

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

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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 α 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 $Drd_1^{-/-}$ mice were explicitly conducted in females because of baseline sex differences in LI previously identified by us; male and female $Drd_2^{-/-}$ mice and female $Drd_1^{-/-}$ mice show comparable LI, but male $Drd_1^{-/-}$ 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 repeated-measures factor. *Post hoc* comparisons were performed as described for LI experiments. The *n* numbers per experiment were: 30 (9F, 21M) $Drd_2^{+/+}$ and 19 (9F, 10M) $Drd_2^{-/-}$ (Figure 1a); 18 (7F, 11M) $Drd_2^{+/+}$ and 12 (6M, 6F) $Drd_2^{-/-}$ (Figure 1b); 16 $Drd_2^{+/+}$ (55F, 61M) and 127 (62F, 65M) $Drd_2^{-/-}$ (Figure 2); 72F $Drd_1^{+/+}$ and 57F $Drd_1^{-/-}$ (Figure 3a); 46 (23F, 23M) $Drd_1^{+/+}$ and 34 (17F, 17M) $Drd_1^{-/-}$ (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 μ l 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

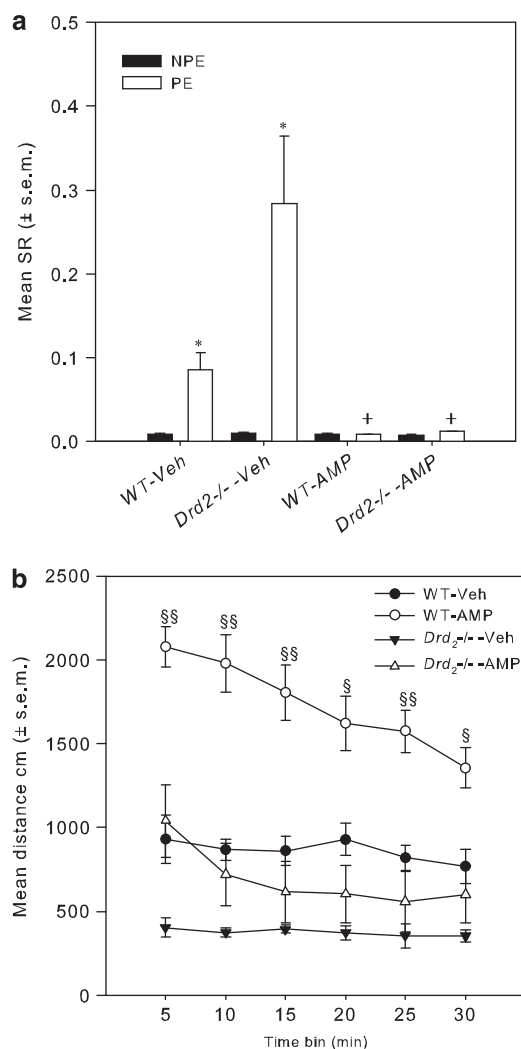


Figure 1 D-Amphetamine (AMP) disruption of latent inhibition (LI) is similar in wild-type (WT) and $Drd_2^{-/-}$ (a). Vehicle-treated WT (WT-Veh) and vehicle-treated $Drd_2^{-/-}$ ($Drd_2^{-/-}$ -Veh) mice show LI (higher suppression ratio (SR) in pre-exposed (PE) vs non-pre-exposed (NPE)), while D-amphetamine-treated WT (WT-AMP) and D-amphetamine-treated $Drd_2^{-/-}$ ($Drd_2^{-/-}$ -AMP) mice do not. AMP locomotor hyperactivity seen in WT is blunted in $Drd_2^{-/-}$ mice (b). * $P < 0.05$ NPE vs PE, same genotype and drug; + $P < 0.05$ vs vehicle same genotype for PE; and § $P < 0.05$, §§ $P < 0.001$ vs vehicle same genotype and time bin. $Drd_2^{-/-}$, D_2 -null mice.

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.

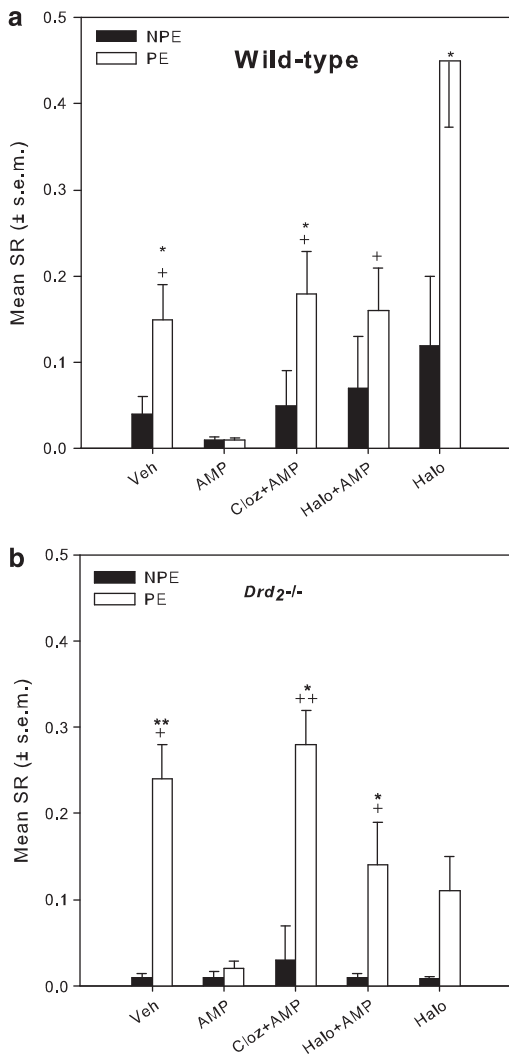


Figure 2 Haloperidol (Hal) and clozapine (Cloz) attenuate disruption of latent inhibition (LI) by D-amphetamine (AMP) in both wild-type (WT; a) and D_2 $-/-$ mice (Drd_2 $-/-$; b); * $P < 0.05$, ** $P < 0.01$, non-pre-exposed (NPE) vs pre-exposed (PE) same genotype and drug; + $P < 0.05$, ++ $P < 0.01$ vs AMP-PE same genotype. Drd_2 $-/-$, D_2 -null mice.

First experiments investigated effects of AMP on LI and locomotor activity in Drd_2 $+/+$ and Drd_2 $-/-$. Subsequently, effects of the typical D_2 antipsychotic haloperidol and the atypical antipsychotic clozapine on AMP disruption of LI were investigated. To evaluate a potential role for D_1 in AMP disruption of LI, a series of experiments investigated AMP effects on LI and locomotor activity in Drd_1 $+/+$ and Drd_1 $-/-$ and of the D_1 antagonist SKF83566 on AMP disruption of LI.

RESULTS

AMP Reduction of LI in Drd_2 $-/-$ Mice

AMP disrupted LI in Drd_2 $-/-$ 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 \times treatment interaction ($F_{(1,41)} = 10.20$, $P < 0.01$). There was a significant difference between AMP and vehicle in the PE

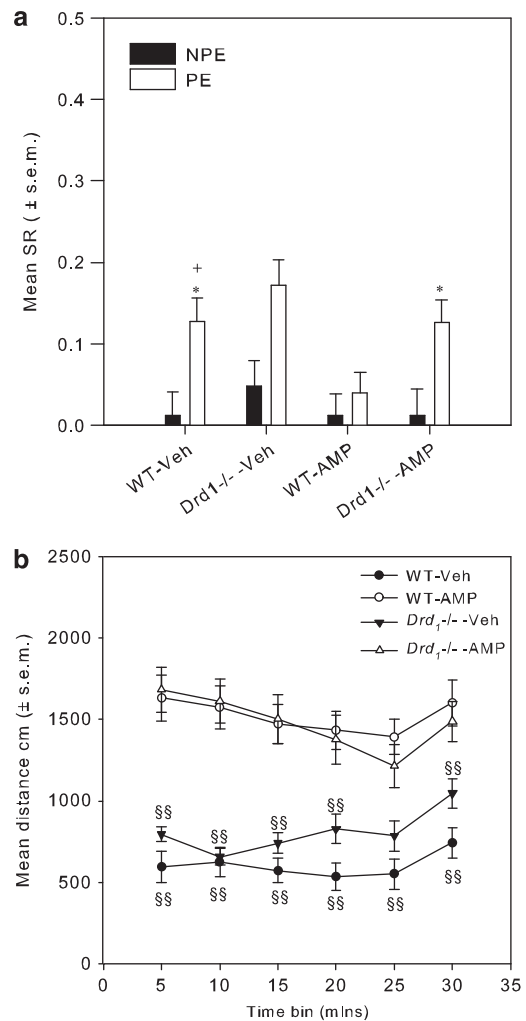


Figure 3 D-Amphetamine (AMP) disruption of latent inhibition (LI) (60 pre-exposed (PE)) is attenuated in Drd_1 $-/-$ mice (a), but locomotor hyperactivity is not (b). * $P < 0.05$ non-pre-exposed (NPE) vs PE same genotype and drug; + $P < 0.05$ vs AMP-PE same genotype; ^ $P < 0.05$ vs WT AMP-PE; §§ $P < 0.001$ vs vehicle same genotype and time bin. Drd_1 $-/-$, D_1 -null mice.

condition ($T_8 = 2.749$, $P < 0.05$) and NPE vs PE ($T_8 = 2.741$, $P < 0.05$) in Drd_2 $-/-$ mice. There was no significant effect of genotype nor interaction between genotype and other factors. In Drd_2 $+/+$ 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 ($T_{24} = -2.7$, $P < 0.05$) but not the AMP group ($T_{21} = 0.4$).

AMP Locomotor Hyperactivity in Drd_2 $-/-$ Mice

AMP locomotor hyperactivity was seen in WT mice, but was blunted in Drd_2 $-/-$ 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 \times 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

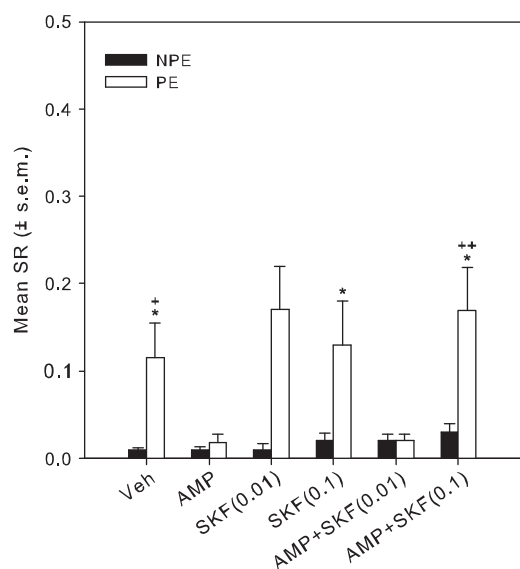


Figure 4 D-Amphetamine (AMP) reduction of latent inhibition (LI) in wild-type (WT) mice is attenuated by pretreatment with SKF83566 at 0.1 mg/kg but not 0.01 mg/kg. * $P < 0.05$ non-pre-exposed (NPE) vs pre-exposed (PE) same drug; + $P < 0.05$, ++ $P < 0.01$ vs AMP-PE.

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 Drd_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 ($F_{(1, 223)} = 38.10$, $P < 0.001$), 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 Drd_2 -/- mice; AMP vs AMP + haloperidol ($T_{21} = -2.60$, $P = 0.01$) and AMP vs AMP + clozapine ($T_{19} = -3.66$, $P < 0.005$) (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 Drd_1 -/- Mice

We found that AMP disruption of LI is reduced in Drd_1 -/- mice compared with WT (Figure 3a). There was a significant effect of drug treatment ($F_{(1,121)} = 4.23$, $P < 0.05$), exposure ($F_{(1,121)} = 21.00$, $P < 0.001$), and genotype ($F_{(1,121)} = 4.05$, $P < 0.05$); the interaction between drug

treatment, exposure, and genotype was not significant. There was a significant difference between AMP-WT and AMP- Drd_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 Drd_1 -/- ($T_{28} = -2.5$, $P < 0.05$), suggesting LI in these groups but not in AMP-treated Drd_1 +/+ or vehicle-treated Drd_1 -/- groups.

AMP Locomotor Hyperactivity in Drd_1 -/- Mice

AMP locomotor hyperactivity was maintained in both WT and Drd_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 ($F_{(5,380)} = 9.44$, $P < 0.001$).

D_1 Antagonist SKF83566 Effect on AMP Reduction of LI in C57/BL/6 Mice

Figure 4 shows that pretreatment with SKF83566 at 0.1 mg/kg but not 0.01 mg/kg prevented AMP disruption of LI. These data suggest that D_1 antagonism can be sufficient to prevent AMP disruption of LI. There was a significant effect of exposure ($F_{(1, 116)} = 21.38$, $P < 0.001$) and drug treatment ($F_{(5, 116)} = 2.65$, $P < 0.05$) and a drug treatment \times exposure interaction ($F_{(5, 116)} = 2.33$, $P < 0.05$). There was a significant difference between PE groups AMP + SKF 0.1mg/kg and AMP ($T_{21} = 2.6$, $P < 0.05$). There was a significant difference between NPE and PE conditions in vehicle ($T_{21} = -2.37$, $P < 0.05$), AMP + SKF 0.1 mg/kg ($T_{21} = -2.4$, $P < 0.05$), and SKF 0.01 mg/kg ($T_{22} = -2.7$, $P < 0.01$) groups but not in SKF 0.1 mg/kg ($T_{21} = 1.9$) or AMPH-treated group (T 's < 0.5).

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.

Understanding the biological basis of the cognitive effects of AMP and how it affects the selection of information are

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 sensorimotor gating in a variety of species (Angrist *et al*, 1980; Cole, 1978; Hutchison 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 devolved 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 D₂ antagonist (Cohen and Lipinski, 1986; Shi *et al*, 2000). Most antipsychotic drugs have affinity at the noradrenaline α 1 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-HT_{2A} receptor antagonists have been shown to reverse AMP disruption of LI in rats (Weiner and Arad, 2009); 5-HT_{2A} antagonism is a putative feature of 'atypical' antipsychotics and polymorphisms in 5-HT_{2A} genes have been reported in schizophrenia (Maier *et al*, 2008; Miyamoto *et al*, 2005). It is worth noting that 5-HT_{2A} receptors modulate activated but not basal mesolimbic DA function (Schmidt and Fadayeel, 1996;

Schmidt *et al*, 1995). However, while clozapine has significant affinity for 5-HT_{2A} 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 GlyT1inhibitor 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 D₁ 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*−/−; hence, a dissociation would be found. It is also possible that *Drd2*−/− mice differ in metabolism or neural activity consequent to developmental absence of D₂ 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 (Vezina, 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 identify 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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