Methylphenidate can reduce selectivity in associative learning in an aversive trace conditioning task

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Abstract
There are good grounds to expect that methylphenidate (MP) should enhance cognitive function. However, experimental evidence on this point is scant. The present study therefore examined the effects of MP on learning the association between a conditioned stimulus (CS, in this case, noise) and an unconditioned stimulus (UCS, in this case, footshock) in an aversive variant of a trace conditioning procedure. Learning was measured off-the-baseline as conditioned suppression of drinking (both latencies to drink, expressed as suppression ratios, and the amount drunk, expressed as the number of licks, in the presence of the CS). In addition to the measures of discrete cue conditioning, MP effects on contextual conditioning were measured as suppression to apparatus cues and an experimental background stimulus. MP was administered at 1 or 5 mg/kg prior to conditioning sessions. As attention deficit hyperactivity disorder (ADHD) has been characterized as involving a ‘wide attentional window’ (e.g. Shalev and Tsal, 2003), it was predicted that MP, as the treatment of choice for ADHD, should increase selectivity (narrowing the attentional window). This outcome would show as reduced levels of conditioning (compared to control rats) to less informative trace and contextual cues present during conditioning.

Contrary to prediction, both 1 and 5 mg/kg MP increased learning about all the available stimuli, including the less informative trace CS and the background stimulus. These findings are consistent with reduced rather than increased selectivity in learning (because of increased rather than decreased conditioning to weak cues) under MP.

Keywords
aversive conditioning, trace interval, contextual conditioning, rat, methylphenidate, dopamine

Introduction
The dopamine (DA) hypothesis and related amphetamine model of schizophrenia (Ellison and Eison, 1983) have prompted considerable interest in how drugs that influence the brain DA system may affect basic cognitive processes in animal models. Consequently, effects of amphetamine and other DA-ergic drugs on associative learning processes in rats have been extensively studied, particularly in relation to selectivity in learning. However, methylphenidate (MP) has not commonly been tested in such tasks. This is surprising since amphetamine and MP both increase extracellular DA in the striatum (Chiueh and Moore, 1975a, 1975b; Hurd and Ungerstedt, 1989; Giros et al., 1996). Moreover, both amphetamine and MP are used to treat disorders of attention such as attention deficit hyperactivity disorder (ADHD). MP is the current drug of choice in the treatment of ADHD and the prescription of MP for ADHD (and other disorders) has risen dramatically over recent years (see Biederman and Faraone, 2005).

Attentional impairment matters most when cognitive and behavioural systems are inappropriately engaged by environmental cues. Here we used a Pavlovian conditioning procedure to test for effects of MP on attentional learning in rats. We have previously used this procedure to examine the effects of the indirect DA agonist d-amphetamine (Norman and Cassaday, 2003) and lesions to the DA rich nucleus accumbens (Cassaday et al., 2005). It is by now very well established that both d- and dl-amphetamine can increase conditioning to poor (irrelevant, redundant) predictors of reinforcement in two-stage selective learning tasks, e.g. latent inhibition (LI: Solomon et al., 1981; Weiner et al., 1981, 1988; Gray et al., 1992; Kumari et al., 1999) and blocking (Crider et al., 1982; Ohad et al., 1987; Jones et al., 1997). However, in such procedures, it is not clear how a treatment such as amphetamine is effective and precisely through what mechanism(s) the influence of earlier associations on subsequent conditioning is reduced. We therefore developed one-stage procedures to compare drug effects on conditioning but without invoking the need explicitly to compare competing associative representations.

Specifically, we used a conditioned stimulus (CS) that is rendered less informative through the introduction of a time interval between CS and unconditioned stimulus (UCS) presentation...
(Kamin, 1965) and measured learning about this (trace) CS compared to a (contiguous) CS that was immediately followed by the UCS. We used an aversive off-the-baseline conditioning procedure, like that typically used in LI experiments, with footshock as the UCS (Nayak and Cassaday, 2003; Norman and Cassaday, 2003). For both trace and contiguously conditioned groups an experimental flashing light background stimulus was presented continuously throughout the conditioning session and provided an alternative conditioning stimulus.

In addition to testing conditioning to the discrete trace and contiguous CSs and to the background stimulus, we also measured contextual conditioning to the apparatus cues (‘box context’) as lick suppression at the start of the first post-conditioning drinking session in the aversive procedure. Therefore as well as providing an alternative means (to pre-exposure) of presenting animals with a weakly predictive discrete CS, the trace conditioning procedures used here can assess contextual conditioning both to the experimental background stimulus and the cues provided by the conditioning chambers.

This procedure has shown a clear role for DA in the associative learning processes under test but the effects of d-amphetamine have not been consistent with any improvement in the selectivity aspect of associative learning (Norman and Cassaday, 2003; Kantini et al., 2004). Whilst we have no basis to predict that the effects of MP would be different in this respect, clearly any demonstrable improvement in selectivity under MP would suggest a mechanism for its therapeutic actions in ADHD. To ensure the translational relevance of our findings, we tested the effects of two doses of MP: 1 and 5 mg/kg (in rats), which evidence suggests are comparable to human therapeutic doses (Brandon et al., 2001). If treatment with MP reduced the range of cues conditioned to, this would be consistent with the evidence to suggest that disorders like ADHD are characterized by a ‘wide attentional window’ (Shalev and Tsal, 2003) that should be narrowed by effective treatments (see also Taranowski et al., 1986; Richards et al., 1990; Sonuga-Barke et al., 2004). Conversely, if the ability to restrict associative learning to more reliable predictors was reduced under MP (as was most clearly the case under d-amphetamine in the aversive procedure, Norman and Cassaday, 2003) this would arguably be a paradoxical effect. Decreased selectivity in learning would suggest that ADHD might be improved by the alternative mechanism of increased attention to, and/or subsequent associability of, a wider range of cues.

Methods

Animals

A total of 72 naive male Wistar rats (Charles Rivers, UK) were used, of weight range 216–286 g at the start of water deprivation. All animals were caged in pairs on a 12:12 h light/dark cycle (lights on 8.00 AM to 8.00 PM) and handled over two weeks before the start of the experiment. All training and testing took place during the light phase (between 9.00 AM and 5.00 PM).

Rats were water deprived, receiving one hour of access to water per day following testing. Food was freely available in the home cage throughout the duration of the experiment.

All procedures were carried out in accordance with the United Kingdom Animals Scientific Procedures Act 1986, Project Licence number PPL 40/2648.

Drugs

Methylphenidate hydrochloride (Sigma Aldrich, Dorset, UK) was dissolved in physiological saline and injected in 1 ml/kg body weight, at doses 1 and 5 mg/kg s.c. 15 min prior to the conditioning phase. Extinction tests were conducted drug free.

Apparatus

Four identical fully automated conditioning chambers, housed within sound-attenuating cases containing ventilation fans (Cambridge Cognition, Cambridge, UK), were used. Each of the inner conditioning chambers consisted of a plain steel box (25 x 25 x 22 cm high) with a Plexiglas door (19 x 27 cm) at the front. The floor was a shock grid with steel bars 1 cm apart and 1 cm above the lip of a 7 cm deep sawdust tray. Mounted in one wall were two retracted levers (that were not in use), three stimulus lights and a waterspout.

The spout was 5 cm above the floor and connected to a lickometer supplied by a pump. Licks were registered by the breaking of the photo beam within the spout, which also triggered water delivery of 0.05 ml per lick. The waterspout was illuminated when water was available. A loudspeaker for the presentation of auditory stimuli was set in the roof.

Two stimuli were used in this procedure; a mixed frequency noise set at 70 dB (including background) of 5 s duration as the trace or contiguous target stimulus (i.e. the CS paired with the UCS) and a continuously presented flashing light as the alternate or background stimulus, provided by the three wall mounted stimulus lights and the house light flashing on (0.5 s) and off (0.5 s) throughout the session. Footshock of 1 s duration and 1 mA intensity was delivered through the grid floor by a constant current shock generator (pulsed voltage: output square wave 10 ms on, 80 ms off, 370 V peak under no load conditions, MISAC Systems, Newbury, UK). Stimulus control and data collection was by a Acorn Archimedes RISC computer programmed in Basic with additional interfacing using an Arachnid extension (Cambridge Cognition).

Procedure

Rats were handled for on average 10 minutes per day, on each of 7 days, over the 2 week settling period before experimental procedures commenced. Water deprivation was introduced the day before shaping to drink in the experimental chambers. The one stage conditioning procedure was preceded by 10 days of baseline sessions and followed by reshaping before the test phase.

Preconditioning  Rats’ responses were shaped (over 2 days) until all the animals drank from the waterspout. Rats were then individ-
ually assigned to a conditioning box for the duration of the experiment, counterbalanced for both drug and behavioural condition.

There then followed 10 days of preconditioning, in which rats drank in the experimental chamber for 15 minutes each day (timed from first lick). The drinking spout was illuminated throughout, but there were no other stimuli presented in this phase. Latency to first lick was measured as an indicator of habituation to the experimental context. Total number of licks was also recorded each day to assess pre-existing differences in drinking (prior to any conditioning).

**Conditioning** Conditioning was conducted in 1 day following the last preconditioning day. There was a continuous background stimulus (flashing lights) onto which pairings of the 5 s target (noise CS) and footshock were superimposed. There were two such conditioning trials. The first pairing of CS and UCS was presented after 5 minutes of background stimulus had elapsed, and the second pairing was at 5 minutes after the first, with a further 5 minutes left in the apparatus following the second shock presentation.

Depending on experimental group, the footshock followed either 0 s (for the contiguous groups) or 30 s (for the trace groups) after target CS offset. The flashing light stimulus was presented throughout the 15 minute session, including the 30 s inter-stimulus-interval (ISI) that simply added to the overall duration of the session in the trace groups. In the absence of drinking (no water was available within the chamber during conditioning and the waterspout was not illuminated), there was nothing to record.

The conditioning parameters (1 mA shock level presented over two conditioning trials) selected to test the effects of MP in the current study corresponded exactly with those that showed heightened conditioning over a trace interval under d-amphetamine (Norman and Cassaday, 2003).

**Reshaping** On the day following conditioning, animals were reshaped following the same procedure as in preconditioning sessions. This was in order to re-establish drinking after conditioning. Reshaping also provided measures of conditioning to the box context: latency to first lick and the pattern of licking over the first 5 min of the session.

**Test** There were two test days, one for each type of stimulus, with the order of testing counterbalanced such that half the rats were first tested for conditioning to CS (noise) and half the rats were tested for conditioning to the background stimulus (light). The time taken to complete 50 licks prior to any stimulus presentation (the A period) provided a measure of any individual variation in baseline lick responding, to be compared with the time taken to complete 50 licks during stimulus presentation (the B period) in a suppression ratio (measured as A/(A+B), to assess conditioning whilst taking baseline variation into account). The closer the ratio gets to zero, the higher the learning. These were extinction tests and both stimuli were presented continuously throughout the session, so in each case the B period was a maximum of 900 s, for rats that did not complete 50 licks within the 15 min session. An additional measure of conditioning was provided by the pattern of drinking over the first 5 min of the test session.

**Design and analysis**

The experiment was run in a 3 x 2 factorial design for later analysis of variance (ANOVA). Thus 72 rats were assigned to six experimental conditions, counterbalanced for box. The between subjects factors were Drug (at levels saline, 1 mg/kg and 5 mg/kg MP) and trace (at levels 0 s and 30 s). The dependent variables to assess conditioning at test were the suppression ratio and the number of licks made over the first 5 minutes of stimulus presentation, broken down into five 1 minute bins. The full duration of stimulus presentation was 15 minutes; all analyses are restricted to the first five bins (cf. Tai et al., 1995, Experiment 1; Cassaday et al., 2001). The correspondence between results obtained with licks measures and the suppression ratio has to depend on the level of suppression seen in any particular experiment, because of the way the ratio is calculated. To anticipate, in the present study, there is justification for focusing on early drinking because this is where we would expect effects on associative learning to be seen at relatively low levels of suppression (as were seen here). The rationale is that we should in each case evaluate treatment effects on the dependent variable(s) that show the behavioural (here ‘trace’) effect of interest in the control treated groups: high conditioning in the 0 s group; at the same time low conditioning in the corresponding 30 s group.

Preconditioning drink levels were also assessed in a 3 x 2 mixed design ANOVA with the repeated measures factor of days (at ten levels) to check for any pre-existing differences in total amount drunk. The reshape latencies provided a measure of conditioning to the apparatus contextual cues (in the absence of any experimental stimulus) by drug and trace condition: measured as latency to first lick and the pattern of drinking over the first 5 min of the session (Cassaday et al., 1991, 2005). As above, when in the session the rats start drinking determines which of the lick bins will be informative, and in the present study they drank early.

All ANOVAs used an alpha level of 0.05. Significant main effects and interactions were explored by two-tailed t-tests. When exploring significant main effects in the absence of interactions, the t-tests are collapsed across groups. To explore intersections we made only the relevant pair-wise comparisons that were necessary, in order to determine the basis for any difference in the level of conditioning supported by trace and contiguous Cs’s under MP. Given that comparisons were planned, and were only a small subset of the possible comparisons, then the inflation of family-wise Type 1 error rate was not very large (Howell, 2002). The licks measure generated the largest number of possible comparisons (because of the repeated measures factor). However, there are clear a priori grounds to focus on the earliest bins in which behavioural differences are apparent (Tai et al., 1995, Experiment 1; Cassaday et al., 2001) and this further limits the number of comparisons required (Ableson, 1995). Moreover, in each case, the licks analyses supported the conclusions to be drawn from the latency analyses.
Results

Baseline responding

Analysis of the total amount drunk on baseline days showed that the groups were well matched prior to drug treatment and conditioning, with no main effects for drug or trace group allocation and no interaction between these factors (all Fs < 1).

Reshape

Latency measure  Times to first lick showed no evidence of any overall effects of MP or trace group on conditioning to apparatus cues [maximum F (2,66) = 1.92].

Licks measure  There was a progressive reduction in drinking over the session consistent with satiation indicated by a main effect of Bins [F (4,264) = 27.49, p < 0.001] which was also unaffected by Prior Drug or Trace [maximum F (4,264) = 2.11]. The Prior Drug × Trace × Bins interaction was significant [F (8,264) = 2.12, p < 0.05]. Based on previous work, the early drinking shown at reshape (Fig. 1) should be used to assess MP effects on contextual conditioning (Tai et al., 1995, Experiment 1; Cassaday et al., 2001). Planned comparisons showed that the earliest differences to which we could attribute this interaction were in the second minute of drinking on the reshape test (i.e. bin 2). These differences were confined to the trace conditioned groups: under both 1 mg/kg MP [t (22) = 2.12, p < 0.05] and 5 mg/kg MP [t (22) = 2.75, p = 0.01] rats drank significantly less than the 30s conditioned saline group. This is consistent with an increase in contextual conditioning under MP in trace conditioned groups, for which the cues provided by box context should potentially provide a more reliable predictor of footshock. Further comparison showed that, in the fourth minute of drinking (i.e. bin 4) the 0s conditioned group treated with 5 mg/kg MP made significantly fewer licks than any other group at this stage of the reshaping session [minimum t (22) = 2.19, p < 0.05]. However, this relatively late effect does not necessarily reflect increased contextual conditioning in this group, because the onset of satiety can compromise the extinction measure of learning provided by the later bins (Tai et al., 1995, Experiment 1). Otherwise there were no significant differences [maximum t (22) = 1.94].

Test: target stimulus

Latency and suppression ratio measures  Analysis of latencies to make first 50 licks (A periods) prior to presentation of the target

![Figure 1](00_JOP668_067381_000-000.pdf)
noise CS showed no significant main effects for prior drug or trace or interactions between these factors [maximum F (2,66)=1.21]. The suppression ratios showed that, as expected, contiguously conditioned rats were significantly more suppressed than those conditioned with a 30 s trace [F (1,66)=6.35, p < 0.05]. There was a significant effect for prior drug [F (2,66)=5.18, p<0.05]: both doses of MP resulted in greater suppression compared to saline controls. The Prior Drug × Trace interaction was not significant [F (2,66)=0.72]; however, means indicated that treatment at both doses of MP produced the largest increase in conditioning to the 30 s CS (see Fig. 2). Pair-wise comparisons showed that the differences between MP (at both doses) and saline in the 30 s group were significant [minimum t (22)=2.12, p<0.05], but that in the contiguous groups, these differences were not significant [maximum t (22)=1.58]. Thus the main effect of prior drug came about because of increased conditioning that was primarily restricted to the 30s trace group. This means that under MP rats nonetheless conditioned to a stimulus that would normally be treated as uninformative.

**Licks measure** Differences in conditioning (shown by the suppression ratio measure) were confirmed by the amount drunk over time as follows: analysis of the pattern of drinking over bins 1–5, showed a significant effect for bins [F (4, 264)=34.57, p<0.001]. Bins also interacted (independently) with trace and with prior drug [minimum F(4, 264)=2.55, p<0.05]. The trace × bins interaction was explored further with independent t-tests; these confirmed that in bin 1, contiguously conditioned rats licked less overall (showing higher learning in line with the lower suppression ratio results) than those conditioned with a trace [t (70)= 2.11, p<0.05]. Pair-wise
comparisons during the first minute of drinking – which reliably reflect differences in learning at these levels of conditioned suppression (Tai et al., 1995, Experiment 1; Cassaday et al., 2001), and where the trace effect was demonstrated in the present study - showed that there was an overall reduction in licking at both doses of MP compared to saline (minimum t (46)=2.34, p<0.05). No other differences by drug were significant (maximum t (46)=1.49).

The prior drug × trace × bins interaction (Fig. 3) did not reach significance [F (8, 264)=1.66] but there was some evidence for a differentially greater effect in trace conditioned groups in bin 1 (where the trace effect was demonstrated, see above): pair-wise comparisons showed that rats in the 5 mg/kg dose group [t (22)=2.15, p<0.05], but not in those the 1 mg/kg dose group [t (22)=1.64], were significantly more suppressed than the corresponding saline group. No significant differences by drug were seen in the contiguous group [maximum t (22)=1.91].

In summary, tests of responding to the target (noise) CS showed that, as expected, conditioning was more effective in the 0s compared to the 30 s trace group overall. Prior treatment with MP resulted in heightened aversive conditioning most prominently in the 30 s trace group. The trace × bins interaction in this experiment arises from clear differences in the first minute of drinking, when (overall) continguously conditioned rats licked significantly less than those conditioned with a trace. Therefore, it is appropriate to focus on early drinking to evaluate MP effects on conditioning to the trace CS. In line with the suppression ratio results, in the first minute of drinking, there was statistical support for the view that MP increased conditioning the trace conditioned groups (in this case at 5 mg/kg).

Test: background stimulus

Latency and suppression ratio measures Analysis of A periods prior to presentation of the flashing light background stimulus showed no significant main effects for prior drug or trace or interactions between these factors [maximum F (2,66)=1.10]. On the suppression ratio measure of responding during presentation of the background stimulus, there was no significant main effect of trace, nor did trace interact significantly with prior drug [maximum F (1,66)=1.36 – see Fig. 4]. There was however a significant main effect of prior drug [F (2, 66)=4.63, p<0.05]. At both doses, MP groups were significantly more suppressed than saline treated rats [minimum t=2.23,p<0.05] so there was increased conditioning to the background stimulus under MP.

Licks measure Analysis of the pattern of drinking over Bins 1–5 showed a significant interaction between prior drug × bins [F (8,264)=2.12, p<0.05]. Independent t-tests showed that in bin 1, at the 5 mg/kg dose, there was a reduction in licking compared to saline [t (46)=3.44, p<0.001]. This confirms the result shown on the suppression ratio measure in Fig. 4 (licks data not shown) that there was increased conditioning to the background stimulus under MP. The reduction was dose related but not statistically significant at the 1 mg/kg dose [t (46)=1.77]. The trace × bins and the prior drug × trace × bins interactions were not significant [maximum F (8,264)=1.57].

Discussion

Learning that a CS (e.g. noise) predicts biologically relevant outcomes (UCSs) such as food or footshock is normally reduced when these events are separated in time. This effect was seen in the aversive trace conditioning variant tested. There was also clear evidence that MP affected the associative learning processes under test.

Prior treatment with MP (at both 1 mg/kg and 5 mg/kg doses) had the effect of generally increasing conditioning to the target CS. Statistically, this effect was seen irrespective of whether the CS preceded the UCS by a 0 s or 30 s interval, however further analysis demonstrated that this increase was primarily restricted to the MP groups conditioned with a 30 s trace. Thus the effect of MP shown here was a general facilitation of conditioning but most particularly to a cue that was rendered less informative through separation in time from the UCS. Consequently, MP treatment resulted in a loss of selectivity in learning because of potentiated conditioning to the weak 30 s CS.

These effects shown first on the suppression ratio are confirmed on the licks measure of learning. There is justification for focusing on early drinking because this is where we would expect effects on associative learning to be seen at the relatively low levels of suppression shown here (cf. Tai et al., 1995, Experiment 1; Cassaday et al., 2001) and indeed this is where the trace effect was demonstrated in the present study. The drug effect of increased conditioning under 5 mg/kg MP seen in the trace conditioned groups was demonstrated in the first minute of drinking when (overall) continguously conditioned rats licked significantly less than those conditioned with a trace.

An increase in contextual conditioning was also observed; this was shown by greater levels of conditioning to the flashing light background stimulus at test. This was clear at both MP doses on
suppression ratios and confirmed on the licks measure for the high dose during the first minute of drinking. There was also some evidence from the licks reshaping data for increased contextual conditioning to the cues provided by the experimental apparatus.

**Do baseline levels of activity or motivation confound interpretation of the results?**

Before firm conclusions can be drawn with respect to the effects of MP treatment on associative learning, pre-existing levels of baseline activity and responding need to be eliminated as possible confounds. Baseline lick rates were measured prior to drug treatment and conditioning: analyses verified that groups were well-matched prior to conditioning. After conditioning on the drug-free reshaping day, there were some group differences consistent with MP-induced increased conditioning to contextual cues. However, this effect (shown as suppression to a box context) was transient and all the rats were licking freely for the drug-free extinction tests. Separate analysis of responding in the A periods demonstrated no significant effects by prior drug treatment or behavioural condition that would confound interpretation of effects on the level of suppression shown to the experimental stimuli. Moreover, suppression ratios were calculated in order to adjust for potential differences in individual lick rates on a case by case basis.

**MP increased conditioning to discrete and contextual cues**

There was a loss of selectivity in the MP-treated groups because of increased conditioning to the weakly predictive 30 s CS. This is of particular theoretical relevance to the proposition that those with ADHD may be intolerant of delay (e.g. Sonuga-Barke, 2002; Sagvolden et al., 2005). Studies in humans have shown that participants with ADHD show a greater bias (than controls) for smaller immediate rewards compared to larger rewards in the long term (Rapport et al., 1986; Sonuga-Barke et al., 1992; Tripp and Alsop, 2001). Moreover in rats, treatment with amphetamine increased animals’ capacity to choose larger long-term rewards over smaller short-term rewards whereas lesions to the core subterritory of the nucleus accumbens reduced this capacity (Wade et al., 2000; Cardinal et al., 2001). Here MP increased conditioning to a predictor that was rendered less effective by the introduction of a time interval between CS offset and UCS onset in trace conditioned groups. This suggests that MP (like d-amphetamine) somehow increased the rats’ capacity to nonetheless form an association over a trace interval. However, the present study was not designed to determine precisely how such effects of MP on conditioning were mediated, an increase in the capacity to make the association between CS and UCS over a trace might occur in a number ways including drug effects on the CS (e.g. CS salience), on the UCS (e.g. reinforcer impact) and/or the timing of the trace interval.

Rats treated with MP also showed increased learning about the context provided by the flashing light background stimulus, irrespective of whether the CS had been presented at 0 s or 30 s trace. In untreated animals this stimulus was too weak to support good levels of learning. As increased conditioning has been observed to both the discrete 30 s CS and also to a background contextual CS, this suggests that the mechanism by which MP exerts its therapeutic effects might be broader: generally enhancing learning about a wider range of available cues, rather than through specifically strengthening associations over a trace interval.

**Dose-related effects**

In designs of this kind, where there are two behavioural conditions that require quite large numbers of subjects per experimental group it is usual to justify testing a couple of doses that are of a priori interest. This means that we cannot exclude the possibility that we would have found different results had it been possible to test a wider range of doses. The doses used here were selected for comparability with typical human clinical doses (Brandon et al., 2001) although therapeutically MP is given chronically. At neither dose tested was there any indication of non-specific effects of drug treatment such as hyperactivity that might be observed at higher doses (e.g., 10 mg/kg acute dose MP increases locomotor activity: Gaytan et al., 1997; Crawford et al., 1998). Moreover, the increased suppression that we observed was manifest as reduced activity (though this was not a direct effect of treatment with MP as rats were tested drug free). As hyperactivity is not usually a consequence of therapeutic MP treatment (Brandon et al., 2001), then this provides a further indication that the doses tested here in rats may be of relevance to effects of MP in humans.

However effects of MP seen here in a highly motivating aversive task variant might not necessarily transfer to differently motivated task variants as the effect of MP is likely to interact with task difficulty, the reinforcing capacity of the UCS and with baseline level of arousal. In humans, clinical doses of MP have been shown to exert greater neurochemical effects during more highly motivated tasks (Volkow et al., 2004).

**How do these effects compare with those previously reported for amphetamine?**

In aversive trace conditioning, d-amphetamine has been shown to increase conditioning to discrete and contextual cues (Norman and Cassaday, 2003). Here MP, like d-amphetamine, increased conditioning to both discrete and contextual stimuli. MP led to a loss of selectivity in the present study because the increase in conditioning was primarily seen to the weaker 30 s trace CS. Treatment with d-amphetamine has the same effect (Norman and Cassaday, 2003: Experiment 3). Both d- and dl-amphetamine similarly enhanced aversive conditioning to the flashing light background stimulus (Norman and Cassaday, 2003: Experiments 1, 2 and 3) irrespective of whether the target CS was trace or contiguously conditioned. Overall, the effects of MP and d-amphetamine on aversive trace conditioning are very similar, consistent with similar mechanisms of action. Both amphetamine and MP increase DA in the striatum (Kuczenski, 1983; Rosa-Neto et al., 2005) and they both act on the DA transporter (DAT), although MP also inhibits DA reuptake (Chiu et al., 1975a, 1975b; Hurd and Ungerstedt, 1989; Giros et al., 1996; Volkow et al., 1998). This suggests that the therapeutic properties of these drugs in ADHD...
may arise from a common action of increasing extracellular DA availability to post-synaptic receptors (probably in the nucleus accumbens) possibly via effects on DAT. Moreover, recent PET studies have shown a correlation between methylphenidate-induced changes in striatal dopamine and behavioural measures of ADHD (Rosa-Neto et al., 2005).

Conclusions and implications

We found that MP generally promoted conditioning to a range of cues that are predictive of footshock UCS, and most particularly to weaker predictors: here, increased conditioning to a 30 s trace CS and an experimental background stimulus was observed. This was contrary to the prediction that MP might act to focus, rather than broaden attention and is consistent with a loss of selectivity. Usually a loss of selectivity is considered to be detrimental to cognition, for example, the loss of LI that can be produced by both d- and dl-amphetamine (Solomon et al., 1981; Weiner et al., 1981, 1988; Gray et al., 1992; Kumari et al., 1999). However, whether the effects under MP seen here in a trace conditioning procedure should be thought of as ‘impairment’ is arguable. ADHD sufferers have been characterized as delay-averse (Sonuga-Barker 2002, 2005) and argued to have a steeper delay-of-reinforcement gradient (Sagvolden et al., 1998, 2005). In the present study, MP increased conditioning over a trace interval, consistent with the potential to improve this aspect of cognition. Moreover, identifying cognitive enhancement is complex in that, in line with the Yerkes–Dodson law, effects of stimulants in ADHD have been shown to depend on the baseline level of arousal and task difficulty (Rapport and Kelly, 1991).

The direction of effects of MP on the attentional window may be less relevant to its mechanism of action than the fact an effect was clearly demonstrated.

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