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ORIGINAL INVESTIGATION

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Attenuation of *d*-amphetamine-induced disruption of conditional discrimination performance by α-flupenthixol

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Abstract *Rationale:* Previous evidence suggests that manipulation of forebrain dopamine (DA) systems may impair the use of conditional information to inform goal-directed performance, and this may be related to impairments in the ability to use task-setting cues in schizophrenia. *Objective:* To investigate, using the indirect DA agonist *d*-amphetamine and the D_1/D_2 receptor antagonist α-flupenthixol, the influence of DAergic manipulation on discrimination performance that requires the use of conditional information to inform goal-directed performance. *Methods:* Both instrumental and Pavlovian conditional discriminations were employed in which rats learned to respond appropriately according to the presence of auditory conditional stimuli, and results from these experiments were contrasted with a control Pavlovian-instrumental transfer task. *Results:* Experiment 1 showed a disruption of instrumental conditional discrimination performance by *d*-amphetamine at 1.5 mg/kg and attenuation of correct responding following 1.0 mg/kg. Disruption with both doses was observed in experiment 2 using a conditional discrimination based on Pavlovian, conditioned responding. Results from a control Pavlovian-instrumental transfer task (experiment 3) revealed that *d*-amphetamine (0.5, 1.0 and 1.5 mg/kg) did not have any detrimental effect on subjects' basic sensory, motor or motivational processes. Experiment 4 showed that *d*-amphetamine disruption of instrumental conditional discrimination was attenuated by pre-treatment with the D_1/D_2 receptor antagonist α-flupenthixol. *Conclusion:* These results demonstrate that tasks dependent on conditional relationships are highly sensitive to manipulation of DAergic systems.

Keywords Schizophrenia · Context · Conditional discrimination · Dopamine · *d*-Amphetamine · α-Flupenthixol

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Introduction

A number of studies have suggested that changes in dopaminergic (DAergic) function may lead to selective deficits in performance of conditional tasks—that is, tasks in which performance of a particular response will be rewarded if made in the presence of specific conditional cues and will not be rewarded if made at other times. For example, Robbins and Sahakian (1983) reported that systemic treatment with D-amphetamine at doses in the range 0.4 mg/kg to 1.6 mg/kg disrupted performance of a biconditional discrimination in which the temporal frequency of lights or tones (slow versus quick intermittent presentations) provided the cues directing rats to respond on a left or right lever for reward (e.g. slow: press left; quick: press right). Similarly, Sarter (1990) reported that D-amphetamine (0.3–1.0 mg/kg) reduced the number of correct responses in a conditional visual discrimination task, whereas previous reports indicated that D-amphetamine produced disruptions of performance in two-component multiple schedule tasks, in which reward for responding in the presence of stimuli depended on the presence of conditional cues, in both pigeons (Moerschbaecher et al. 1979) and monkeys (Moerschbaecher and Thompson 1980).

Whilst the reports above suggest a consistent pattern of findings, there has been little systematic analysis of this effect. However, recent theoretical and empirical findings suggest these results may warrant detailed examination. Converging evidence has suggested that a core cognitive deficit in schizophrenia is a reduced capacity to make use of task-setting cues (i.e. cues that dictate how to interpret

ambiguous stimuli within the current task) to guide goal-directed behaviour (Cohen and Servan-Schreiber 1992; Stratta et al. 1998), an effect that may be dopamine (DA) dependent. According to this hypothesis, deficits in aspects of cognitive processing that superficially appear to be heterogeneous, i.e. signal detection, language processing, memory and problem solving, may all be derived from a single degraded ability to construct, maintain and update task-setting rules in order to inform appropriate behavioural responses. In support of these claims, work has demonstrated that patients with schizophrenia demonstrate difficulties in a variety of tasks, many of which assume prefrontal mediation, including the Stroop task (Wysocki and Sweet 1985), the lexical ambiguity task (Cohen et al. 1988) and continuous performance test (CPT) (Cornblatt et al. 1989).

Each of these tasks has been presented as being dependent on the processing of task-setting information to guide behaviour (Cohen and Servan-Schreiber 1992). Although at first sight it may appear that these tasks are rather removed from the solution and performance of conditional discriminations, a simple formal analysis suggests a closer analogy. For example, in the CPT-AX task, a correct response is conditional on the preceding cues; participants must respond to an X if preceded by an A, and to respond to a Y if preceded by a B (Javitt et al. 2000; Umbricht et al. 2000). In a biconditional discrimination task, subjects might be required to produce a left lever-press response (X) if preceded by stimulus A (e.g. a tone) and a right lever-press response (Y) if preceded by stimulus B (e.g. a light). In the lexical ambiguity task, participants faced with words with ambiguous meanings are required to derive the appropriate meaning, guided by conditional cues provided by the semantic context. Although these analogies are acknowledged to be purely formal and abstract, and other unique specific demands are clearly important in each individual task, their fundamental relationship is made apparent by the fact that performance on these various tasks, and solution of conditional discriminations, are accounted for by an identical connectionist model (Cohen et al. 1990). Although this model was originally developed to account for the Stroop task (Cohen et al. 1990), it has also been used to account for the modulatory role of DA perturbations in schizophrenia in relation to lexical ambiguity (Cohen and Servan-Schreiber 1992) and the CPT-AX task (Barch et al. 1995, 2001). This framework therefore suggests that disruptions of conditional task performance by DAergic manipulations may well reflect similar processes to those observed in disruptions of cognitive tasks in patients with schizophrenia. If this is the case, then disruptions of conditional task performance may well be able to give insight into brain mechanisms that are altered in schizophrenia.

Therefore, we sought in experiment 1 to confirm that systemic administration of D-amphetamine would disrupt conditional task performance using an instrumental lever-pressing task, similar to those reviewed above, in which a choice between two responses (both of which have been rewarded and non-rewarded) is disambiguated by conditional task cues. Experiment 2 extended the generality of these results to examine performance in a purely Pavlovian biconditional discrimination that made use of the experimental contexts (the nature of the training chambers) as the biconditional cues. Experiment 3 made use of a Pavlovian-instrumental transfer (PIT) task to examine the possible confounding influence of systemic D-amphetamine treatment on motivational, motor or sensory processes. This PIT task is unrelated to conditional discrimination tasks in logical structure, but requires the simultaneous solution of two simple discriminations that are assessed in a test session identical to that used to assess conditional task performance. Finally, experiment 4 made use of the instrumental procedure to begin the process of establishing the pharmacological specificity of the D-amphetamine effect, examining the ability of the DA D_1/D_2 receptor antagonist α-flupenthixol to reverse the D-amphetamine-induced deficit in performance.

Materials and methods

Experiment 1

Animals

Male Lister Hooded rats were used (experiments 1, 2 and 4a: n=8; experiment 4b: n=24; Charles River, UK; experiment 3: n=8; Harlan, UK Ltd) and were housed in pairs in a humidity- and temperature-controlled vivarium on a 12-h/12-h light/dark cycle (lights on at 0800 hours). At the start of behavioural testing, animals weighed between 270 g and 425 g and were maintained on a mild food deprivation schedule (food available for 1.5 h/day) for the duration of training and experimental procedures. Water was available ad libitum. Training and test phases were conducted at the same times each day (during the light phase). All rats were handled prior to behavioural testing. Animal husbandry and experimental procedures were conducted according to "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985), and the UK Animals (Scientific Procedures) Act (1986).

Drugs

Experiments 1, 2 and 3 D-Amphetamine sulphate (Sigma-Aldrich, UK) was dissolved in 0.9% saline to achieve appropriate concentrations and administered at doses of 0, 0.5, 1.0 and 1.5 mg/kg. Injections were given intraperitoneally (i.p.) c. 5 min prior to each test session. Saline (0.9%) served as control vehicle solution.

Experiments 4a and 4b α-Flupenthixol (Sigma-Aldrich, UK) was dissolved in 0.9% and administered i.p. c. 20 min prior to test at doses of 0, 0.125, 0.25 and 0.5 mg/kg (experiment 4a) and 0.25 mg/kg (experiment 4b), with 0.9% saline used as control vehicle. D-Amphetamine

sulphate was dissolved in 0.9% saline and administered at a dose of 1.5 mg/kg i.p. c. 5 min prior to test (experiment 4b).

Stimuli and apparatus

Experiments 1, 3, 4a and 4b: instrumental biconditional discriminations and PIT Eight identical operant chambers (30×24×22 cm, Med-Associates, Vermont) were used. The chamber to which an animal was originally assigned remained constant throughout lever training, discrimination training, extinction tests and recovery sessions. Operant chambers were housed in individual light- and sound-attenuating chambers and were equipped with a single 4.2-W house light fixed on the upper rear wall of each chamber. A pellet dispenser delivered 45-mg food pellets (Noyes Precision Formula A/I, PJ Noyes Company, Inc., NH) into a recessed food tray located centrally in the front wall. The food magazine was itself positioned between two retractable levers. Two auditory stimuli were available in the chamber: a 3-kHz tone (85 dB) produced by a lab-manufactured tone generator, delivered through a speaker located in the top rear corner of the chamber and a 10-Hz (73–75 dB) train of clicks produced by operation of a heavy-duty relay mounted adjacent to the speaker behind the rear wall. A Dantum II/B computer equipped with Med-PC software (Med-Associates, St Albans, VT) controlled the experimental equipment and recorded data.

Experiment 2: Pavlovian biconditional discrimination Two sets of four operant chambers were used, creating two equivalent counterbalanced contexts for training and testing. The chambers and contextual stimuli were identical to those reported by Good and Honey (1991). In brief, one set of four chambers made up context A, distinguished from the second set of four chambers, context B, by odour cues and distinctive wall patterns within the chambers. Time of day provided a further contextual cue—for example, animals were run in context A in the morning and context B in the afternoon (all features counterbalanced). Two discrete stimuli were available as counterbalanced conditional stimuli (CSs) in each chamber. These were a 3-kHz tone at 80 dB and a change in illumination from light to dark (provided by switching a 12-W fluorescent strip light mounted above the translucent Perspex ceiling of each chamber). In all chambers, food-pellet reinforcement could be delivered into a recessed magazine that was located centrally in the left-hand wall of the chamber, which could be accessed by the rats via displacement of a hinged Perspex flap.

Training and testing procedures

Experiment 1: instrumental biconditional discrimination Lever-press training: prior to lever-press and conditional discrimination training, each animal received two, 30-min magazine training sessions. Food pellets were presented according to a variable time (VT) 30-s schedule. After ensuring that all animals reliably approached the magazine, lever training commenced. A progression of variable-interval (VI) schedules were used whereby animals on session 1 were introduced to a VI7-s schedule of reinforcement on a single lever. In the following sessions, the alternative lever was presented and the VI schedule was extended to VI15 and then to VI30. All sessions were 30 min in duration, and the order of lever training was counterbalanced across animals and sessions. For the final session, both levers were presented with reinforcement available on independent VI30 schedules. As a prerequisite to conditional discrimination training, animals had to demonstrate successful and consistent responding on both levers.

Conditional discrimination training: all conditional discrimination training sessions comprised ten 5-min trials—five presentations of tone and five presentations of clicks. The initial stimulus presentation (tone or click) was randomly generated by the computer, after which stimuli were presented in alternation. Both levers were present throughout the sessions and animals had to learn that reinforcement was contingent (VI30) on pressing a specific lever during each of the auditory stimuli, e.g. tone: left press, click: right press. The conditional relationship between levers and auditory stimuli was counterbalanced across animals. Subjects were given ten conditional discrimination training sessions in order to exceed a predetermined discrimination ratio criterion of 0.70. The discrimination ratio was computed in the following manner: total correct responses/[total correct + incorrect responses].

Extinction testing: following conditional discrimination training, a within-subject Latin-square design was employed in order to produce a dose–response analysis of the effects of D-amphetamine on conditional task performance. All animals received systemic treatments with control vehicle and 0.5, 1.0 and 1.5 mg/kg D-amphetamine, in four separate extinction tests over a 7-day period. A recovery training session was given between test sessions in order to reinstate criterion conditional discrimination performance. Extinction tests were identical to training sessions, except that no pellets were delivered and the number of trials was reduced from ten to six, three with each auditory stimulus. The length of each test session was therefore 30 min as the behavioural effects of D-amphetamine have generally dissipated by 45 min post-injection. The rationale for testing in a single, short extinction session is not to examine extinction processes per se, but rather to provide a test of conditional task performance that is unconfounded by the established influence of amphetamine on reinforcement processes.

Experiment 2: Pavlovian biconditional discrimination Prior to conditional discrimination training, all animals received two, 30-min magazine training sessions in each set of chambers. In each session, pellets were delivered according to a VT60 schedule. After all animals were reliably entering the magazines in both contexts and eating

the pellet reward, conditional discrimination training began. Again, this followed the protocol used by Good and Honey (1991), and continued for 14 days. In each 30-min session (one in each context per day, run morning and afternoon), animals received eight trials with each CS. One CS was reliably followed by delivery of a single food pellet in each context. That is, the tone CS (but not dark CS) might be followed by reward in context A but not context B, whereas the dark CS (but not tone CS) was followed by reward in context B, but not context A. Hence, animals were solving a conditional discrimination in which the Pavlovian contingency between CSs and reward was determined by the contextual stimuli present at the time. CSs were 30 s long, and magazine entries were measured both during reinforced and non-reinforced CS presentations and during equivalent 30-s preCS periods. CSs, contexts and conditional relationships were counterbalanced across animals. Trials were equally spaced across each session. After 14 training sessions, all animals received a series of extinction tests following treatment with D-amphetamine or control vehicle.

Extinction testing: once again, extinction tests were conducted following a within-subject Latin-square design of drug administration. Each animal was tested in one of the contexts immediately following i.p. injection (5 min pre-test) with one of the three doses of D-amphetamine or control injection of vehicle. A single day of recovery training (one session in each context) was given between successive stages of the Latin-square design. A total of eight extinction tests (four in each context) were run. Extinction tests were identical to training sessions except that food reward was not presented following either CS in either context. Once again, CS and preCS magazine entries were recorded.

Experiment 3: Pavlovian-instrumental transfer The procedure for magazine training was identical to that described in experiment 1. Following this, the experiment consisted of three parts: Pavlovian conditioning, instrumental training and test. The Pavlovian conditioning and instrumental training took place on alternate days and order was counterbalanced across animals.

Pavlovian conditioning: each Pavlovian conditioning session lasted 50 min and comprised ten 5-min trials. During each trial, one of two auditory stimuli (either tone or clicks) was continuously presented. At the start of each session, the stimulus to be presented during the first trial was randomly selected, after which the stimuli alternated across trials (in exactly the same manner as experiment 1). During one of the stimuli, food pellets were delivered into the magazine on a VT-30 schedule. That is, one auditory stimulus was reinforced throughout the Pavlovian conditioning phase, whilst the other stimulus was never paired with the delivery of food. The particular stimulus reinforced was counterbalanced, so that half the animals experienced the tone as the reinforced stimulus; the others, the clicks. The number of magazine entries during each stimulus was recorded. Animals received five Pavlovian conditioning sessions.

Instrumental training: on alternate days to the Pavlovian conditioning sessions, animals were trained to perform an instrumental response for food reward. Each instrumental training session took 50 min to complete. Each animal received five training sessions, during which both the left and right levers were present in the experimental chamber. For the entire duration of each training session, responding to one of the levers was reinforced with food-pellet delivery under a VI30 schedule, whilst pressing the other lever had no programmed consequences. Animals did not receive initial individual lever training, but acquired discriminated lever pressing directly on the VI30 schedule of reinforcement. The position of the reinforced lever (either left or right) was counterbalanced across animals, but was fixed throughout each session and remained the same for individual animals across all sessions.

PIT testing: PIT tests were identical to the extinction tests used in experiment 1. That is, animals received extinction tests in which both levers were present, and 5-min presentations of the two auditory stimuli occurred in alternation. As in experiment 1, there were six trials in each session; three with each of the auditory stimuli. The length of each session was therefore 30 min. Although the final tests for experiments 1 and 3 were identical, the preceding training differed; in experiment 1, animals were required to use conditional relationships whereas in experiment 3 they were not. The assessment in experiment 3 was of the ability of rats to demonstrate the impact of Pavlovian stimuli on concurrent instrumental responding. Subjects were administered control vehicle and 0.5, 1.0 and 1.5 mg/kg D-amphetamine i.p. c. 5 min before the start of each of four extinction tests in accordance with the Latin-square design described in experiment 1. Retraining sessions (one Pavlovian and one instrumental) were given on intervening days.

Experiments 4a and 4b: instrumental biconditional discrimination Training and test procedures were identical to those outlined in experiment 1. During testing in experiment 4, a Latin-square, within-subjects dose–response analysis was conducted using three doses of α-flupenthixol and control vehicle. Thus, all animals received control vehicle injections and doses of 0.125, 0.25 and 0.5 mg/kg. Three recovery sessions were interspersed between the four extinction tests. Experiment 4b used a between-subjects design in which two groups of eight subjects were injected with 1.5 mg/kg D-amphetamine i.p. c. 5 min prior to test. Prior to this treatment, one group received pre-treatment (c. 20 prior to test) with 0.25 mg/kg α-flupenthixol, whereas the other group received control vehicle pre-treatment. The final group of eight rats received control vehicle injections at both time points before testing.

Statistical analyses

Analyses were conducted on response rates (lever presses, experiments 1, 3, 4a and 4b; magazine approach, exper-

iment 2) using analysis of variance (ANOVA). Significant interactions were examined using simple effects analysis and, where appropriate, post-hoc Newman–Keuls pairwise comparisons. In order to assess the impact of amphetamine on cue discrimination and response bias, data from experiment 1 were subject to additional analyses in terms of signal detection theory, using one-way ANOVAs examining drug-related changes in discriminability (d') and response criterion (c). Further, to assess the potential impact of amphetamine on behavioural switching in experiment 1, we examined the percentage of responses that occurred as a result of a switch of lever, again by ANOVA.

Results

Experiment 1

Instrumental conditional discrimination training

Following the first conditional discrimination training session, animals performed 31.2 correct and 28.9 incorrect lever presses, and at the conclusion of training all subjects were successfully and consistently performing more correct than incorrect lever presses (correct=48.3, incorrect=21.20). By session 10, animals had exceeded the predetermined discrimination ratio criterion (0.70) which was maintained during the recovery sessions interspersed between extinction tests. A paired t-test conducted on responses from session 10 revealed that the number of correct responses was significantly higher than the number of incorrect responses ($t_{1,7}$=19.3, P<0.01).

Extinction testing

Figure 1 shows the mean number of correct and incorrect responses made during the four, 30-min extinction tests following administration with each of the three doses of D-amphetamine and control vehicle. Systemic treatment with 1.5 mg/kg D-amphetamine clearly disrupted performance of the conditional discrimination task and treatment with 1.0 mg/kg attenuated correct lever-press performance.

A 4×2 within-subject ANOVA, with factors of drug dose (0, 0.5, 1.0 and 1.5 mg/kg) and lever (correct/incorrect) revealed significant effects of both drug ($F_{3,21}$=11.2, P<0.01) and lever ($F_{1,7}$=55.9, P<0.01). There was also a significant interaction between drug and lever ($F_{3,21}$=7.8, P<0.01). Analysis of simple effects from this interaction indicated that the number of correct responses was significantly greater than the number of incorrect responses following treatment with control vehicle and 0.5 mg/kg D-amphetamine and, although numerically much smaller, also following treatment with 1.0 mg/kg D-amphetamine (minimum $F_{1,7}$=30.4, P<0.01). In contrast, this difference was not significant following treatment with 1.5 mg/kg D-amphetamine ($F_{1,7}$=1.7, P<0.01). Simple-effects analysis also revealed a significant effect of drug dose on the number of correct ($F_{3,21}$=12.7, P<0.01) and incorrect lever presses ($F_{3,21}$=12.0, P<0.05). Newman–Keuls pairwise comparisons revealed that the number of incorrect responses made following administration of control vehicle did not differ from the number of incorrect responses given after treatment with 1.0 mg/kg or 1.5 mg/kg of D-amphetamine (P values>0.05), but that performance after these three treatments all differed from performance after 0.5 mg/kg D-amphetamine (P values<0.05). In contrast, Newman–Keuls tests revealed that performance on the correct lever did not differ between control treatments and treatment with 0.5 mg/kg D-amphetamine, and that performance in these two conditions differed from that following treatment with 1.0 mg/kg and 1.5 mg/kg D-amphetamine (P values<0.05), which in turn did not differ from one another (P>0.05). That is, higher doses of D-amphetamine significantly attenuated performance on the correct lever. These results clearly indicate that treatment with 1.5 mg/kg D-amphetamine disrupted performance of an instrumental conditional discrimination. Treatment with 1.0 mg/kg D-amphetamine dramatically reduced correct performance, although some evidence of correct discrimination was still evident. Discrimination was intact following treatment with 0.5 mg/kg D-amphetamine, although overall response rates were elevated.

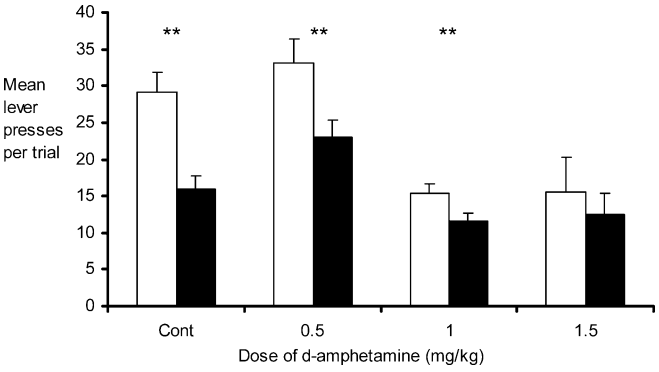

Fig. 1 Experiment 1: correct (*open bars*) and incorrect (*filled bars*) lever-press responses in an extinction test following administration of D-amphetamine or saline control vehicle. Administration of 1.5 mg/kg D-amphetamine resulted in a marked deficit in instrumental conditional discrimination performance, with attenuated performance on the correct lever at 1.0 mg/kg. Mean±SEM. **Significant difference between correct and incorrect responses (P<0.01)

Signal detection analysis

This interpretation is further supported by an analysis of these results in terms of signal-detection theory, which revealed a steady decline in d' with an increasing dose of D-amphetamine (saline control d'=0.80; 0.5 mg/kg d'=0.46; 1.0 mg/kg d'=0.41; 1.5 mg/kg d'=0.07) that was reflected in a main effect of drug dose in a one-way ANOVA ($F_{3,21}$=5.6, P<0.01). A similar analysis of changes in criterion (c) failed to reveal any effect of

drug (saline control c=0.11; 0.5 mg/kg c=−0.18; 1.0 mg/kg c=0.04; 1.5 mg/kg c=−0.16; [$F_{3,21}$=1.8, P>0.1]).

Response switching

Examination of lever-press performance revealed that following D-amphetamine treatment there was no change in the level of switching (percentage of responses that occurred as a result of a switch: SAL=33.6%; 0.5 mg/kg=38.1%; 1.0 mg/kg=35.3%; 1.5 mg/kg=34.3%), and ANOVA with factors of drug dose and trial revealed no main effect of dose ($F_{3,21}$=1.2, P>0.3), trial ($F_{5,35}$=1.4, P>0.2) or an interaction (F<1), suggesting that impairments were in the performance of the previously learned conditional relationships rather than due to increased response switching.

Experiment 2

Pavlovian conditional discrimination training

All animals successfully solved the Pavlovian contextual conditional discrimination during the 14 sessions of training. As preliminary analysis revealed no influence of counterbalancing factors of context (A or B) or CS (tone and light/dark) (F values <1), data were combined across CSs and contexts to represent preCS and CS magazine entries before and during reinforced CS presentations (correct responses) and non-reinforced CS presentations (incorrect responses). Conditional discrimination was assessed by examining CS–preCS correct and incorrect responses across the 14 sessions of training. On average, animals made 4.3 correct and 8.8 incorrect responses per CS during the first session, but 12.1 correct and 1.5 incorrect responses per CS during the final training session. Within-subject ANOVA with factors of session (1–14) and response (correct/incorrect) revealed a main effect of response ($F_{1,7}$=32.0, P<0.01) and a response × session interaction ($F_{13,91}$=3.7, P<0.01).

Extinction testing

The results of the extinction tests following control vehicle or D-amphetamine administration are shown in Fig. 2. Whereas animals clearly continued to solve the conditional discrimination following vehicle treatment or administration of 0.5 mg/kg D-amphetamine, performance of the discrimination was completely disrupted following treatment with D-amphetamine at doses of 1.0 mg/kg and 1.5 mg/kg. This interpretation was confirmed using within-subjects ANOVA with factors of drug dose (0, 0.5, 1.0 and 1.5 mg/kg) and response (correct/incorrect). As the variance of the data was found to increase with the mean, data were subject to a square-root transformation (Howell 1992). ANOVA revealed a main effect of response ($F_{1,7}$=16.8, P<0.01) and, more importantly, an interaction of drug treatment and response ($F_{3,21}$=3.6, P<0.05). Analysis of the simple effects from this interaction revealed an effect of response following treatment with control vehicle and 0.5 mg/kg D-amphetamine ($F_{1,7}$ values=6.1 and 9.5, respectively, P values<0.05), but not following treatment with the two higher doses of D-amphetamine ($F_{1,7}$ values=0.9 and 2.3, respectively, P values>0.05). Similarly, drug administration influenced correct performance ($F_{3,21}$=5.5, P<0.05) but not incorrect performance (F<1).

Experiment 3

Pavlovian conditioning

Although the animals continued to enter the magazine during the non-reinforced stimulus across all five training sessions, it was clear that by the final conditioning session the animals were confining most of their magazine entries to the reinforced-stimulus. On session 1, 60% of total magazine entries occurred during the reinforced stimulus. This had risen to 68% at the conclusion of session 5 (59.5 entries per rewarded CS versus 28.7 entries per non-rewarded CS). Therefore, by the end of the conditioning phase, magazine responding was greater during the reinforced CS, indicating that animals successfully discriminated the reinforced CS from the non-reinforced CS. However, as performance during training was confounded by reward delivery during the CS, no formal analysis of discriminated magazine approach was conducted at this stage.

Instrumental training

All animals demonstrated rapid acquisition of responding on the reinforced lever, whilst responding to the non-reinforced lever remained low throughout training (first

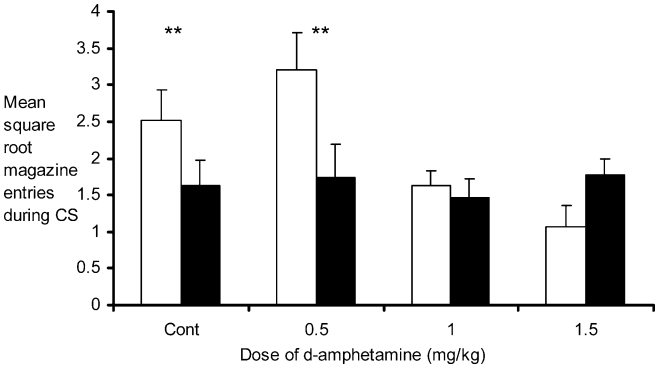

Fig. 2 Experiment 2: mean number of correct (*open bars*) or incorrect (*filled bars*) magazine entries during CS presentations following treatment with D-amphetamine or saline control. Administration of 1.0 mg/kg and 1.5 mg/kg D-amphetamine resulted in marked disruption of Pavlovian conditional discrimination performance. Mean±SEM. **Significant difference between correct and incorrect responses (P<0.01)

training session: reinforced=117, non-reinforced=20; final training session: reinforced=389, non-reinforced=7).

Extinction testing

The data from the extinction test are presented in Fig. 3, averaged across trials. Lever presses made during presentations of the reinforced stimulus are displayed in the left-hand panel, and during the non-reinforced stimulus in the right-hand panel. A comparison of responding on reinforced and non-reinforced levers during both reinforced and non-reinforced stimuli indicates that rats maintained a clear discrimination between levers, irrespective of the dose of D-amphetamine administered. Similarly, the level of responding on the reinforced lever was higher during the reinforced stimulus than during the non-reinforced stimulus across all drug doses, indicating that the animals were also able to discriminate between the reinforced and non-reinforced stimulus, showing robust PIT. A 4×2×2 within-subject ANOVA, with factors of drug dose (0, 0.5, 1.0 and 1.5 mg/kg), lever (reinforced and non-reinforced) and stimulus (reinforced and non-reinforced) was conducted. This revealed no main effect of drug dose (F<1), but a significant main effect of lever ($F_{1,7}$=137.7, P<0.01) and stimulus ($F_{1,7}$=83.9, P<0.01), and a significant lever×stimulus interaction ($F_{1,7}$=157.8, P<0.01). No other interactions were significant. Analysis of simple main effects from the lever×stimulus interaction demonstrated that the reinforced lever was significantly preferred over the non-reinforced lever during both the reinforced stimulus and non-reinforced stimulus ($F_{1,7}$=158.8, P<0.01 and $F_{1,7}$=79.5, P<0.01, respectively). It also revealed that there was significantly more responding on the reinforced lever during the reinforced stimulus than during the non-reinforced stimulus ($F_{1,7}$=124.4, P<0.01), but that the level of responding on the non-reinforced lever did not differ significantly between stimuli ($F_{1,7}$=1.0, P>0.05).

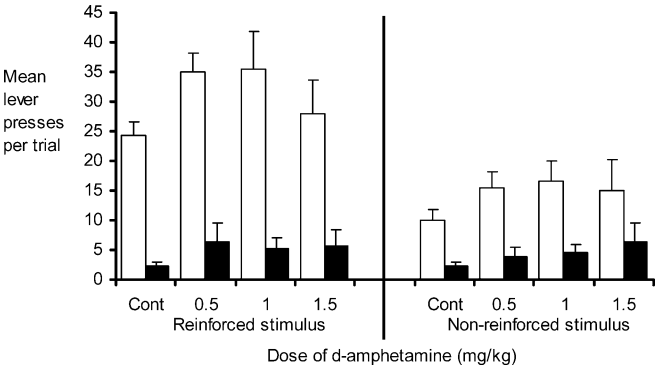

Fig. 3 Experiment 3: Pavlovian-instrumental transfer after treatment with three doses of D-amphetamine or saline control vehicle. *Left panel* mean responding during the reinforced CS, *right panel* mean responding during the non-reinforced CS. This highlights two distinct effects: (1) D-amphetamine does not disrupt the ability to discriminate between the previously reinforced (*open bars*) and non-reinforced (*closed bars*) levers; (2) responding on the reinforced lever during the non-reinforced stimulus is markedly lower than that during the reinforced stimulus, suggesting that D-amphetamine does not impair discrimination of auditory stimuli. Mean±SEM

Experiments 4a and 4b

Training

At the conclusion of session 6, subjects had exceeded the 0.70 discrimination ratio criterion. Rats in experiment 4a made on average 50.4 correct and 20.0 incorrect responses. A paired t-test revealed that this difference was significant ($t_{1,7}$=10.7, P<0.01). Rats in experiment 4b that were subsequently treated with α-flupenthixol prior to D-amphetamine administration demonstrated a significant difference between correct (mean=55.5) and incorrect (mean=22.8) lever presses ($t_{1,7}$=6.5, P<0.01). This level of discrimination was also seen in rats that were to be treated with control vehicle prior to D-amphetamine treatment (correct mean=54.1; incorrect mean=21.0; ($t_{1,7}$=5.5, P<0.01) and in rats that received two control vehicle injections (correct mean=51.6; incorrect mean=17.4; $t_{1,7}$=5.7, P<0.01).

Conditional discrimination testing

Experiment 4a Figure 4a shows the mean number of correct and incorrect lever presses made during four 30-min extinction tests following treatment with each of three doses of α-flupenthixol and control vehicle. Animals showed successful conditional discrimination performance in all conditions. A 4×2 within-subjects ANOVA, with factors of drug dose (0, 0.125, 0.25 and 0.5) and lever (correct/incorrect) revealed a significant main effect of drug ($F_{3,21}$=5.3, P<0.01) and lever ($F_{1,7}$=52.5, P<0.01), but importantly no drug×lever interaction was evident (F<1). Hence, α-flupenthixol had no effect on conditional discrimination performance, but higher doses did reduce overall response rates. Simple main-effects analysis confirmed the initial interpretation by demonstrating that the number of correct responses exceeded incorrect responses in all conditions (minimum $F_{1,7}$=12.1, P<0.01). Furthermore, irrespective of lever, there was no difference between performance following treatment with control vehicle and that following the lowest dose of α-flupenthixol, but control performance differed from that following both of the higher doses (P values<0.05). Hence a dose of 0.25 mg/kg was selected for use in experiment 4b; although there was some evidence that this dose decreased response rates, there was no effect of this treatment on discrimination performance.

Experiment 4b Figure 4b shows the results from the three experimental groups (control/control, control/amphetamine, α-flupenthixol/amphetamine) together with the 0.25 mg/kg α-flupenthixol-treated group from experiment 4a. Although this group was not run at precisely the same

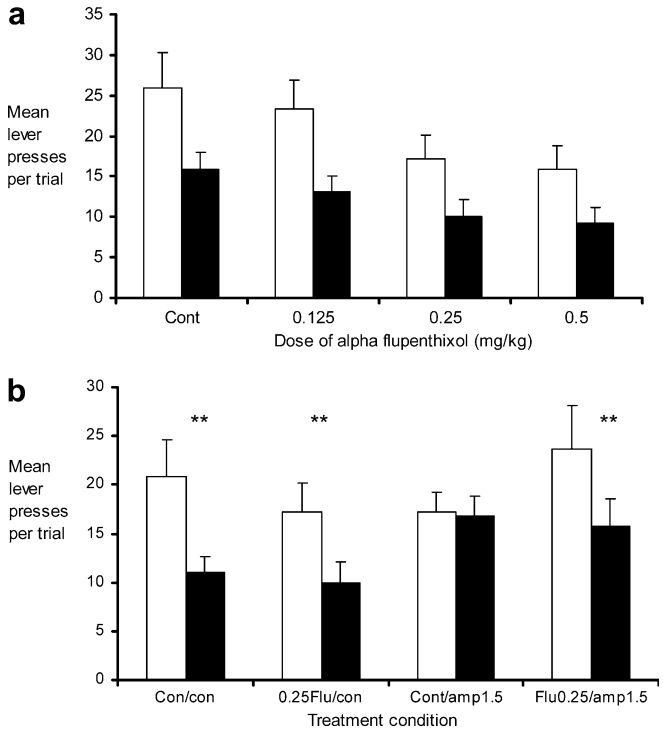

Fig. 4 **a** Experiment 4a: mean number of correct (*open bars*) and incorrect (*filled bars*) lever-press responses made following treatment with three doses of α-flupenthixol and control vehicle. Mean ±SEM. **b** Experiment 4b: mean number of correct (*open bars*) and incorrect (*filled bars*) lever-press responses on conditional discrimination extinction test from the three experimental groups (control/control, control/amphetamine, α-flupenthixol/amphetamine) together with the 0.25 mg/kg α-flupenthixol-treated group from experiment 4a. The disruption of conditional discrimination by systemic D-amphetamine (1.5 mg/kg) is attenuated by pre-treatment with α-flupenthixol. Mean±SEM. **Significant difference between correct and incorrect responses ($P<0.01$)

time as the others (a period of 7 days separated testing), they were run in an identical manner, and pre-test rates of responding and discrimination did not vary amongst groups (4×2 mixed ANOVA of pre-test performance with factors of group and lever revealed no effect of group [$F<1$], a main effect of lever [$F_{1,28}=280.2$, $P<0.01$] and no interaction [$F<1$]). These four groups afforded a mixed 2×2×2 ANOVA with between-subject factors of amphetamine treatment (control versus amphetamine), α-flupenthixol treatment (control versus α-flupenthixol) and a within-subject factor of lever (correct/incorrect). This analysis yielded a main effect of lever ($F_{1,28}=69.7$, $P<0.01$) but not of either drug treatment (maximum $F_{1,28}=1.7$). More importantly, there was a significant two-way interaction of lever and amphetamine treatment ($F_{1,28}=8.1$, $P<0.01$), but not of lever and α-flupenthixol treatment ($F_{1,28}=24$, $P>0.1$), and a significant three-way interaction of all factors ($F_{1,28}=11.2$, $P<0.01$). Analysis of the simple effects from this interaction revealed an effect of lever (correct > incorrect) for the control group, the α-flupenthixol-treated group and the group receiving combined treatment of amphetamine and α-flupenthixol (minimum $F_{1,28}=15.7$, $P<0.01$). However, this effect was not present in the group receiving D-amphetamine alone prior to test ($F<1$). Hence treatment with α-flupenthixol reversed the D-amphetamine-induced deficit in conditional discrimination performance.

To ensure that the effect was robust within a single experiment, we conducted a further analysis making use of only those animals that received D-amphetamine treatment in experiment 4b (i.e. vehicle/amphetamine and α-flupenthixol/amphetamine). This yielded a 2×2 mixed ANOVA, with factors of pre-treatment (α-flupenthixol or control vehicle) and lever (correct or incorrect). This revealed no main effect of pre-treatment ($F<1$) but a main effect of lever ($F_{1,14}=21.2$, $P<0.01$) and, crucially, a pre-treatment×lever interaction ($F_{1,14}=16.8$, $P<0.01$). Analysis of the simple main effects from this interaction showed that, although there was no significant effect of pre-treatment in the number of correct or incorrect lever presses (minimum $F_{1,14}=1.7$, P values>0.2), there were significantly more correct than incorrect responses in the α-flupenthixol pre-treated amphetamine group ($F_{1,14}=37.9$, $P<0.01$) but not in the vehicle pre-treated amphetamine group ($F<1$).

Discussion

Experiment 1 revealed deficits following D-amphetamine treatment on performance of a conditional discrimination task that required the use of discrete cues to guide goal-directed lever pressing. That is, animals treated with the psychotomimetic D-amphetamine appear to be incapable of using past training of task requirements (i.e. of the sort: if A do X; if B do Y) to inform their current lever-press responding. A dose of 1.5 mg/kg D-amphetamine resulted in marked disruption of conditional performance, and a dose of 1.0 mg/kg significantly reduced performance on the correct lever.

In experiment 2, systemic treatment with D-amphetamine at doses of 1.0 mg/kg and 1.5 mg/kg disrupted performance of a conditional discrimination task that was based on Pavlovian rather than instrumental conditioning. Here, correct performance relied on reflexive performance of Pavlovian conditioned magazine approach rather than goal-directed lever presses, and the discrimination was based on the ability of contextual, rather than discrete, stimuli to act as conditional cues. These results make it unlikely that the observed influence of D-amphetamine on conditional task performance is due to any non-specific influence on either Pavlovian or instrumental conditioning. Further, the fact that incorrect responding following D-amphetamine treatment did not differ significantly from performance following vehicle treatment highlights the absence of any major motoric impairments that might account for reductions in correct responding. That this is unlikely to be the result of a simple rate-dependent effect (e.g. amphetamine selectively reducing high rates of responding) is demonstrated by the results of experiment 3, where similar high rates of responding are increased by amphetamine treatment.

The results of the PIT task in experiment 3 also showed that D-amphetamine at these doses did not significantly disrupt animals' capacity to show successful simple discriminations between reinforced cues and non-reinforced cues (either auditory stimuli or levers). Additionally, despite treatment with D-amphetamine, animals were able to successfully combine information about the auditory CSs and levers (i.e. responding was highest on the reinforced lever when the reinforced CS was presented). This strongly suggests that the disruption of performance observed in experiment 1 was not a product of a drug-induced deficits in basic perceptual or motor discrimination processes, and indicate the selectivity of this deficit for conditional task performance by eliminating possible motor, sensory or motivational confounds. If, for example, the deficits observed following treatment with the higher doses of D-amphetamine were due to general changes in locomotor activity (e.g. lever presses came to be made on the basis of whichever lever was closer to the animal at any given time) then incorrect responding would have been significantly higher during the PIT test during both reinforced and non-reinforced stimulus presentations. Therefore, D-amphetamine does not appear to impair general sensory or motor discrimination processes, but rather selectively disrupts performance in tasks that involve the use of conditional cues to direct performance.

A further possibility is that the deficits observed in experiments 1 and 2 occurred as a result of the effects of D-amphetamine on task switching (Robbins and Evenden 1984; Evenden and Dogget 1989), rather than any detrimental effects on conditional discrimination per se. However, direct analysis of this possibility in experiment 1 rejected this account. Furthermore, it is unlikely that switching could account in a straightforward manner for deficits in the Pavlovian conditional discrimination task in experiment 2. This examined only magazine approach as a response, assessed during discrete rewarded and non-rewarded CSs. If increased behavioural switching increased the likelihood of other interfering behaviours in the testing chamber, this would decrease responding in both CS+ and CS− trials, an effect that was clearly not observed (Fig. 2).

Experiment 4a demonstrated that performance of conditional discrimination was not influenced to any major degree by treatment with α-flupenthixol, suggesting that at these doses the D_1/D_2 antagonist has little effect on animals' abilities to make use of conditional relationships. This is not to say that other doses might not influence performance (Robbins et al. 1990), but the purpose of experiment 4a was to establish doses that could antagonise the effects of D-amphetamine without interfering with performance per se. Selection of a dose that in itself has no effect on behavioural performance is important not only to emphasise the pharmacological specificity of the effect, but also because results from previous studies have shown that sufficiently high doses of D_1/D_2 receptor antagonists can attenuate both lever pressing for primary reinforcers (Wise et al. 1978; Cousins et al. 1994) and conditioned reinforcers (Robbins et al. 1983; Fletcher and Higgins 1997; Killcross et al. 1997) and the impact of PIT (Dickinson et al. 2000). Experiment 4b showed that the disruptive effect of D-amphetamine on conditional discrimination performance was clearly attenuated by prior treatment with α-flupenthixol at a dose that did not itself unduly influence performance.

Given that α-flupenthixol possesses high affinity for both D_1 and D_2 receptors (Murrin 1983), this strongly suggests that the disruptive effect of D-amphetamine is mediated by alterations in the normal functioning of forebrain DA systems and that changes in D_1 and/or D_2 receptor activation were responsible for impaired conditional discrimination performance. However, the nature and locus of possible changes in DA receptor activity remain uncertain. Previous research examining the neural bases of conditional discriminations has revealed the importance of multiple systems, with changes in conditional task acquisition following manipulations of the cholinergic innervation of the anterior cingulate cortex (Marston et al. 1994; Muir et al. 1996), lesions of the PFC (Petrides 1987), ibotenic acid lesions of the ventral and dorsolateral striatum (Reading et al. 1991) and 6-hydroxydopamine lesions of the dorsal striatum (Robbins et al. 1990). This suggests that several interactive systems may modulate acquisition and performance of conditional tasks, and it seems likely that these systems are each involved (directly or indirectly) in modulation of behaviour by cortical and subcortical DA systems. Furthermore, both hyper-DAergic and hypo-DAergic activity in the PFC are associated with impairments of cognitive performance in rats and non-human primates (Arnsten 1998; Murphy et al. 1996; Zahrt et al. 1997; Arnsten and Goldman-Rakic 1998), and it appears that optimal levels of DA activity are required for normal functioning. Robbins et al. (1990) have reported that DA receptor antagonism by systemic treatment with α-flupenthixol can produce deficits in a conditional visual discrimination, suggesting that disruptions in conditional tasks may also be produced by both under-stimulation and over-stimulation of forebrain DA systems. The present systemic studies do not directly address this issue but we would note the probable reciprocal relationship of cortical and subcortical DA activity (Wilkinson et al. 1998).

Recent theoretical and empirical studies have suggested that a deficit in the ability to make use of task-setting cues to direct behaviour may be a core cognitive deficit in schizophrenia, and further evidence suggests that this may depend on disrupted DAergic function in patients. The present findings represent a very preliminary assessment of this cognitive deficit in an animal model. Solution of biconditional discriminations requires the use of task-setting cues to guide behaviour, and this is selectively disrupted by treatment with D-amphetamine, and reversed by treatment with the D_1/D_2 DA receptor antagonist and antipsychotic α-flupenthixol. Clearly, a large number of issues remain to be examined. The pharmacological selectivity of the effects needs to be established and extended, examining the influence of other psychotomimetics (such as phencyclidine, PCP) that may have greater

relevance for cognitive deficits in schizophrenia, other non-psychotomimetic neuroactive agents, and the effects of both typical and atypical antipsychotic drugs, administered both acutely and chronically, as well as putative antipsychotics from other neurotransmitter classes (e.g. 5-HT). These studies have been conducted, the results of which will be published in companion articles indicating that a similar disruption of performance is seen following acute and chronic treatment with PCP and related glutamatergic agents MK-801 and ketamine, that these effects can be reversed by antipsychotic treatment in a manner that depends on treatment regime, and finally that non-psychotomimetic agents are without effect in this task. The experiments presented here, in combination with data reviewed, suggest that both increases and decreases in forebrain DA activity can disrupt biconditional task performance. We are currently examining the precise neural location of these opposing effects (or, for example, whether they both result from disruptions of the balance of DAergic activity in cortical and subcortical regions) and whether typical and atypical antipsychotics show differential effects depending on the nature of the DAergic disruption that caused the initial deficit.

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