Behavioural and pharmacological specificity of the effects of drugs on punished schedule-induced polydipsia

Ángeles Pérez-Padilla and Ricardo Pellón

Wistar rats were exposed to a multiple fixed-time 30-s food delivery schedule, with an on/off tone signalling the two components. Animals were matched in accordance with the levels of schedule-induced polydipsia. Drinking was then punished in one of the components: half of the rats received lick-dependent 10-s signalled delays and the other half lick-dependent electric shocks. The intensities of the shocks were adjusted to reduce behaviour by the same amount as the delays in food presentation. Unpunished components were used as yoked-control conditions, by presenting delays or shocks independently of the animals' behaviour. D-Amphetamine (0.3–2.0 mg/kg) and cocaine (1.0–10.0 mg/kg) dose-dependently increased (although only slightly) and then decreased schedule-induced polydipsia punished with lick-dependent delays in food presentation, a result not observed in control conditions or when the behaviour was suppressed by lick-dependent electric shocks. Diazepam (1.0–17.0 mg/kg) and pentobarbital (3.0–17.0 mg/kg) dose-dependently increased and then decreased only the schedule-induced drinking punished with lick-dependent shocks. Buspirone (0.1–1.0 mg/kg) and morphine (2.0–5.6 mg/kg) showed either no specific effects or further suppressed schedule-induced drinking. Results of these and previous experiments suggest that the antipunishment effects of drugs depend not only on the precise nature of the drug, but also on the manner in which the behaviour is maintained. *Behavioural Pharmacology* **18:681–689**

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Animal Behaviour Laboratories, Department of Basic Psychology I, Faculty of Psychology, National University for Distance Education (Universidad Nacional de Educación a Distancia), Madrid, Spain

Correspondence to Dr Ricardo Pellón, PhD, Departamento de Psicología Básica I, Facultad de Psicología, Universidad Nacional de Educación a Distancia, C/Juan del Rosal 10, Ciudad Universitaria, 28040-Madrid, Spain
E-mail: rpellon@psi.uned.es

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Introduction

The behavioural phenomenon of schedule-induced polydipsia was first reported by Falk (1961), who observed that food-deprived rats exposed to an intermittent food-reinforcement schedule developed a robust pattern of excessive water intake. The excessive consumption resulted from the rats drinking small quantities after, rather than before, the presentation of each food pellet (see, however, Gilbert, 1974; Avila and Bruner, 1994; López-Crespo *et al.*, 2004). The rats' ingestion of water was anomalous because the animals had not been water deprived, and because they did not drink to obtain the food reinforcer. Despite the absence of an explicit contingency between drinking and food delivery, the degree of schedule-induced polydipsia is strongly determined by the food-reinforcement frequency (Falk, 1966; Flory, 1971; Brown and Flory, 1972; Yoburn and Cohen, 1979) and by the level of food deprivation (Falk, 1969). Theories of schedule-induced polydipsia (and adjunctive behaviour in general) have linked the behaviour to the dynamics resulting from the intermittent presentation of food; however, there is still no consensus on the exact mechanisms involved (see reviews by Falk, 1971; Wetherington, 1982; Reid and Staddon, 1990; Pellón *et al.*, 1998).

Drug studies have been important for understanding the nature of adjunctive behaviour, by comparing the effects of compounds acting on different systems on schedule-induced polydipsia and conventional operant lever pressing (see review by Pellón and Flores, 2007). Particularly relevant are studies on the punishment of schedule-induced polydipsia. Schedule-induced polydipsia is susceptible to being modified by the scheduling of contingent environmental consequences, a defining characteristic of operant behaviour. The amount of adjunctive drinking can be increased or decreased, respectively, by arranging licking-explicit contingencies of reinforcement or punishment (Bond *et al.*, 1973; Flory and Lickfett, 1974; Reberg, 1980; Pellón and Blackman, 1987). For example, Bond *et al.* (1973) reported the suppression of schedule-induced polydipsia by introducing electric shocks contingent upon licking (a positive punishment procedure), and Pellón and Blackman (1987) reported the suppression of schedule-induced polydipsia by presentations of lick-contingent delays (a negative punishment procedure).

Pellón *et al.* (1992) exposed rats to a fixed-time (FT) 60-s food-delivery schedule that presented a single food pellet every 60 s independently of the animals' behaviour: they

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observed that D-amphetamine increased schedule-induced polydipsia, which had been reduced by a negative punishment procedure in which each lick initiated a resetting 10-s delay in the delivery of the next food pellet. D-Amphetamine had little or no effect on unpunished schedule-induced polydipsia. Moreover, D-amphetamine increased adjunctive drinking neither in yoked-control rats that had received the delays dictated by the experimental rats irrespective of their own licking, nor in experimental rats that had received the delays from the onset of training rather than after the behaviour had developed (Pérez-Padilla and Pellón, 2003). The benzodiazepine diazepam slightly increased unpunished adjunctive drinking, but had no effect on schedule-induced polydipsia punished with lick-initiated delays in food presentation (Pellón *et al.*, 1992).

Flores and Pellón (1998) also exposed rats to a FT 60-s food-delivery schedule, and showed that diazepam strongly increased punished schedule-induced polydipsia when the punishment procedure was the administration of foot shocks (0.10 mA, 0.3 s) contingent on every fifth lick at the water spout. Behaviour maintained by the presentation of noncontingent shocks was not increased by diazepam. D-Amphetamine and buspirone (a serotonergic anxiolytic) failed to increase schedule-induced polydipsia punished by shock presentation. The antipunishment effect of diazepam depended on the degree of suppression of schedule-induced polydipsia by contingent shocks (Flores and Pellón, 2000); to the same extent, the antipunishment effect of amphetamine depended on the degree of suppression of schedule-induced polydipsia by contingent delays (Pérez-Padilla and Pellón, 2006).

In view of all these earlier findings, there seems to be a certain pharmacological specificity according to the type of punishment procedure used to reduce schedule-induced polydipsia. Stimulants such as amphetamine increase suppressed adjunctive drinking only if the punishment procedure involves withdrawal of positive reinforcers (such as lick-contingent delay in food presentation); benzodiazepine anxiolytics increase suppressed adjunctive drinking only after punishment with presentation of aversive events (such as lick-contingent shock delivery). Parallel results have been reported on operant behaviour (see Miczek, 1973; Branch *et al.*, 1977; Evenden and Ko, 2005). The purpose of these experiments was to investigate further this pharmacological and behavioural specificity by implementing a procedure in which to test, in a single study and within the same animals, the effects of a variety of drugs on schedule-induced polydipsia punished by shock delivery or by delays in food presentation. The compounds tested were the stimulants D-amphetamine and cocaine, the anxiolytics diazepam and pentobarbital, and buspirone and morphine. The opioid analgesic morphine was included to evaluate any antinociceptive contribution to the increases produced by the drugs on suppressed schedule-induced polydipsia.

Animals developed schedule-induced polydipsia in both the FT 30-s components of a multiple schedule of food presentation, in which the components alternated regularly and were signalled by on/off tone. Licking in one of the components was then suppressed via punishment, the licking of the other component remained unpunished (though this was used as a yoked-control condition). Half of the rats received lick-dependent 10-s delays in food delivery, the other half lick-contingent shocks (animals received, respectively, noncontingent shocks or noncontingent delays in the unpunished component). Shock intensities were adjusted to reduce schedule-induced polydipsia to the same degree as that achieved by the 10-s food delays, thus equating within the same experimental session and across animals the rates of punished schedule-induced polydipsia (something that could not be done before because of the independent execution of previous studies). Rate-dependent effects on behaviour have been shown to be a very general finding of drug action (see review by Robbins, 1981), an effect also reported for amphetamine on schedule-induced polydipsia (Flores and Pellón, 1995). If stimulants were to increase low rates of punished schedule-induced polydipsia by delays in food presentation more than the equivalent low rates of punished schedule-induced polydipsia by shock presentation, and if anxiolytics were to increase punished schedule-induced polydipsia by shocks to a greater extent than equivalent rates of punished adjunctive drinking by food delays, this would provide a gross differentiation for these two classes of drugs. It would also be a potential method for characterizing novel pharmacological agents as being stimulant-like or anxiolytic-like, which might complement currently used procedures.

Methods

Subjects

Twelve experimentally naive male Wistar rats, obtained from Charles River (Lyon, France), were used. All animals were monitored and fed daily, and they began the experiment at 90 days of age. They were individually housed in a room with controlled environmental conditions (21°C ambient temperature, 60% relative humidity and an 08.00 h/20.00 h light/dark cycle). The rats' weights were gradually reduced to and maintained at 80–85% of their ideal free-feeding weights through food restriction, on the basis of a growth curve. The mean weight at the beginning of the experiment was 401 g (range 370–450 g). The 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 the 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 identical 29 × 24.5 × 35.5 cm, Letica Instruments LI-836 rodent-conditioning chambers (Barcelona, Spain). Each chamber was enclosed in soundproofed housing, which was 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 were permanently withdrawn during the course of the experiment. A water bottle was attached to the external side of the right wall, with its spout accessible to the animal 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 it but not maintain permanent contact with it. Licks at the spout were detected when the electric circuit between the 16 parallel metal bars encircling the grid floor and the drinking bottle spout was completed via contact with the animal's tongue. Letica LI 100-20 generators (Barcelona, Spain) supplied scrambled electric shocks individually through the grid floor of each box. 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, Frenchtown, New Jersey, USA) 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 IBM Pentium 133 personal computer installed with the MED-PC for Windows software package (MED Associates Inc., Georgia, Vermont, USA).

Behavioural procedure

On being stabilized at 80–85% of their free-feeding weight, all the rats were subjected to a water-intake test in their own home cages. For two consecutive days, 80 food pellets of 45 mg each were deposited at once in a tray, and the amount of water consumed over 40 min was measured. This procedure provided a baseline against which to compare the level of schedule-induced drinking observed subsequently in the 40-min experimental sessions, which called for the 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 20 min, with 80 food pellets being previously deposited in the food tray, and with ventilation and illumination being provided, but with no experimental contingency in operation. During this session, no water bottles were installed.

Thereafter, the rats were exposed to 65 sessions of 40 min each: each session consisted of a multiple FT 30-s FT 30-s schedule of food pellet presentation, in which the food pellets were regularly delivered into the food tray at 30-s intervals regardless of the animal's behaviour. Before each session, bottles containing 100 ml of fresh tap water were installed in each chamber as described above. Components alternated every 10 min, lights being turned on at the beginning and turned off at the end of each component. A 10-s timeout separated the components. In addition, the second component was signalled by a tone (70 dB, 40 Hz). The order of components was counterbalanced across the animals.

The animals were matched into pairs according to the number of licks recorded at the end of the previous phase; then the rats within each pair were assigned to one of two groups, which resulted in equivalent response rates. For the first group, food continued to be delivered according to the multiple FT 30-s FT 30-s schedule, but now every lick given during the unsignalled component initiated a 10-s delay in the delivery of the next food pellet. These delays were signalled by a blackout. In the component signalled by the tone, animals of group 1 received electric shocks that were noncontingent upon their own licking, but which were dictated by the licking of the rats of group 2 (see below).

In the second group, and during the component signalled by the tone, food continued to be delivered according to a FT 30-s schedule, but every fifth lick was now followed by a 0.3-s electric foot shock (a fixed-ratio 5-shock schedule). The intensity of shocks was adjusted for each rat to suppress licking in the same proportion as that obtained with the delays in the first group (shock intensities were between 0.04 and 0.20 mA). During the unsignalled component, the animals of the second group were subjected to the same food delays as their counterparts in the first group but independently of their own licking.

When the rates of punished and unpunished licking became stabilized after 30 experimental sessions, all animals received drug administrations as described below.

Pharmacological procedure

Each rat of both groups was exposed initially to the administration of S(+)-amphetamine sulphate (D-amphetamine sulphate) at doses of 0.3, 1.0 and 2.0 mg/kg;

thereafter they were given (−)-cocaine hydrochloride (ecgonine methyl ester benzoate) at doses of 1.0, 3.0, 5.6 and 10.0 mg/kg; this was followed by diazepam at doses of 1.0, 3.0, 10.0 and 17.0 mg/kg; sodium pentobarbital at doses of 3.0, 10.0 and 17.0 mg/kg; buspirone hydrochloride at doses of 0.1, 0.3 and 1.0 mg/kg and finally, by morphine sulphate at doses of 2.0 and 5.6 mg/kg. D-Amphetamine was obtained from RBI/Sigma (Natick, Massachusetts, USA); buspirone was obtained from Sigma-Química (Madrid, Spain); cocaine, diazepam, pentobarbital and morphine were supplied by Servicio de Restricción de Estupefacientes (Ministerio de Sanidad y Consumo, Madrid, Spain). D-Amphetamine, cocaine, pentobarbital, buspirone and morphine were dissolved in 0.9% saline, whereas diazepam was suspended in a solution composed of distilled water and three drops of Tween 80 (Sigma-Química). Drugs were administered by intraperitoneal injection in a volume of 1 ml/kg body weight 10 min before the experimental sessions, except for the doses of morphine that were administered by subcutaneous injection. For each drug, doses were administered following a random order, but all the rats received the same dose on the same day. Drug sessions (Tuesdays and Fridays) were preceded by a control session, during which the animals were either not injected (Mondays), or were injected with saline at a volume of 1 ml/kg 10 min before the experimental session (Thursdays). Wednesdays were control sessions without injection, but were not taken into account for data analysis.

For each rat and each experimental session, the number of licks for each component was recorded separately. The number of licks was converted into licks/min. Licks/min were subjected to analysis of variance and Newman–Keuls post hoc tests when conditions permitted, using the Statistica 5.0 package.

Results

The animals drank small amounts of water during the two baseline days in their home cages before the start of the experimental sessions. The rats in the lick-contingent shock condition consumed on average 6.50 ± 0.67 ml, and those in the lick-contingent delay condition consumed 7.08 ± 0.58 ml. Mean water intake increased up to 24.43 ± 0.31 ml during the final 5 days of exposure to the multiple FT 30-s food schedules for the lick-contingent shock rats and to 30.17 ± 0.93 ml for the lick-contingent delay rats, increases of around four orders of magnitude above baseline levels. This water consumption is typical of schedule-induced polydipsia.

Table 1 shows the data for the licks/min at the end of the acquisition and punishment phases for each of the two components of the multiple schedule and for each of the two groups of animals. Both groups of rats developed high rates of licking in the FT 30-s components of the multiple schedule at the end of the acquisition phase. When licks in one of the FT 30-s components was followed by electric shocks (left-hand in Table 1) or by food delays (right-hand in Table 1) during the punishment phase, the rate of licking decreased considerably. Suppressed licking rates were similar for both punishment procedures, thus showing the effectiveness of both shocks and delays to punish adjunctive drinking. Decreases were also observed in the condition in which shocks were presented noncontingently upon licking (column under control shock), although to a much lesser degree than in the lick-contingent shock condition. Presentations of noncontingent delays (column under control delay) had little effect on behaviour; if anything there was a small increase in licking.

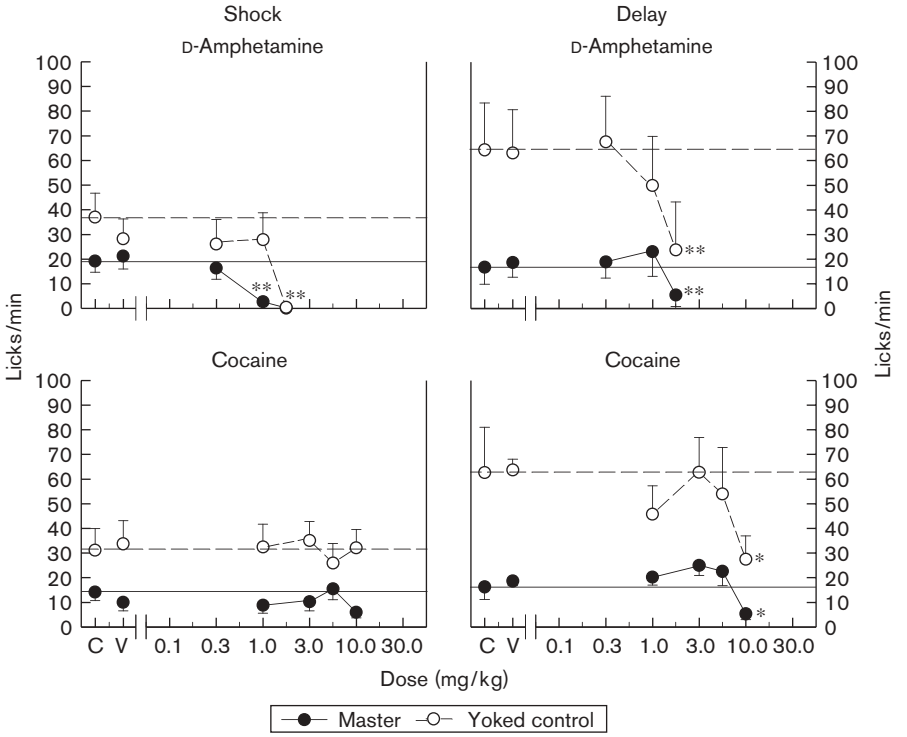
Figure 1 depicts the effects of the different doses of D-amphetamine (upper panels) and of cocaine (lower panels) on the rates of schedule-induced licking for the two shock conditions (left-hand panels) and for the two delay conditions (right-hand panels). Filled circles correspond to the data in which shocks or delays were presented contingently upon licking, and open circles to those in which shocks or delays were presented noncontingently. Control data without injection (C), vehicle data (V) and behavioural effect of the drug are represented as the means and SEs of licks/min. Dotted lines intercepting the *y*-axis denote the noninjection effect on unpunished components, and the solid lines correspond to the noninjection effects on punished components. Vehicle administration had no effect (or nonsignificant decreases) on the rate of licking with respect to noninjection sessions in any group, experimental condition or drug treatment.

D-Amphetamine had dose-dependent effects on punished and unpunished schedule-induced polydipsia in both shock and delay conditions [$F(4,20) = 7.88$, $P < 0.01$ for master shock; $F(4,20) = 3.70$, $P = 0.02$ for control shock; $F(4,20) = 7.96$, $P < 0.01$ for master delay; $F(4,20) = 9.94$,

Table 1 Mean licks/min (± SE) during the last five sessions of the acquisition and punishment phases for the two schedule components in each of the two experimental groups of rats

	Lick-contingent shock group		Lick-contingent delay group	
	Master (shock)	Control (delay)	Control (shock)	Master (delay)
Acquisition	49.75 ± 1.55	58.81 ± 4.70	51.42 ± 2.10	67.49 ± 3.55
Punishment	18.35 ± 1.54	61.17 ± 0.90	28.62 ± 4.56	14.24 ± 5.46

Fig. 1



Effects of different doses of D-amphetamine and cocaine on the licking rates in the two experimental groups. Values are mean + SE. Filled circles correspond to the effects when lick-contingent shocks or delays were presented; open circles correspond to the effects when shocks or delays were presented noncontingently. $^{**}P<0.01$; $^{*}P<0.05$, compared with control without injection (C). V, vehicle data.

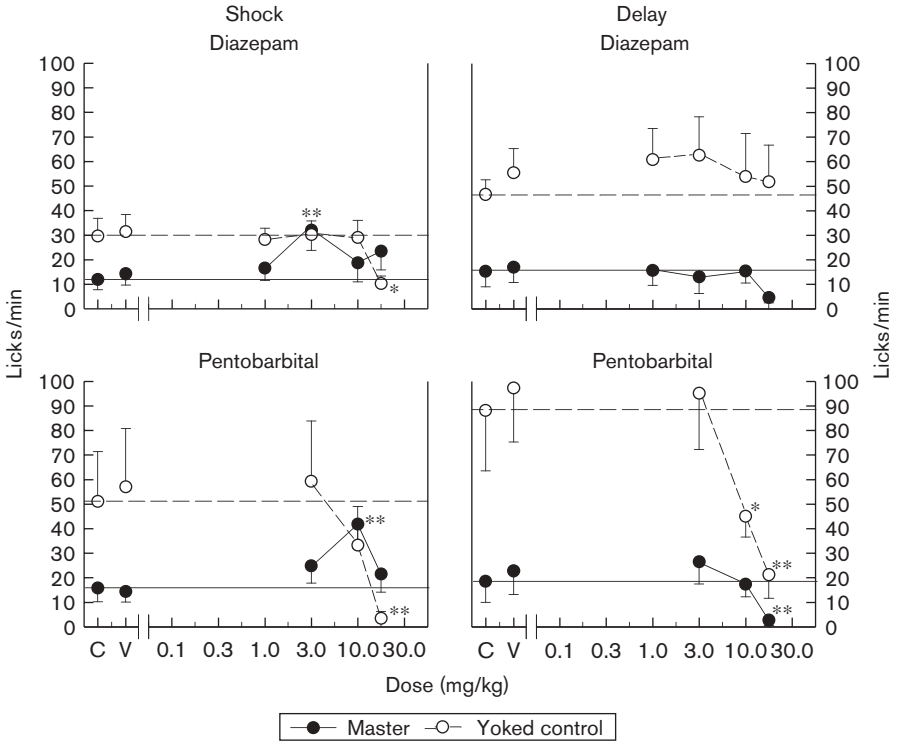
$P < 0.01$ for control delay], with 0.3 mg/kg having no general effect on licking and 2.0 mg/kg suppressing all rates of licking ($P < 0.01$). D-Amphetamine at the 1.0 mg/kg dose also had no significant effect on licks/min in yoked-control conditions, but had different effects on lick-contingent conditions as a function of the type of punishment procedure. Licks/min were slightly increased in the lick-contingent delay condition (although not statistically significant) and almost completely suppressed in the lick-contingent shock condition ($P < 0.01$).

Cocaine showed dose-dependent effects on punished [$F(5,25) = 3.02$, $P = 0.02$] and unpunished [$F(5,25) = 2.90$, $P = 0.03$] schedule-induced polydipsia by delays in food presentation, but had no clear dose-dependent effects on the two shock conditions (lower left-hand panel). Increases were obtained at 1.0, 3.0 and 5.6 mg/kg doses in the contingent-delay condition but not in the noncontingent condition; however, the results were not statistically significant. The dose of 10.0 mg/kg reduced licking in both delay conditions ($P < 0.05$).

Figure 2 shows the effects of the different doses of diazepam (upper panels) and of pentobarbital (lower panels) on the rates of schedule-induced licking for the two shock conditions (left-hand panels) and for the two delay conditions (right-hand panels). Filled circles correspond to the data in which the shocks or delays were presented contingently upon licking, and open circles to those in which the shocks or delays were presented noncontingently. As in Fig. 1, no-injection control (C), vehicle (V) and drug effect on behaviour are expressed as the means and SEs of licks/min. Vehicle administration had no effect (or no reliable increases) on the rate of licking with respect to noninjection sessions in any group, experimental condition or drug treatment.

Diazepam showed dose-dependent effects on punished [$F(5,25) = 2.48$, $P = 0.05$] and unpunished [$F(5,25) = 3.39$, $P = 0.01$] schedule-induced polydipsia by shock delivery, but had no statistically significant dose-dependent effects in the two delay conditions (upper right-hand panel). Schedule-induced polydipsia punished by lick-contingent shocks was increased after the administration of diazepam, mainly at the 3.0 mg/kg dose ($P < 0.01$), a result observed neither in the lick-contingent delay condition nor in noncontingent control conditions. The dose of 17.0 mg/kg reduced the licks/min both in the noncontingent shock condition ($P = 0.02$) and in the lick-contingent delay condition.

Fig. 2



Effects of different doses of diazepam and pentobarbital on the licking rates in the two experimental groups. Values are mean + SE. Filled circles correspond to the effects when lick-contingent shocks or delays were presented; open circles correspond to the effects when shocks or delays were presented noncontingently. $^{**}P<0.01$; $^{*}P<0.05$, compared with control without injection (C). V, vehicle data.

Pentobarbital showed very clear dose-dependent effects on all the four conditions of the experiment [$F(4,20) = 2.85$, $P = 0.04$ for master shock; $F(4,20) = 4.08$, $P = 0.01$ for control shock; $F(4,20) = 2.91$, $P = 0.04$ for master delay; $F(4,20) = 5.28$, $P < 0.01$ for control delay]. As the dose of the drug was increased, the rate of responding just decreased in all except the lick-contingent shock condition. Licking was almost eliminated at the dose of 17.0 mg/kg ($P < 0.01$). In the lick-contingent shock condition, the 10.0-mg/kg dose increased the licks/min ($P < 0.01$), a dose that reduced licks/min in yoked-control conditions ($P < 0.05$ in control delay) or had no effect on the lick-contingent delay condition.

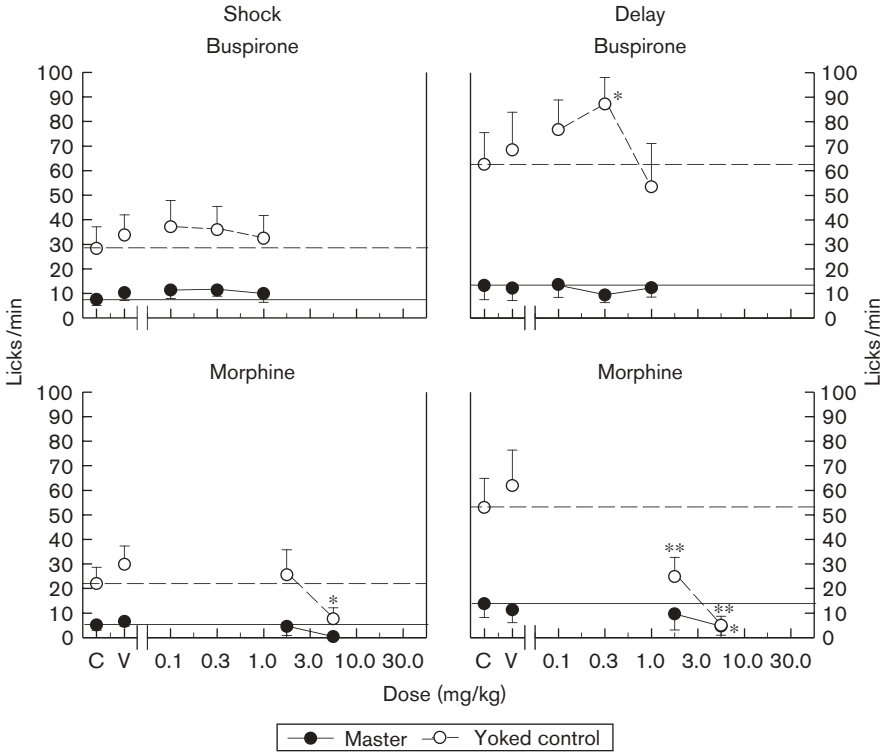
Figure 3 shows the effects of the different doses of buspirone (upper panels) and of morphine (lower panels) on the rates of schedule-induced licking for the two shock conditions (left-hand panels) and for the two delay conditions (right-hand panels). Filled circles correspond to data in which shocks or delays were presented contingently upon licking, and open circles to data in which shocks or delays were presented noncontingently. As in Figs 1 and 2, no-injection control (C), vehicle (V) and drug effect on behaviour are expressed as the means and SEs of licks/min. Vehicle administration had no significant effects on the rate of licking.

Buspirone in general had no significant effect on punished or unpunished schedule-induced polydipsia, except in the yoked-control delay condition [$F(4,20) = 5.84$, $P < 0.01$]. Licks/min increased after the 0.3-mg/kg dose in control delay ($P = 0.02$), but no other effect was observed in any of the four conditions of the experiment, not even a decrease at the highest dose tested. Morphine showed dose-dependent reductions in licks/min [$F(3,15) = 4.78$, $P < 0.01$ for control shock; $F(3,15) = 4.17$, $P < 0.02$ for master delay; $F(3,15) = 16.72$, $P < 0.01$ for control delay], with decreases at the 2.0-mg/kg dose in control delay ($P < 0.01$) and at the 5.6-mg/kg dose in all conditions ($P = 0.03$ for control shock; $P = 0.02$ for master delay; $P < 0.01$ for control delay). Neither buspirone nor morphine selectively increased punished schedule-induced polydipsia by shocks or delays in food delivery.

Discussion

Animals were exposed to a multiple FT 30-s FT 30-s schedule of food pellet presentation; they developed schedule-induced polydipsia, measured in terms of licks/

Fig. 3



Effects of different doses of buspirone and morphine on the licking rates in the two experimental groups. Values are mean + SE. Filled circles correspond to the effects when lick-contingent shocks or delays were presented, open circles correspond to the effects when shocks or delays were presented noncontingently. $^{**}P<0.01$; $^{*}P<0.05$, compared with control without injection (C). V, vehicle data.

min and volume of water intake. Schedule-induced polydipsia was then reduced by lick-dependent 10-s delays in food delivery or by low-intensity electric shocks, but not to the same extent by noncontingent presentations of delays or shocks. These results show again that both lick-contingent delays in the reinforcer (Flory and Lickfett, 1974; Pellón and Blackman, 1987; Pellón and Castilla, 2000) and lick-contingent electric shocks (Bond *et al.*, 1973; Flores and Pellón, 1998) can punish or suppress previously acquired schedule-induced polydipsia. Furthermore, punished schedule-induced polydipsia in this present study was maintained at similar licking rates by delays and shocks, a control that allows comparisons between drug effects on punishment uncontaminated by the potential influence of response rate on drug effects.

D-Amphetamine produced either no effect or decreases, depending on the dose, on the licking by yoked-control rats, thus resembling the effects of the drug on unpunished schedule-induced polydipsia (Byrd, 1973; Sanger, 1978; Williams and White, 1984; Flores and Pellón, 1995). Low and intermediate doses of D-amphetamine have no enhancing effect on the rate of adjunctive drinking maintained by different schedules of food presentation (Flores and Pellón, 1995). In contrast, when schedule-induced polydipsia was suppressed by punishment procedures, the effect of D-amphetamine on punished responding seemed to depend on the type of punishment procedure used to reduce behaviour. When schedule-induced polydipsia was reduced by lick-contingent delays in the delivery of the food pellets, an intermediate dose of D-amphetamine slightly increased adjunctive licking (for stronger results see Pellón *et al.*, 1992; Pérez-Padilla and Pellón, 2003, 2006). When schedule-induced polydipsia was reduced by lick-contingent foot shocks, same dose of D-amphetamine decreased the rate of licking (see also Flores and Pellón, 1998).

Cocaine generally produced no alteration in the licking rates of the control animals and some decreases at the highest dose, being similar to the reported effects of cocaine on unpunished schedule-induced polydipsia (Jones *et al.*, 1994). Cocaine at low-to-intermediate doses slightly increased suppressed schedule-induced polydipsia by lick-contingent delays in food delivery, but did not increase suppressed licking by contingent electric shocks. Therefore, findings with cocaine were in general similar to those with D-amphetamine.

Low doses of benzodiazepines tend to increase unpunished adjunctive drinking, but high doses decrease schedule-induced polydipsia. Increases observed at low doses have been reported in the volume of water intake but not in the frequency of licking (Bacotti and Barrett, 1976; Sanger and Blackman, 1976; Mittleman *et al.*, 1988; Pellón *et al.*, 1992); this is consistent with the current results not only with diazepam, but also with the anxiolytic pentobarbital. With regard to punishment, schedule-induced polydipsia suppressed by lick-contingent shocks has been reported to be increased by moderate doses of diazepam (Flores and Pellón, 1998, 2000) and other benzodiazepines (Pellón *et al.*, 2007). Such increases were not found after the suppression of schedule-induced polydipsia by lick-contingent delays in food delivery (Pellón *et al.*, 1992). The dependency of the antipunishment effect of diazepam on the type of procedure used to reduce schedule-induced polydipsia was replicated in this study using a within-subject design. Furthermore, intermediate doses of pentobarbital also increased punished licking when the animals received a moderate electric shock contingent upon licking, but not when schedule-induced polydipsia was punished by lick-contingent food delays.

Buspirone, a 5-HT_{1A} partial agonist devoid of activity at benzodiazepine receptors and clinically used as an effective anxiolytic, neither specifically increased punished schedule-induced polydipsia by shock delivery as conventional anxiolytics did, nor had any clear dose-dependent effects on punished or unpunished adjunctive drinking (except for increases at an intermediate dose when food delays were noncontingent upon licking). Previous findings have also reported the absence of anxiolytic activity of buspirone on schedule-induced polydipsia (Ryan *et al.*, 1993; Flores and Pellón, 1998).

The opioid agonist morphine produced no increases in punished schedule-induced polydipsia by shock delivery, thus suggesting that the increases in the rates of suppressed responding by diazepam and pentobarbital were not due to an analgesic effect. Morphine led to similar dose–response decreases on both unpunished and punished schedule-induced polydipsia, and in conditions with both shock delivery and delays in food presentation.

The moderate increases seen with amphetamine and cocaine in schedule-induced polydipsia punished by delays in food delivery, as well as the significant increases caused by diazepam and pentobarbital in schedule-induced polydipsia punished by shock delivery, were observed at doses lower than those required to decrease the rates of unpunished schedule-induced polydipsia. This indicates a high specificity in the antipunishment effects of drugs.

Not only do drugs have selective effects on punished behaviour, but also, while increasing punished responding, display a specificity that is a function of the punishment procedure used to reduce schedule-induced polydipsia; previous studies as well as a part of these results suggest that this specificity exists for particular drugs. Anxiolytics such as barbiturates and benzodiazepines tend to increase punished responding suppressed by response-contingent electric shocks (a positive punishment procedure), an effect not so readily seen with stimulants or anxiolytics acting through the serotonergic system. Conversely, stimulants such as amphetamines have been shown to increase punished adjunctive drinking only when the behaviour was suppressed by lick-contingent delays in food delivery (a negative punishment procedure), an effect not shown by the anxiolytics. Similar effects of drugs have been reported on positively reinforced operant behaviour as on adjunctive behaviour (for a fuller analysis see Pellón and Flores, 2007). In the case of psychostimulants, there is still the need for a better characterization of their effects on operant behaviour punished by the withdrawal of positive reinforcers (as opposed to punishment by the contingent presentation of aversive events); however, the behavioural effects of amphetamine or cocaine seem to depend on the type of event that maintains the operant behaviour (e.g. Johanson, 1978).

The procedure used here is valuable for understanding the differential effects of drugs on schedule-induced behaviour punished by the presentation of aversive events or by the withdrawal of positive events: it tested the different drug classes in a single study and within the same animals, under control conditions that minimized the potential contributions of variables such as the baseline rate of responding or the time of testing. The procedure used here might also be a complementary method for classifying compounds as being stimulant-like or anxiolytic-like on the basis of their effects on the two types of punished responding. Finally, the procedure used here is important for the better understanding of adjunctive behaviour in general. The current and previous data suggest that schedule-induced polydipsia and other patterns of adjunctive behaviour show remarkable similarities with operant behaviour maintained by the scheduling of positive consequences on responding. Positive consequences (in the form of food presented at regular intervals) might contribute to the maintenance of adjunctive behaviour; the specific way in which that might operate is still a focus of debate and investigation.

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