable to drug abuse or addiction. The clinical reality is complex and this makes it difficult to specify exactly what intermittent human regimen should be inferred from the present data. However, a potential approach—and one that would be similar to the intermittent model used in the current study—might be regular dosing with short inter-dosing intervals. This has recently been attempted successfully in patients in a study showing that clinical efficacy can be maintained by extending antipsychotic dosing from everyday to every 2 days (Remington *et al*, 2005). The authors of this last study have now replicated these initial findings in a larger-scale, double-blind study (Remington *et al*, 2010). Indeed, intermittent antipsychotic dosing regimens are drawing increasing clinical attention (Remington, 2010).

An important consideration is that we have assessed conditioned reward function at a time when animals were withdrawn from antipsychotic treatment. Altered reward processing (as evidenced for example by compulsive drug use in schizophrenia) can certainly co-occur with antipsychotic treatment. However, the majority of patients with schizophrenia frequently cease antipsychotic treatment for various periods of time (Perkins, 1999), particularly patients who abuse drugs (Owen *et al*, 1996). Our findings are relevant to understanding changes in reward-directed behavior during such periods of withdrawal from antipsychotic drugs. Another issue that arises from the present findings is whether similar alterations in amphetamine-induced conditioned reward function and gene regulation would be seen following chronic exposure to an atypical antipsychotic, as atypical antipsychotics are now more widely used in the clinic. Work is underway in our laboratory to address this question.

In summary, our findings show (a) that exposure to a clinically representative dose and mode of antipsychotic treatment (modeled here by the continuous treatment condition) enhances the ability of amphetamine to potentiate the incentive motivational properties of a reward cue and intensifies amphetamine-mediated gene regulation in striatopallidal and striatonigral circuits, and (b) these neuroadaptations do not occur if antipsychotic treatment is given intermittently, such that disruption of dopamine neurotransmission is regular, but fixed and finite in time. This work has two major implications. First, it suggests that current antipsychotic treatment strategies, which involve chronic and continuous medication, might alter reward processing in ways that could contribute to compulsive drug seeking and taking. Second, our findings suggest that intermittent treatment strategies merit exploration in schizophrenic patients at risk for drug addiction.

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DISCLOSURE

The authors declare no conflict of interest.

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