D-Amphetamine and Antipsychotic Drug Effects on Latent Inhibition in Mice Lacking Dopamine D2 Receptors

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Drugs that induce psychosis, such as D-amphetamine (AMP), and those that alleviate it, such as antipsychotics, are suggested to exert behavioral effects via dopamine receptor D2 (D2). All antipsychotic drugs are D2 antagonists, but D2 antagonism underlies the severe and debilitating side effects of these drugs; it is therefore important to know whether D2 is necessary for their behavioral effects. Using D2-null mice (Drd2/−/−), we first investigated whether D2 is required for AMP disruption of latent inhibition (LI). LI is a process of learning to ignore irrelevant stimuli. Disruption of LI by AMP models impaired attention and abnormal salience allocation consequent to dysregulated dopamine relevant to schizophrenia. AMP disruption of LI was seen in both wild-type (WT) and Drd2/−/−. This was in contrast to AMP-induced locomotor hyperactivity, which was reduced in Drd2/−/−. AMP disruption of LI was attenuated in mice lacking dopamine receptor D1 (Drd1/−/−), suggesting that D1 may play a role in AMP disruption of LI. Further supporting this possibility, we found that D1 antagonist SKF83566 attenuated AMP disruption of LI in WT. Remarkably, both haloperidol and clozapine attenuated AMP disruption of LI in Drd2/−/−. This demonstrates that antipsychotic drugs can attenuate AMP disruption of learning to ignore irrelevant stimuli in the absence of D2 receptors. Data suggest that D2 is not essential either for AMP to disrupt or for antipsychotic drugs to reverse AMP disruption of learning to ignore irrelevant stimuli and further that D1 merits investigation in the mediation of AMP disruption of these processes.

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INTRODUCTION

Dopamine (DA) receptor D2 (D2) blockade is common to all antipsychotic drugs and correlates most closely with clinical efficacy (Howes et al, 2009; Meltzer et al, 1989; Seeman et al, 1976). Antipsychotics, however, have affinities for multiple neurotransmitter and peptide receptors, and it is still unknown whether D2 is essential for their behavioral effects. There is a therapeutic imperative to establish whether activity at D2 is obligatory for antipsychotic drug action, as D2 blockade is also associated with extrapyramidal motor symptoms and tardive dyskinesia, which limit compliance (Fleischhacker et al, 1994; Kapur et al, 2000). There is pharmacological evidence to challenge the view that action at D2 may be solely responsible for antipsychotic drug effects. Newer or ‘atypical’ antipsychotics such as clozapine, while having equivalent clinical efficacy to older ‘typical’ drugs such as haloperidol, have affinity for multiple monoamine and peptide receptors (Meltzer et al, 1989). The therapeutic efficacy of ‘atypical’ antipsychotics can occur at doses that produce much lower occupancy of D2 than typical antipsychotics, although this may be explained in terms of faster rate of dissociation from the receptor (Kapur and Seeman, 2001). For the atypical antipsychotics clozapine and quetiapine, no correlation is found between D2 occupancy and reduction in clinical scores (Yilmaz et al, 2012). There is also dissociation between the motor side effects and clinical efficacy of antipsychotic drugs, despite both being presumed to be mediated by D2 (Natesan et al, 2006). New putative therapies such as metabotropic glutamate receptor agonists intended to circumvent D2 interaction may indirectly interact with D2 (Patil et al, 2007; Seeman and Guan, 2009). The broad pharmacological profile of antipsychotics makes it difficult to study the functional role of individual receptors in specific behavioral effects using drugs and no specific D2 antagonists exist. Here, we used a null mouse approach to investigate whether D2 is necessary for antipsychotic drugs to modify salience allocation processes disrupted by D-amphetamine (AMP), tested using the learning phenomenon of latent inhibition (LI).

Symptoms of schizophrenia such as hallucinations and delusions have been suggested to reflect disruption in
processes that allocate attention or salience to features of the environment. This is considered to be consequent to dysregulated DA transmission and to be remediated by antipsychotic drugs, presumed to be via D₂ blockade, although this remains unknown (Gray et al, 1991; Kapur et al, 2005; Howes et al, 2009). LI is widely used in animal models of schizophrenia as an index of the mechanism through which salience or associability (attention to and readiness to form associations) is conferred on stimuli on the basis of how much experience the animal has had of them (Lubow, 2005; Weiner and Arad, 2009). LI is demonstrated experimentally as reduced learning of a conditioned stimulus (CS)–unconditioned stimulus (US) association in a group pre-exposed to that stimulus without reinforcement (pre-exposed, PE) compared with a group without such pre-exposure (non-pre-exposed, NPE). Many, although not all, studies show that patients with schizophrenia display abnormalities in LI, which may depend on the stage of illness (Weiner and Arad, 2009). In rats and humans, antipsychotic drugs such as haloperidol and clozapine potentiate low LI (Moser et al, 2000; Weiner, 2003; Weiner and Arad, 2009). On the other hand, psychotomimetic drugs that induce psychosis, such as AMP that inter alia increase DA release, disrupt LI. Antipsychotic drugs attenuate this disruption in humans, rats, and mice (Chang et al, 2007; Gray et al, 1992; Moser et al, 2000; Weiner, 2003). Experiments using D₂-null mice (Drd₂−/−) mice suggest that D₂ is essential for the behavioral and neural effects of AMP, such as methamphetamine hyperactivity, disruption of prepulse inhibition of the acoustic startle response, and DA release (Boulay et al, 1999; Kelly et al, 1998; Schmitz et al, 2001). It is unknown whether D₂ is essential for disruption of LI by AMP. Direct stimulation of D₂ by the agonist apomorphine has been shown not to disrupt LI (Lacroix et al, 2000), suggesting that D₂ activation does not play a role in LI disruption, but later reports have shown that apomorphine can disrupt LI (Melo et al, 2009; Shao et al, 2010). This study aimed first to investigate whether D₂ is essential for AMP-induced disruption of LI. The second aim was to investigate whether D₂ is required for antipsychotic drugs to attenuate AMPH disruption of LI, which we found to be intact in Drd₂−/− mice. The third aim was to further determine the mechanism by which AMP disrupts LI and to investigate whether D₁ is important for AMP disruption of LI.

MATERIALS AND METHODS

Animals

The original F₂ hybrid strains were generated as reported previously (Drago et al, 1994; Kelly et al, 1997). Congenic D₁ and D₂ lines were established by repeatedly backcrossing heterozygous mutants to wild-type (WT) C57BL/6 for at least 14 generations; homozygous DA receptor D₁ (Drd₁−/−) and Drd₂−/− mice and WT (Drd₁+/+ and Drd₂+/+ mice) littermates were then bred by heterozygous intermatings of congenic heterozygote mutants (Waddington et al, 2005). Male and female (−/− and +/+ ) littermates were used at 10–20 weeks of age. In experiments involving SKF83566, C57BL/6 mice were used, which were purchased from Charles River UK (Kent, UK) and Charles River (Wilmington, MA). Mice were housed 1–4 per cage under a 12 h light:12 h dark cycle (lights on at 0700 hours) and constant temperature (20 ± 2 °C) and humidity (40–60%), with food available ad libitum. Mice were subjected to daily water restriction periods of 23 h throughout LI experiments, with 1 h free access to water in their home cages after each experimental session. All experiments were carried out in accordance with local and national regulations on animal experimentation, and project license authority under the Animals (Scientific Procedures) Act, UK 1986; UK home Office Project licenses No: 40/2883 and its renewal as 40/3501.

Genotyping

This was performed by PCR using genomic DNA extracted from ear biopsies, as described previously (Bay-Richter et al, 2009).

Latent Inhibition

Experiments were carried out in six identical conditioning chambers and the LI protocol was the same as that described previously in detail (Bay-Richter et al, 2009). For further details of the apparatus used, see Supplementary Information. Briefly it consisted of the following:

Water restriction (Days 1–7): Mice were placed on 23-h water restriction 7 days before and throughout the experiment.

Pretraining (Days 8–13): Mice were placed in chambers for 15 min and the number of licks was recorded.

Pre-exposure (Day 14): Mice were placed in chambers with no water present. They were given 60 presentations of a 5-s 85-dB tone with an interstimulus interval of 15 s (PE group); NPE control mice were placed in the chambers for the same amount of time but received no tone pre-exposures.

Conditioning (Day 15): Mice were placed in chambers with no water present. After 2 min, two tone-footshock pairings were presented. Each tone was of 5-s duration and followed by a 1-s 0.38-mA footshock and an intertrial interval of 2.5 min; mice remained in the chamber for 2.5 min following the second shock presentation.

Re-baseline lick training (Days 16 and 17): Mice were placed in chambers for 15 min and given free access to the water sipper to re-establish licking. Mice that did not complete >300 licks continuously were excluded from the experiment and did not continue to the test day (two mice in experiment 1 and two in experiment 2).

Test (Day 18): Mice were placed in chambers with free access to the water sipper. The number of licks was recorded and time taken to complete licks 80–90 (A) and 90–100 (B) recorded. After completion of 90 licks, the tone was presented until the mouse reached lick 100 or 600 s had elapsed. A suppression ratio (SR) was calculated according to the formula A/(A + B) yielding a scale of 0 to 0.5. As logarithmic transformation did not normalize data in all experiments, SR was therefore considered the most appropriate measure.
Spontaneous Locomotor Activity

This was recorded for 30 min using videotracking of an open field as described previously (Bay-Richter et al., 2009). For further details of the apparatus used and the procedure, see Supplementary Information.

Experimental Design and Statistics

Statistics were performed using SPSS (Versions 16 (2007) and 18 (2009); SPSS Chicago, IL). For LI experiments, analysis of variance (ANOVA) was used. Post hoc tests comprised planned T-tests with Bonferroni correction for a slippage for genotype, drug treatment group, and NPE vs PE comparisons as appropriate. Data were collapsed across sex as there was no significant effect of sex and no interactions with treatment, exposure, or genotype in any experiment. Experiments in Drd1−/− mice were explicitly conducted in females because of baseline sex differences in LI previously identified by us; male and female Drd1−/− mice and female Drd2−/− mice show comparable LI, but male Drd1−/− mice do not show LI; hence, AMP disruption could not be evaluated in the present conditions in males (Bay-Richter et al., 2009). Locomotor activity experiments used split-plot ANOVA with genotype and drug treatment group as between-group factors and 5-min time bin as used split-plot ANOVA with genotype and drug treatment group as between-group factors and 5-min time bin as a repeated-measures factor. Post hoc comparisons were performed as described for LI experiments. The n numbers per experiment were: 30 (9F, 21M) Drd1−/+ and 19 (9F, 10M) Drd2−/− (Figure 1a); 18 (7F, 11M) Drd1−/+ and 12 (6M, 6F) Drd2−/− (Figure 1b); 16 Drd2+/+ (55F, 61M) and 127 (62F, 65M) Drd2−/− (Figure 2); 72F Drd1−/+ and 57F Drd2−/− (Figure 3a); 46 (23F, 23M) Drd2−/+ and 34 (17F, 17M) Drd1−/− (Figure 3b); and 138 C57BL/6 (70F, 68M) (Figure 4). In all LI experiments, groups did not differ in their times to complete licks 80–90 (time A) nor were there any effects of sex or interaction between sex and other variables (all F values <1). Owing to the large number of experimental groups required to allow simultaneous evaluation of clozapine and haloperidol, and requirement for age, sex, and littermate matching, the experiment in Figure 2 was carried out in four matched cohort replications; there were neither significant effects of cohort on SR nor significant interactions with exposure, drug treatment, or genotype (all F values <1.5).

Drugs and Administration

AMP sulfate (Sigma-Aldrich, Dorset, UK) was dissolved in sterile 0.9% (w/v) saline and a dose of 2.5 mg/kg was used in all experiments. SKF83566 (Tocris, Bristol, UK) was dissolved in 0.9% NaCl mixed with a few drops of tartaric acid and buffered to pH 6.5 with NaOH. Doses of 0.01 and 0.1 mg/kg were used. Haloperidol and clozapine (Sigma-Aldrich) were dissolved in 25 µl glacial acetic acid and buffered to pH 6.5 using 0.1 mM NaOH before final dilution in sterile 0.9% saline to appropriate concentrations (0.1 mg/kg for haloperidol; 2.5 mg/kg for clozapine); controls received vehicle to the same injection volumes (10 ml/kg). Doses of AMP (2.5 mg/kg, intraperitoneally) and haloperidol (0.1 mg/kg, intraperitoneally) were based on previously established dosage for LI in mice (Chang et al., 2007; Meyer et al., 2004). The dose of clozapine used was lower than published reports (Lipina et al., 2005), as significant sedation occurred in our hands at published effective doses. For mice receiving AMP in combination with either haloperidol, clozapine, or SKF83566, the drug was administered 5 min before AMP, with control mice receiving a matched number of vehicle injections. All injections were given intraperitoneal 30 min before both pre-exposure and conditioning sessions (LI) or both habituation and testing (locomotion). The two injection regimen is based on a number of studies, which show that two injections of AMPH, one before pre-exposure and one before conditioning, are required to disrupt LI (Weiner et al., 1988; although see Young et al., 2005). The same regimen was then maintained to enable direct comparison at the same doses in locomotor activity experiments.
First experiments investigated effects of AMP on LI and locomotor activity in Drd2+/+ and Drd2−/− mice. Subsequently, effects of the typical D2 antipsychotic haloperidol and the atypical antipsychotic clozapine on AMP disruption of LI were investigated. To evaluate a potential role for D1 in AMP disruption of LI, a series of experiments investigated AMP effects on LI and locomotor activity in Drd1+/+ and Drd1−/− and of the D1 antagonist SKF83566 on AMP disruption of LI.

RESULTS

AMP Reduction of LI in Drd2−/− Mice

AMP disrupted LI in Drd2−/− mice (Figure 1a). There was an effect of pre-exposure (F(1,41) = 10.89, P < 0.01), treatment (F(1,41) = 10.78, P < 0.01), and a pre-exposure × treatment interaction (F(1,41) = 10.20, P < 0.01). There was a significant difference between AMP and vehicle in the PE condition (T8 = 2.749, P < 0.05) and NPE vs PE (T8 = 2.741, P < 0.05) in Drd2−/− mice. There was no significant effect of genotype nor interaction between genotype and other factors. In Drd2+/+ mice, there was no significant difference between AMP and vehicle in the PE condition; however, NPE was significantly different from PE condition, indicating LI in the vehicle (T24 = 2.7, P < 0.05) but not the AMP group (T21 = 0.4).

AMP Locomotor Hyperactivity in Drd2−/− Mice

AMP locomotor hyperactivity was seen in WT mice, but was blunted in Drd2−/− mice using the same dose and regimen of AMP as described for LI experiments (Figure 1b). There was a significant effect of genotype (F(1,22) = 47.23, P < 0.001), drug treatment (F(1,22) = 33.96, P < 0.001), and a genotype × drug treatment interaction (F(1,22) = 8.42, P < 0.01). There was a significant effect of time bin (F(5,110) = 13.07, P < 0.001) and an interaction...
between time bin and treatment ($F_{(5,110)} = 7.46, P < 0.001$) but not genotype.

Antipsychotic Drug Effects on LI Reduction by AMP in $D_{2}−/−$ Mice

AMP disruption of LI in $D_{2}−/−$ mice was attenuated by both drugs tested, haloperidol and clozapine (Figure 2b), demonstrating that clozapine and haloperidol do not require the presence of $D_{2}$ to attenuate AMP disruption of LI. There were significant effects of pre-exposure ($F_{(5, 223)} = 38.10, P < 0.001$), treatment ($F_{(4, 223)} = 5.01, P < 0.001$), and an exposure $\times$ treatment interaction ($F_{(4, 223)} = 2.92, P < 0.05$). There was no significant effect of genotype nor interaction between genotype and other factors ($F'$s $< 1$). In WT PE groups, there was a significant difference between AMP vs AMP + haloperidol ($T_{21} = 2.68, P < 0.05$) and AMP vs AMP + clozapine ($T_{19} = −3.43, P < 0.05$), and these differences were also seen in $D_{2}$ mice; AMP vs AMP + haloperidol ($T_{21} = 2.60, P = 0.01$) and AMP vs AMP + clozapine ($T_{19} = −3.66, P < 0.05$) (Figure 2b). Vehicle vs haloperidol in PE WT group ($T_{19} = 2.2$) was not significant. NPE was significantly different from PE group in vehicle- ($T_{83} = −3.67, P < 0.005$), haloperidol- ($T_{18} = −2.86, P < 0.05$), AMP + haloperidol- ($T_{42} = −2.79, P < 0.05$), and AMP + clozapine-treated groups ($T_{49} = −3.46, P < 0.005$) but not in AMP- ($T_{41} = −0.5$) treated groups.

**AMP Reduction of LI in $D_{1}−/−$ Mice**

We found that AMP disruption of LI is reduced in $D_{1}−/−$ mice compared with WT (Figure 3a). There was a significant effect of drug treatment ($F_{(4,121)} = 4.23, P < 0.05$), exposure ($F_{(1,121)} = 21.00, P < 0.001$), and genotype ($F_{(4,121)} = 4.05, P < 0.05$); the interaction between drug treatment, exposure, and genotype was not significant. There was a significant difference between AMP-WT and AMP-$D_{1}−/−$ in PE groups ($T_{35} = 2.24, P < 0.05$), suggesting that AMP effect is moderated in $D_{1}−/−$ mice. There was a significant difference between NPE and PE groups in vehicle- ($T_{31} = 2.97, P < 0.05$) and AMP-treated $D_{1}−/−$ ($T_{28} = −2.5, P < 0.05$), suggesting LI in these groups but not in AMP-treated $D_{1}+/+$ or vehicle-treated $D_{1}−/−$ groups.

**AMP Locomotor Hyperactivity in $D_{1}−/−$ Mice**

AMP locomotor hyperactivity was maintained in both WT and $D_{1}−/−$ mice (Figure 3b). There was a significant effect of drug treatment ($F_{(1, 76)} = 55.50, P < 0.001$) but no effect of genotype. There was also a significant effect of time bin ($F_{(5,380)} = 13.4, P < 0.001$) and an interaction between time bin and drug treatment ($F_{(5,380)} = 9.44, P < 0.001$).

**DISCUSSION**

These data show first that AMP disrupts LI in mice lacking $D_{2}$ receptors, demonstrating that AMP can influence behavior in the absence of $D_{2}$. This effect of AMP may be specific to processes involved in learning to ignore irrelevant stimuli, as locomotor hyperactivity induced by the same regimen of AMP was blunted in mice lacking $D_{2}$ receptors. Second, it was found that the $D_{2}$ receptor is not essential for antipsychotic drugs haloperidol and clozapine to attenuate LI disruption induced by AMP. This has important therapeutic implications as it suggests that it is possible to modulate impaired ability to ignore irrelevant stimuli induced by a hyperdopaminergic state without interaction with $D_{2}$. Third, AMP disruption of LI was blunted in the absence of $D_{1}$, suggesting that its effects to disrupt LI may require $D_{1}$. Hyperactivity induced by AMP at the same dose was not affected in the absence of $D_{1}$, suggesting dissociation between the effects of AMP on LI and on locomotor activity. AMP disruption of LI was attenuated by the $D_{1}$ antagonist SKF83566, suggesting further that $D_{1}$ merits further investigation in the mediation of AMP disruption of LI.
important for the understanding of both its abuse potential and psychotomimetic effects. AMP produces a wide variety of behavioral effects, including psychosis, locomotor hyperactivity, stereotypy, self-administration, and disruption of sensori motor gating in a variety of species (Angrist et al, 1980; Cole, 1978; Hutchinson and Swift, 1999; Mansbach et al, 1988; Marriott, 1968; Ralph-Williams et al, 2002). A consistent feature of these behavioral effects of AMP is that they are prevented by antipsychotic drugs that block D2, leading to their application as animal models relevant to schizophrenia. Studies in null mice also suggest that D2 plays a crucial role in mediating AMP behavioral effects (Kelly et al, 2008; Ralph et al, 1999). We show that D2 is not essential for AMP to exert its disruptive effect on LI, yet hyperactivity induced by the same dose and regimen of AMP is reduced in the absence of D2. Reduced locomotor activity in Drd2−/− mice replicates previous findings using mixed background strains (Kelly et al, 1998). Data suggest that acute stimulatory effects of AMP using this dose and treatment regimen may require D2. Statistical interaction between genotype and treatment suggests that this is distinguishable from baseline reduction in activity in Drd2−/− mice. This finding is consistent with evidence showing reduced acute stimulatory effects of methamphetamine in Drd2−/− mice (Kelly et al, 2008).

Preserved AMP disruption of LI in Drd2−/− is consistent with pharmacological studies indicating no effect of direct D2 agonist apomorphine on LI in rats (eg Broersen et al, 1999, Lacroix et al, 2000). It is notable that in some cases where LI has been disrupted by apomorphine, it is NPE disruption that produced the loss of LI, making effects on LI per se difficult to interpret (eg Melo et al, 2009, Shao et al, 2010).

As AMP acts via presynaptic mechanisms to increase the release of a number of neurotransmitters, including noradrenaline and serotonin, as well as DA, it is possible that its primary DAergic mechanism is devoted to a different mechanism in compensation for developmental absence of D2 in Drd2−/− mice. There is electrophysiological evidence that firing of DA neurons induced by AMP in the ventral tegmental area switches to a noradrenergic mechanism in the pharmacological absence of D2, that is, in the presence of a D3 antagonist (Cohen and Lipinski, 1986; Shi et al, 2000). Most antipsychotic drugs have affinity at the noradrenaline x1 receptor (Cohen and Lipinski, 1986) as well as D2 and other DA receptor subtypes, making action through noradrenergic modulation one possible mechanism.

Remarkably, we found that AMP disruption of LI is attenuated by haloperidol and clozapine and does not require the presence of D2. This surprising outcome suggests that attenuation of AMP-induced abnormal salience allocation by antipsychotic drugs can occur in the absence of D2. There are a number of possible candidate biological mechanisms. Drugs such as 5-HT2A receptor antagonists have been shown to reverse AMP disruption of LI in rats (Weiner and Arad, 2009); 5-HT2A antagonism is a putative feature of ‘atypical’ antipsychotics and polymorphisms in 5-HT2A genes have been reported in schizophrenia (Maier et al, 2008; Miyamoto et al, 2005). It is worth noting that 5-HT2A receptors modulate activated but not basal mesolimbic DA function (Schmidt and Fadayel, 1996; Schmidt et al, 1995). However, while clozapine has significant affinity for 5-HT2A receptors, haloperidol does not, particularly at the dose used in this study. Interaction with the cholinergic system is also a possible mechanism. Muscarinic receptor M4 agonists have been suggested to have antipsychotic potential (Shekhar et al, 2008; Dencker et al, 2011) and have been shown to reverse AMP disruption of LI (Barak and Weiner, 2011). Abnormal glutamatergic neurotransmission, particularly at N-methyl-d-aspartate (NMDA)-type glutamate receptors, has also been implicated in schizophrenia (Olney et al, 1999). NMDA receptors are regulated by the amino-acid glycine and drugs that interact with transporters for glycine (GlyT1) may have antipsychotic potential (Javitt, 2012). The GlyT1 inhibitor SSR103800 has furthermore been shown to reduce AMP disruption of LI (Black et al, 2009). The mechanism of the D2-independent action of these drugs has yet to be identified; however, AMP-disrupted LI in Drd2−/− mice may be of use as a novel model system to identify D2-independent effects of these drugs. Their identification could suggest neural strategies to remediate hyperdopaminergia-related disruption in a behaviorally specific manner without interaction with D2.

One interpretation of these findings is that AMP effects in Drd2−/− mice differ from AMP effects in APD-treated mice, broadly suggesting dissociation between pharmacological and genetic manipulations of D2 in the presence of AMP. We have shown previously that in the absence of AMP both potentiate low levels of LI and effects on locomotor activity are consistent, indicating that this dissociation is not a general phenomenon or even specific for LI (Moser et al, 2000; Bay-Richter et al, 2009). One possible explanation is that if AMP interacts with D2 to disrupt LI as later experiments suggest, then the D1 antagonist action of APD would reverse AMP disruption of LI by pharmacological antagonism. This would not be seen in Drd2−/− mice. One possible dissociation would be found. It is also possible that Drd1−/− mice differ in metabolism or neural activity consequent to developmental absence of Drd1 and this becomes unmasked in the presence of AMP. We cannot determine from this study whether that is the case or not, but this possibility does not alter the conclusion from the study that D2 is not essential for AMP disruption of LI.

Our findings indicate an attenuation of AMP disruption of LI in female Drd1−/− mice. This is consistent with the observation that the diverse pharmacological actions of psychotomimetic drugs, such as AMP, LSD, and PCP, include effects on D1-mediated function (Watts et al, 1995). Supporting a role for Drd1−/−, we showed that SKF83566 attenuated the effects of AMP on LI. In contrast, we show that AMP effects on locomotor activity are not reduced in Drd1−/− mice. AMP hyperlocomotor activity has previously been shown to be blunted in Drd1−/− mice on mixed background strains following acute and repeated administration (Xu et al, 2000; Crawford et al, 1997). We cannot determine whether this difference is due to background strain difference, dose, or dosing regimen of AMP. However, intact locomotor stimulation by AMP in Drd1−/− mice is clearly dissociable from both attenuated locomotor stimulation seen in their Drd2−/− counterparts and attenuated disruption of LI in Drd1−/− female mice.
using the same dose and regimen of AMP. There is a possibility that this finding is sex-specific as male Drd1−/− could not be evaluated in these conditions, as they do not show robust LI. Recently, it has been shown in male rat studies that the D1 antagonist SCH23390 can reverse AMP effects on LI (Nelson et al, 2012), suggesting that these effects may not be specific to mice or females. Previous rat studies have shown that LI disruption by nicotine is also reversed by D1 antagonists. Nicotine (like AMP) is thought to disrupt LI via mesolimbic DA release and is reversed by antipsychotic drugs (Joseph et al, 1993; Moran et al, 1996; Young et al, 2005). Taken together with the present data, we suggest that the role of D1 in drug effects to disrupt and potentially improve salience allocation merits further investigation. It has been suggested that D1 antagonism may be important for the behavioral effects of antipsychotics and may be secondary to D2 antagonism (Josselyn et al, 1997; Miller, 1990, 2009). A potential role for D1 in AMP disruption of LI is consistent with studies in rats implicating D1 in overshadowing, a related measure of salience allocation, other behavioral effects of AMP in other species, as well as a more general role for midbrain D1 in attentional accuracy (Liu et al, 2010, 2011; O’Tuathaigh and Moran, 2002; Zelikowsky and Fanselow, 2010).

Translation of the outcome of experiments using animal model systems to human psychosis and its treatment must include the caveat of species and environmental differences from the human condition. In these studies, mice were water restricted; there is a possibility that this may be important for demonstration of the effects we have shown. In rats, it has been shown that drinking in water-restricted rats can increase midbrain DA release (Young et al, 1992). It is possible that cross-sensitization may have occurred between effects of water restriction and AMP effects on DA release in key brain regions such as the nucleus accumbens. Sensitization of the locomotor response induced by AMP has been shown specifically to involve D1 (Veznia, 1996); further experiments would be required to test this possibility.

CONCLUSION

We have demonstrated the principle that AMP, clozapine, and haloperidol can exert behavioral effects in the absence of D2 in mice. These D2-independent effects may be behaviorally specific to the process of learning to ignore irrelevant stimuli and allocating salience appropriately as measured in LI. D1 merits further investigation in the mediation of these effects. Identification of this D2-independent mechanism may constitute a novel behavior-driven approach to identifying existing and candidate antipsychotic drug actions that are behaviorally specific and independent of D2.

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DISCLOSURE

The authors declare no conflict of interest.

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