Amphetamine increases schedule-induced drinking reduced by negative punishment procedures

Abstract Rationale: d-Amphetamine has been reported to increase schedule-induced drinking punished by lick-dependent signalled delays in food delivery. This might reflect a drug-behaviour interaction dependent on the type of punisher, because no such effect has been found when drinking was reduced by lick-contingent electric shocks. However, the anti-punishment effect of amphetamine could be mediated by other behavioural processes, such as a loss of discriminative control or an increase in the value of delayed reinforcers. Objectives: To test the effects of d-amphetamine on the acquisition and maintenance of schedule-induced drinking reduced by unsignalled delays in food delivery. Methods: Rats received 10-s unsignalled delays initiated by each lick after polydipsia was induced by a fixed-time 30-s food reinforcement schedule or from the outset of the experiment. Yoked-control rats received these same delays but independently of their own behaviour. d-Amphetamine (0.1–3.0 mg/kg) was then tested IP. Results: d-Amphetamine dose-dependently increased and then decreased punished schedule-induced drinking. The drug led to dose-dependent reductions when the delays were not contingent or when they were applied from the outset of training. Conclusions: These results support the contention that d-amphetamine has an increasing effect on schedule-induced drinking that has been previously reduced by a negative punishment procedure. This effect cannot be attributed to other potentially involved processes, and therefore support the idea that drug effects on punished behaviour depend on punishment being delays in food or shock deliveries.

Keywords Schedule-induced drinking · Lick-dependent delays · d-Amphetamine · Rats
delays in the presentation of the reinforcer had the effect of attenuating the acquisition of schedule-induced polydipsia as compared to drinking levels attained by yoked control rats.

Pellón et al. (1992) examined the effects of amphetamine and diazepam on schedule-induced polydipsia punished by the occurrence of 10-s lick-dependent signalled delays in food delivery. d-Amphetamine had little or no effect on unpunished behaviour, but increased punished schedule-induced drinking. Diazepam slightly increased unpunished adjunctive drinking, but had no effect on punished behaviour. Flores and Pellón (1998) punished schedule-induced polydipsia with electric shocks and observed that diazepam, but not amphetamine or buspirone, increased punished schedule-induced drinking (also see Flores and Pellón 2000). The effect of any given drug on punished schedule-induced polydipsia seems to depend to a marked degree on the type of punishment procedure used to reduce the behaviour (Pérez and Pellón 1998). Similar results have been observed with respect to operant behaviour (Branch et al. 1977).

Some variables could be mediating amphetamine’s behavioural effects, however, and could be reflecting an apparent anti-punishment effect of the drug. Delays in food presentation were signalled by a blackout, and amphetamine might therefore be simply affecting the degree of such discriminative control (Katz 1988; Wenger et al. 1995). The effects of the drug could also be mediated by a change in reinforcement frequency, since any increase in the behaviour was inevitably accompanied by a decrease in the rate of food delivery. Amphetamine may also increase the value of the delayed reinforcers (LeSage et al. 1996; Richards et al. 1999); stimulants tend to increase the preference for large delayed reinforcers over small immediate ones (Wade et al. 2000), an effect that in the case of amphetamine depends critically on delays being signalled (Cardinal et al. 2000). Finally, amphetamine leads to a leftward shift in the temporal distribution of adjunctive behaviour at doses that have no effect on the overall response rate (e.g. Sanger 1978; Williams and White 1984; Pellón and Blackman 1992; Flores and Pellón 1997). Such a response-distribution effect might reflect a subjective shortening of the time that has to pass in order to receive the next food pellet (Mariq et al. 1981), thus reducing the aversiveness of delayed reinforcers.

The experiments described below sought to determine whether amphetamine exerts specific anti-punishment effects in cases where adjunctive drinking has been reduced by the withdrawal of food presentation. The effects of the drug after suppression of the behaviour by contingent delays (maintenance condition) were compared with the effects of the drug on behaviour that had developed with contingent delays from the outset of training (acquisition condition). If the drug acts through the general effect of reducing the effectiveness of the delays to control behaviour, then increases in schedule-induced polydipsia following administration of d-amphetamine would be expected in all conditions. If the drug has specific anti-punishment effects, then, notwithstanding the delays, no increases in adjunctive behaviour should be observed in the acquisition condition, in contrast to what should occur in the maintenance condition. Delays were not signalled in any case and experiments incorporated control animals yoked with respect to food presentation but with delays being not contingent upon their licking. The drug was tested simultaneously on these animals and on the experimental rats, but also d-amphetamine was administered to yoked control rats on sessions in which experimental rats were tested without the drug.

Materials and methods

Subjects

Twenty-four experimentally naïve male Wistar rats, obtained from IFFA-CREDO (Lyon, France) were used. All animals were monitored and fed daily, and at 90 days of age began the experiment. They were individually housed in comfortable home cages, and placed in a room with rigorously controlled environmental conditions (ambient temperature 21°C, 60% humidity, and an 8 a.m.:8 p.m. light/dark cycle). The rats were gradually reduced to 80–85% of their ideal free-feeding weight through food restriction and then maintained at this level of deprivation on the basis of a growth curve. Mean weight at the beginning of the experiment was 421 g (range 318–468 g). Animals were weighed daily at the beginning of each experimental session, had their diet calculated and were fed a minimum of 30 min after the end of the session. Water was freely available in all animals’ home cages. All animal use procedures were in accordance with the European Communities Council Directive 86/609/EEC and the Spanish Royal Decree 223/1998 on minimizing stress and discomfort in animals.

Apparatus

The experiment was conducted in six, 29×24.5×35.5 cm, Letica Instruments LI-836 rodent conditioning chambers (Barcelona, Spain). The chambers were enclosed in a soundproofed housing, equipped with a ventilation system and a small observation window in the left panel. The front panel of each chamber was made of aluminium, the left-hand wall and roof of transparent Plexiglas, and the remaining sides of black Plexiglas. Levers in all chambers were permanently withdrawn. A water bottle was attached to the external side of the right wall, with its spout accessible to the subject through a 3.2×3.9 cm aperture, located 20 cm from the front wall and 7 cm above the floor. The spout was positioned 2 cm from the wall aperture, in such a way that the rat could lick but not maintain permanent contact with it. Licks at the spout were detected when the electric circuit between the 16 parallel metal bars comprising the grid floor and the drinking bottle spout was completed via contact with the animal’s tongue. The chambers were illuminated by two internal 3-W bulbs, placed on the upper part of the front panel to either side of the food hopper, and a 25-W ambient light fitted to the external housing. The ambient noise produced by the ventilation was 60 dB, which served to mask any other possible external sounds. With the aid of a Letica Instruments dispenser fitted to the outside of the front panel, 45-mg food pellets (Bio-Serv) could be dropped into a small internal receptacle, situated on the front wall at a height of 3.7 cm above the floor, which served as a food tray. Events were scheduled and recorded using an Archimedes microcomputer (Paul Fray Ltd) installed with the Arachnid software package.
Behavioural procedure

On being stabilised at 80–85% of their free-feeding weight, all rats were subjected to a water-intake test. For 2 consecutive days, 60 food pellets (45 mg) were deposited into the home cages and then the total amount of water consumed over 30 min was measured. This procedure furnished a baseline rate against which to compare the level of schedule-induced drinking observed in the planned 30-min experimental sessions, which called for intermittent delivery of the same number of food pellets as had been received by the animals in their home cages over the test period (Roper 1981; Cohen and Looney 1984; Pellón and Blackman 1987).

The rats were then exposed to an adaptation session in the conditioning chambers for 30 min, with 60 food pellets previously deposited in the food tray, ventilation and illumination provided, but no experimental contingency in operation. During this session no water bottles were installed.

Maintenance with lick-contingent delays

Twelve rats were exposed to 30-min sessions with an FT 30-s (FT30) schedule of food-pellet presentation. Each food pellet was regularly deposited in the food tray at 30-s intervals, regardless of the animal’s behaviour. Prior to each session, bottles containing 100 ml of fresh water were installed as described above. Lights were switched on at the beginning and switched off at the end of each session. Each session concluded 30 s after delivery of the 60th food pellet.

When, after 35 sessions and based on visual inspection, schedule-induced drinking had become stabilised for each rat, the animals were matched into six pairs according to the amount of water consumed and number of licks recorded at the end of exposure to the FT30 schedule. In each pair, one rat was randomly designated as “experimental” and the other as its “yoked control” for the remainder of the experiment. Food continued to be delivered according to the FT30 schedule. Now, however, every lick given by an experimental rat triggered an unsignalled 10-s delay in the delivery of the next food pellet. When no lick was given for 10 s, the FT30 schedule was renewed from the point at which it had been interrupted. Parallel sessions were conducted for controls in conjunction with and subject to those for the experimental rats. Control rats were subjected to the same delays as their experimental counterparts but independently of their own drinking behaviour, with food pellets being simultaneously administered to both control and experimental animals. For each pair of animals, the session concluded 30 s after the last food pellet had been administered within the permitted 30-min period.

Acquisition with lick-contingent delays

The remaining 12 rats were matched on the basis of their water intake levels during test sessions in their own home cages. In each pair, one rat was randomly designated as “experimental” and the other as its “yoked control”. In the absence of licks on the part of the experimental rats, a food pellet was released every 30 s in accordance with the FT30 schedule. Concurrently, each lick of the experimental rats initiated a 10-s delay in the administration of the food pellet.

Pharmacological procedure

Each rat in the maintenance experiment was exposed to administration of s(+)-amphetamine sulphate (dextroamphetamine sulphate) at doses of 0.1, 0.3, 1.0 and 3.0 mg/kg; and then, but just yoked control rats, again to d-amphetamine at doses of 0.3 and 1.0 mg/kg. Rats in the acquisition experiment were administered with d-amphetamine at doses of 0.1, 0.3 and 1.0 mg/kg.

The drug was dissolved in 0.9% saline solution and administered (IP) at a volume of 1 ml/kg, 10 min prior to the experimental session. Drug doses were administered following a random order. All rats received the same dose on the same day, and the yoked procedure remained in place throughout all drug-based sessions. Each drug administration session was preceded by a control session, during which the animals were either not injected or were injected with saline at a volume of 1 ml/kg, 10 min prior to the experimental session. Drug-based sessions were held on Tuesdays and Fridays, the vehicle was administered on Thursdays, and Mondays and Wednesdays were sessions without any injection, but only Monday sessions served as control.

For each rat in each session, the following data were recorded: the number of licks, the amount of water consumed, the number of food pellets obtained and the total duration of the session. The number of licks was converted into licks per minute, and the dose-based percentage change in the lick rate calculated for sessions with injection vis-à-vis the control sessions without injection. The percentage of the control rate was calculated using the following formula: rate of response in the presence of any given drug dose divided by mean response rate in control sessions, multiplied by 100.

Statistical analysis

Licks per minute and volume of water consumed were subjected to two-factor analyses of variance (ANOVA), with a between-group factor (Group or Sequence of Administration) and a within-subject factor (Dose). When necessary, the post-hoc comparisons were calculated by the Newman-Keuls test. All these analyses were performed using the Statistica 5.0 package. The significance level was set at minimum P<0.05.

Results

Maintenance with lick-contingent delays

Table 1 shows the means and standard errors for both experimental and yoked control animals with respect to delays were also applied to the yoked control rats, but in this case were not contingent on their own licks.

When suppressed schedule-induced drinking failed to show systematic variation from one session to another in terms of number of licks and water intake per animal (something that occurred after 30 experimental sessions), all animals received administrations of d-amphetamine as described below under Pharmacological procedure.

Table 1 Maintenance with lick-contingent delays. Means (±SE) for water intake (ml) and licks per minute for rats in the experimental and yoked control groups for the 2 test days in the home cages and the last five sessions in the acquisition and punishment phases

<table>
<thead>
<tr>
<th></th>
<th>Experimental</th>
<th>Yoked control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water (ml)</td>
<td>Licks/min</td>
</tr>
<tr>
<td>Baseline</td>
<td>5.75±1.69</td>
<td>–</td>
</tr>
<tr>
<td>Acquisition</td>
<td>24.83±5.16</td>
<td>80.50±16.45</td>
</tr>
<tr>
<td>Punishment</td>
<td>10.10±4.07</td>
<td>25.46±15.20</td>
</tr>
</tbody>
</table>
volume of water consumed over the 2 test days in home cages prior to the start of the experimental sessions, and volume of water consumed and licks per minute for the last five experimental sessions in the acquisition and punishment phases.

The experimental and control groups consumed almost equally small amounts of water during the two baseline days in their home cages. After exposure to the FT30 schedule, a similar marked increase in water intake was observed for both groups, of approximately 4 orders of magnitude above the baseline level. These increases in total water intake were accompanied by a high lick rate, of around 80 licks per minute, indicating that, overall, the animals in this experiment developed schedule-induced polydipsia through intermittent presentation of food at regular 30-s intervals. With the introduction of contingent delays, the experimental group of subjects were observed to decrease their drinking level as against the level registered at the end of the acquisition phase, in terms both of water intake and of licks per minute. The procedure proved effective to punish adjunctive drinking. During this experimental phase, the control group maintained their behaviour at the level registered in the preceding phase.

Figure 1 depicts the effects of the different doses of d-amphetamine on the lick rate and volume of water consumed during punishment with unsignalled delays. The drug’s effect on behaviour is expressed as the mean and standard error for the percentage change on the control rate without injection. Vehicle (V) data correspond to the mean for licks per minute or millilitres of water consumed of the total number of sessions that preceded drug sessions and in which saline was administered.

Administration of the vehicle failed to result in significant changes with respect to control sessions without injection. In contrast, d-amphetamine led to a dose-dependent increase and subsequent decrease in licks per minute and water intake among the experimental rats. The increasing effect was more pronounced in terms of licks per minute. The drug had no effect on drinking among controls, except at a dose of 3.0 mg/kg, which reduced the licks per minute and the ensuing volume of water consumed. Analyses of variance revealed a group effect \(F(1,10)=7.60, P<0.05\), a dose effect \(F(5,50)=10.98, P<0.01\), and a group\times dose interaction \(F(5,50)=7.36, P<0.01\) with respect to the lick rate. In terms of volume of water consumed, statistical analyses likewise revealed a significant group effect \(F(1,10)=9.69, P<0.01\), dose effect \(F(5,50)=13.76, P<0.01\), and group\times dose interaction \(F(5,50)=6.33, P<0.01\). Post-hoc analysis confirmed that the 1.0 mg/kg dose led to a statistically significant increase \(P<0.01\) in the lick rate and volume of water consumed by the experimental rats, and that the 3.0 mg/kg dose led to decreased drinking by both experimental and yoked control rats \(P<0.05\).

Figure 2 depicts the effect exerted by the second administration of d-amphetamine on adjunctive drinking in the yoked controls during application of delays in food delivery. The filled circles denote the effect on the control animals of the first exposure to doses of 0.3, 1.0 and 3.0 mg/kg of d-amphetamine (see Fig. 1) and the open circles the effect of the second administration of the drug. The vehicle had no appreciable effect on adjunctive drinking. Administration of d-amphetamine led to a dose-dependent decrease in licks per minute \(F(4,20)=22.03, P<0.01\) and water intake \(F(4,20)=33.53, P<0.01\). The interaction of sequence of administration\times dose was also statistically significant, both in licks per minute \(F(4,20)=3.04, P<0.05\) and water consumption \(F(4,20)=3.17, P<0.05\). The reductions in drinking measures occurred at a dose of 3.0 mg/kg after the first administration of the drug and at a dose of 1.0 mg/kg on the second occasion \(P<0.05\).

Acquisition with lick-contingent delays

Table 2 shows the means and standard errors for the following: volume of water consumed over the 2 test days in their home cages prior to the start of the experimental
sessions; and water intake and licks per minute for the last five experimental sessions in the training phase.

The experimental and control groups consumed almost equally small amounts of water during the 2 baseline days in their home cages. In the last five sessions of the training phase, there was a very slight increase in the volume of water consumed by the group of experimental rats exposed to the lick-delay contingency. The yoked controls, which were subjected to the delays non-contingently, drank a large amount of water at the end of the training phase, an increase in the order of 6 over their intake under control conditions in their home cages.

On termination of training, the differences between the experimental and yoked control rats were also observable in the lick rate. The mean licks per minute for controls was approximately 60, almost 7-fold the value registered by the experimental rats.

Figure 3 shows the effects of different doses of d-amphetamine on the lick rate and volume of water consumed at the end of the training phase with unsignalled delays. The drug’s effect on behaviour is expressed as the mean and standard error for the percentage change on the control rate without injection. Vehicle (V) data correspond to the mean for licks per minute or millilitres of water consumed over the total number of sessions in which saline was administered.

Administration of the vehicle failed to result in significant changes compared with control sessions without injection. However, d-amphetamine led to dose-dependent reductions in experimental rats and yoked controls, in terms both of licks per minute and of water intake. The main dose effect was statistically significant in both cases [F(4,40)=12.27, P<0.01, for licks per minute; and F(4,40)=5.82, P<0.01, for volume of water consumed].

**Discussion**

Rats were subjected to an FT30 food delivery schedule. In the absence of lick-contingent delays in the presentation of the food pellet, the animals developed schedule-induced polydipsia measured in terms of licks per minute and water intake. Acquisition of schedule-induced polydipsia could be attenuated if each lick initiated a 10-s delay in the presentation of the following food pellet. Normally acquired polydipsia was reduced with the subsequent introduction of lick-contingent delays. These results demonstrate yet again that lick-contingent delays in food presentation can serve to reduce normal development of schedule-induced polydipsia or punish previously acquired polydipsia (Flory and Lickfett 1974; Moran and Rudolph 1980; Pellón and Blackman 1987, 1991; Lamas and Pellón 1995a, 1995b, 1997; Pellón and Castilla 2000).

Administration of 1.0 mg/kg d-amphetamine increased adjunctive drinking punished by lick-contingent delays in food delivery. This effect was not observed in cases where the delays were non-contingent or where contingent delays were applied from the outset of training. As a whole, these results demonstrate that d-amphetamine has an increasing effect on schedule-induced polydipsia in cases where this has been previously reduced by a negative punishment procedure. These findings are in line with previous results (Pellón et al. 1992) and rule out several alternative explanations.

The fundamental differences between this study and that undertaken by Pellón et al. (1992) were as follows: delays in the presentation of food were not signalled; yoked control animals were included; and the effects of d-amphetamine were tested on controls in the absence of effects of same on the behaviour of the experimental rats. All these variations are extremely important for a better understanding of the nature of the anti-punishment effect exerted by d-amphetamine.

Adjunctive behaviour reduced by the contingent introduction of unsignalled delays in food presentation (a negative punishment procedure) proved to be selectively increased by administration of d-amphetamine. This effect was not observed in the yoked control rats that were subjected to the same delays as their respective
experimental counterparts, albeit non-contingently. The reduction in the frequency of food presentation caused by drug-induced increases in punished drinking can in no way account for the observed effect, since similar increases should otherwise have been seen in the control rats after administration of d-amphetamine. Indeed, when the drug was administered solely to controls and not to the experimental animals, d-amphetamine was likewise observed to have no increasing effect.

The increasing effect of amphetamine likewise would not appear to be mediated by drug-induced disruptions in the degree of stimulus control exerted by the non-reinforcement signals. Similar results have been reported, not only where the delays in the reinforcer were signalled, as in the case of Pellón et al. (1992), but also where they were not signalled, as in the case of the present study.

It has been repeatedly shown that amphetamine some times increases (Richards et al. 1999; Cardinal et al. 2000) and at other times decreases (Charrier and Thiébot 1996; Evenden and Ryan 1996) the reinforcing efficacy of delayed outcomes. Whatever the direction of the effect, however, similar results should have been observed for the drug in the two studies reported here, in which identical delays were presented, albeit in different contexts. The fact that d-amphetamine’s increasing effect is only felt in cases where the behaviour has been acquired and subsequently punished would support the contention that the drug’s anti-punishment effect cannot be explained by a mechanism which involves any alteration in the strength of the delayed reinforcers.

There would seem to be a certain degree of pharmacological specificity dependent on the type of punishment procedure used to reduce behaviour, at least in the case of adjunctive behaviour studies (Pérez and Pellón 1998). Where schedule-induced polydipsia is punished by delay in the presentation of the food reinforcer, behaviour is increased after administration of psychostimulants (Pellón et al. 1992); and where schedule-induced polydipsia is punished by contingent presentation of electric shocks, behaviour is increased after administration of anxiolytics (Flores and Pellón 1998, 2000). This behavioural dependence on the anti-punishment effect of drugs emphasizes once again the need for appropriate functional analysis of the conditions that maintain behaviour in order to predict drug effects (see Barrett 1985).

Furthermore, insight into the effects of drugs on punished adjunctive behaviour has important theoretical implications for a better understanding of the nature of adjunctive behavior itself. While better characterisation is still needed of the effects of psychostimulants on operant behaviour punished by the withdrawal of the positive reinforcer, in general there are strong analogies between the respective effects of drugs on adjunctive and operant behaviour (see Pellón et al. 1998). There is evidence to show that the effects of d-amphetamine sometimes depend upon the type of event that maintains a given positive reinforced operant behaviour (Johanson 1978; but see Barrett 1987). Amphetamine also affects negatively reinforced operant behaviour as a function of the event to be postponed. The drug selectively increases the response rate maintained for a time-out period of avoidance at doses that do not affect the response rate maintained by postponing an electric shock (Galizio and Allen 1991).

Such similarities allow for a conceptualization of adjunctive behavior in terms similar to operant behaviour, as well as questioning certain concepts about the inhibition mechanisms underlying the application of punishment procedures. Gray (1981) defended the concept of equivalence in behavioural inhibition resulting from punishment on the one hand, and non-reinforcement signals on the other. Some pharmacological studies have supported this suggestion; for instance, anxiolytics have similar effects on punished operant behaviour and on the low rates of operant behaviour maintained by differential reinforcement of low rate schedules (e.g. Stephens and Voet 1994). However, the fact that anxiolytics and stimulants may have differential effects on positively or negatively punished adjunctive behaviour tends to argue against such a premise. Indeed, it would appear to indicate that, contrary to Gray’s contention, the reduction in behaviour resulting from the presentation of an aversive stimulus and that resulting from withdrawal of an appetitive stimulus may not be similar.

Acknowledgements The research was funded by grant PB98-0010-C02-01 from Dirección General de Enseñanza Superior e Investigación Científica, Ministerio de Educación y Cultura, Spain (Ricardo Pellón, Principal Investigator). d-Amphetamine was supplied by Servicio de Restricción de Estupefacientes (Ministerio de Sanidad y Consumo, Madrid, Spain). We thank M.A. Villajos for technical support.

References

varying delays of reinforcement. Psychopharmacology 128:161–170
Reber D (1980) Reinforcing the occurrence or nonoccurrence of interim drinking. Anim Learn Behav 8:120–128