Chapter 6.
The effects of *d*-amphetamine, chlordiazepoxide, alpha-flupenthixol and behavioural manipulations on choice of signalled and unsignalled delayed reinforcement

Abstract. Inability to tolerate delays to reward is an important component of impulsive behaviour, and has been suggested to reflect dysfunction of dopamine systems. The present experiments examined the effects of signalling a delayed, large reward on rats' ability to choose it over a small, immediate reward, and on the response to amphetamine, a dopamine receptor antagonist, and a benzodiazepine. Three groups of Lister hooded rats were tested on a two-lever discrete-trial delayed reinforcement task in which they chose one pellet delivered immediately or four pellets delivered after a delay. This delay increased from 0 to 60 s during each session. Trials began with illumination of a houselight: in the Houselight group, this remained on during the delay and feeding period. In the No Cue group, the houselight was extinguished at the moment of choice. In the Cue group, a stimulus light was illuminated during the delay. Once trained, the rats were challenged with *d*-amphetamine (0.3, 1.0, 1.6 mg/kg), chlordiazepoxide (1.0, 3.2, 5.6, 10 mg/kg), α-flupenthixol (0.125, 0.25, 0.5 mg/kg), and various behavioural manipulations. Subjects' choice became and remained sensitive to the delay; the cue speeded learning. Amphetamine decreased choice of the large reinforcer in the No Cue group and increased it in the Cue group. α-Flupenthixol and chlordiazepoxide generally decreased preference for the delayed reinforcer; flupenthixol reduced the cue's effects, but chlordiazepoxide did not interact with the cue condition. It is concluded that signals present during a delay can enhance the ability of amphetamine to promote choice of delayed rewards.

INTRODUCTION

Among the many features of impulsivity, one is 'impulsive choice', exemplified by the inability of an individual to choose a large delayed reward in preference to a small immediate reward (Ainslie, 1975). Impulsive choice has been suggested to reflect an alteration in reinforcement processes, namely that delayed reinforcers have lost their effectiveness, and has been suggested to underlie attention-deficit/hyperactivity disorder (ADHD; Sagvolden *et al.*, 1998; Sagvolden & Sergeant, 1998). ADHD is amenable to treatment with psychomotor stimulant drugs (Bradley, 1937; see Solanto, 1998 for a recent review), suggesting that they might promote the choice of delayed rewards. However, in laboratory models of impulsive choice, the effects of acute administration of psychostimulants have varied: some studies have found that they promote choice of delayed reinforcers (Sagvolden *et al.*, 1992; Richards *et al.*, 1999; Wade *et al.*, 2000), while others have found the opposite effect (Charrier & Thiébot, 1996; Evenden &

Ryan, 1996), and it has been shown that the same psychostimulant can have opposite effects in different tasks designed to measure impulsivity (Richards *et al.*, 1997a).

In studies of delayed reinforcement, it has been demonstrated that signalled delays generally maintain higher rates of free-operant responding than unsignalled delays (see Lattal, 1987 for a review), and signals present during the delay can have an important role in discrete-trials choice (Mazur, 1997). A signal or cue that is associated selectively with a reinforcing outcome may become a conditioned reinforcer (Figure 67). Conditioned reinforcement can affect choice behaviour, perhaps the best demonstration being that of Williams and Dunn (1991), in which pigeons preferred a key associated with a conditioned reinforcer despite this leading to fewer presentations of food. Since amphetamine-like drugs potentiate the effects of conditioned reinforcers (Hill, 1970; Robbins, 1976; Robbins, 1978; Robbins *et al.*, 1983), amphetamine may promote choice of signalled delayed reinforcement.

Evenden and Ryan (1996) developed a model of impulsive choice in which food-restricted rats chose between a small, immediate reward and a large, delayed reward in discrete trials, the delay to the large reinforcer being increased in steps as the session progressed. The present study examined the effects of the psychostimulant *d*-amphetamine, the benzodiazepine chlordiazepoxide, and the mixed dopamine D_1 / D_2 receptor antagonist α-flupenthixol on performance of a modified version of this task, with particular emphasis on the effects of a signal present during the delay to reinforcement. Subsequently, to characterize the basis of performance on the task, the effects of this signal itself, of removing the delays, reversing the order of the delays, of satiation, and of extinction were examined.

Three groups of animals were trained on variations of the task, differing only in the signalling conditions. In the Cue condition, illumination of a stimulus light during the delay provided a signal that was unambiguously associated with the large reinforcer only. This design is commonly used to establish stimuli as conditioned reinforcers in delay-of-reinforcement experiments (for reviews, see Williams, 1994a; Mazur, 1997). In the No Cue condition, the rats awaited and collected the reinforcers in darkness, with no signal present during the delay. This closely resembles the situation in Evenden and Ryan's (1996) study. The Houselight condition was intermediate between these: in this condition, the houselight was illuminated at the start of the trial and remained on until 6 s after the subject had collected the reward. The houselight therefore preceded and accompanied delivery of the large and small reinforcers.

Given that the effect of amphetamine on performance of this task in the absence of differential cues was to increase preference for the small immediate reward (reduced tolerance of delay, Evenden & Ryan, 1996), the addition of a conditioned reinforcer would be expected to reduce or reverse this effect. (The Houselight group were predicted to be intermediate or equivalent to the Cue group, in that the houselight is a weak predictor of food.) Chlordiazepoxide was used as a positive control; its effects were not expected to differ in the presence of a cue because benzodiazepines do not affect the action of appetitive conditioned reinforcers (Killcross *et al.*, 1997a), while the dopamine receptor antagonist α-flupenthixol was predicted to have opposite effects to amphetamine in the cue condition as it attenuates the effects of conditioned reinforcers (Robbins *et al.*, 1983; Killcross *et al.*, 1997a).

METHODS

Subjects, apparatus, and behavioural task

Subjects were 24 experimentally naïve male Lister hooded rats maintained at 90% of their free-feeding mass and housed in pairs (for details of housing conditions, see Chapter 2).

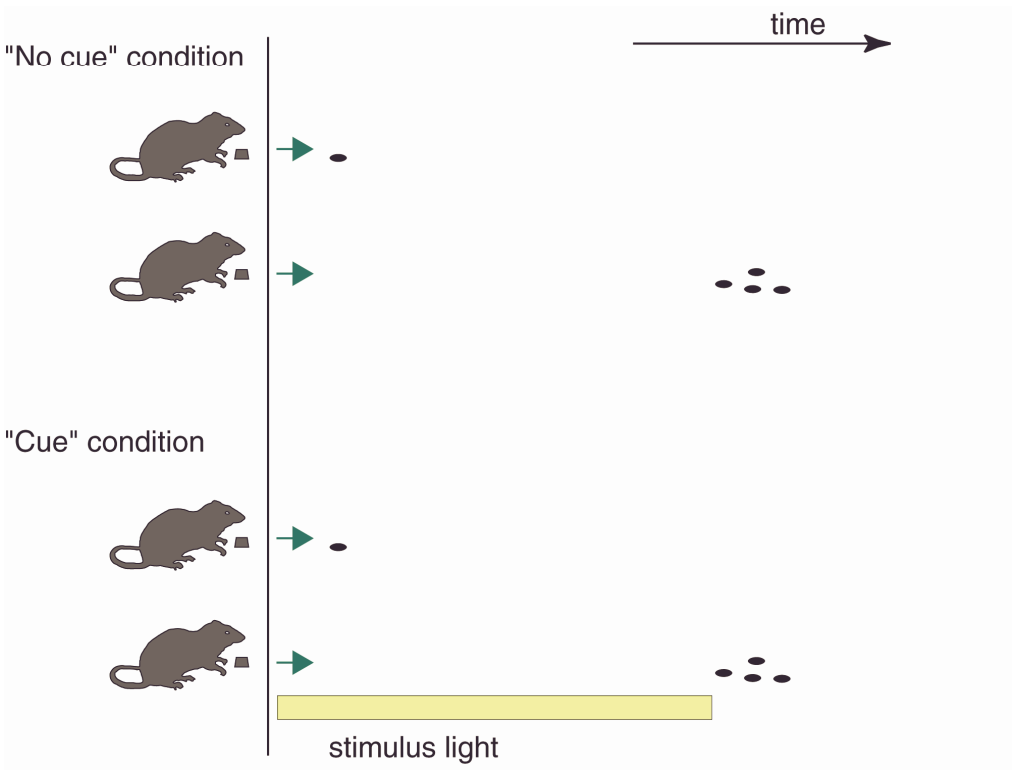

Figure 67. Choice of signalled and unsignalled delayed reinforcement. Subjects may choose between a small, immediate reward and a large, delayed reward. In the 'Cue' condition, a stimulus light is illuminated during the delay to reinforcement; this stimulus is therefore paired with the large reinforcer and may become a conditioned reinforcer.

Systematic technique for assessment of preference for delayed reinforcement

The standard operant chambers described in Chapter 2 were used, with 45-mg sucrose pellets (Rodent Diet Formula P, Noyes, Lancaster, NH) as the reinforcer.

Training. Subjects were first trained under an FR1 schedule to a criterion of 50 presses in 30 min, first for the left lever and then for the right. They were then trained on a simplified version of the full task. The session began with the levers retracted and the operant chamber in darkness. Every 40 s, a trial began with illumination of the houselight and the traylight. The subject was required to make a nosepoke response within 10 s, or the current trial was aborted and the chamber returned to darkness. If the subject nosepoked within this time limit, the traylight was extinguished and a single lever presented. If the rat failed to respond on the lever within 10 s, the lever was retracted and the chamber darkened, but if it responded, a single pellet was delivered immediately and the traylight was illuminated until the rat collected the pellet (or a 10-s collection time limit elapsed, whereupon the chamber was darkened). In the Houselight condition, the houselight was left on until 6 s after the food had been collected; in the Cue and No Cue conditions it was switched off at the moment the lever was pressed.

In every pair of trials, the left lever was presented once and the right lever once, though the order within the pair of trials was random. Rats were trained to a criterion of 60 successful trials in one hour (the maximum possible with a 40-s period being 90).

Behavioural procedure. The task was based on Evenden and Ryan's (1996) procedure and is illustrated in Figure 68. Aside from the use of an extra signal during the delay, the present task differs from that of Evenden and Ryan in a number of ways; in particular, the subjects were required to initiate the trials and choose a lever within a limited time, and a forced-choice trial on each lever was given at the start of each block of choice trials at a given delay. Additionally, in their procedure the houselight was always on, whereas in the present studies the houselight was extinguished during the intertrial interval (ITI), making it an informative stimulus (in that food was delivered

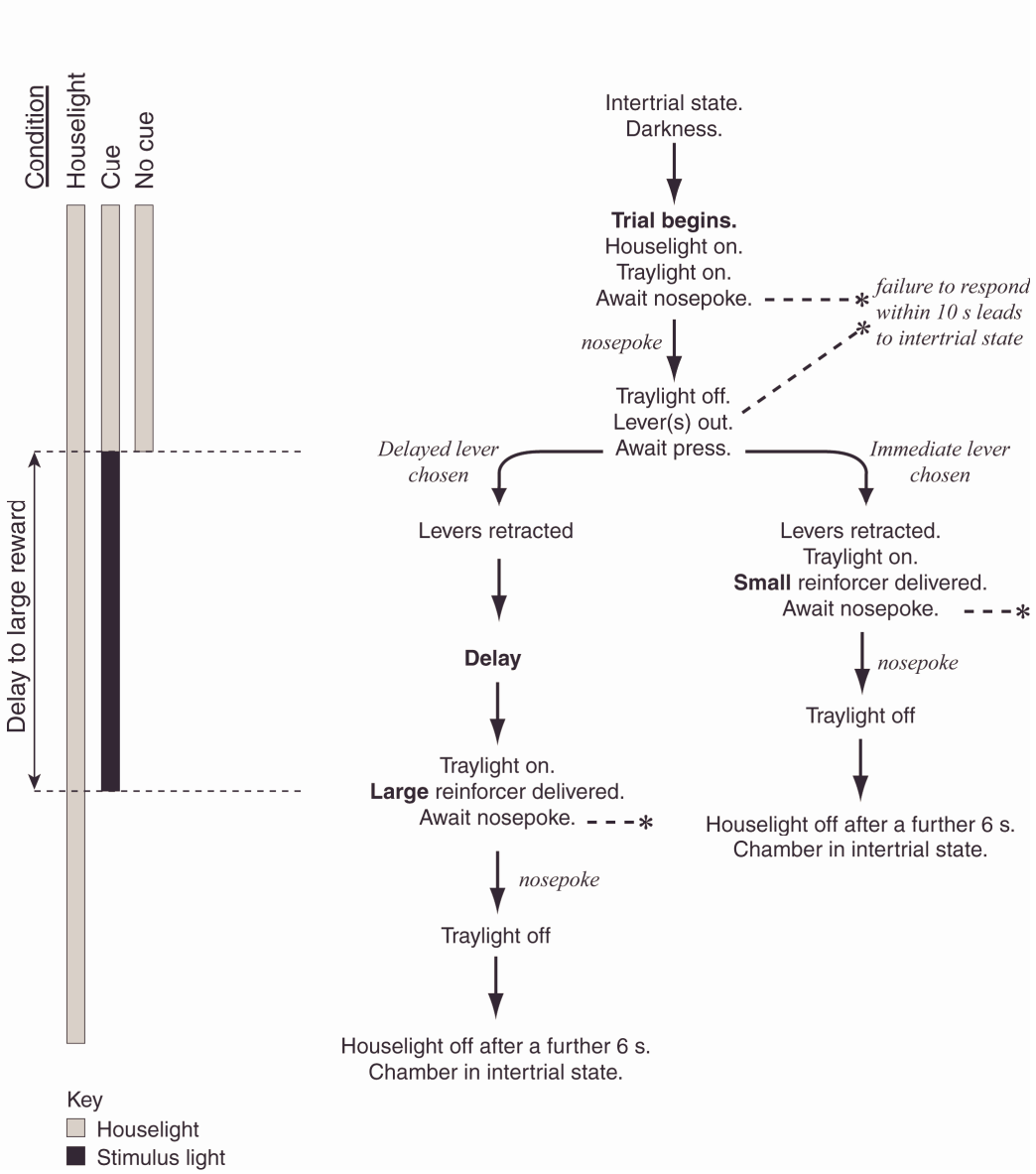

Figure 68. Schematic of the task. On the *right-hand side*, the format of a single trial is shown. This diagram shows in detail the Houselight condition, in which the houselight remains on from the start of the trial until 6 s after the subject has collected the reward. On the *left-hand side*, the differences between the three lighting conditions are illustrated. In the No Cue condition, the houselight is switched off at the moment of choice. In the Cue condition, the houselight is similarly switched off when the subject responds on a lever, but a stimulus light is illuminated during the delay that precedes delivery of the large reinforcer.

when the houselight was on, but never when it was off). Finally, subjects were not given exposure to the large reinforcer before delays were introduced into the task.

The session began in darkness with the levers retracted; this was designated the intertrial state. Trials began at 100-s intervals. Each trial began with the illumination of the houselight and the traylight. The rat was required to make a nosepoke response, ensuring that it was centrally located at the start of the trial (latency to poke was designated the initiation latency). If the rat did not respond within 10 s of the start of the trial, the operant chamber was reset to the intertrial state until the next trial began and the trial was scored as an omission. If the rat was already nosepoking when the trial began, the next stage followed immediately.

Upon a successful nosepoke, the traylight was extinguished and one or both levers were extended. One lever was designated the Delayed lever, the other the Immediate lever (counterbalanced left / right). The latency to choose a lever was recorded. (If the rat did not respond within 10 s of lever presentation, the chamber was reset to the intertrial state until the next trial and the trial was scored as an omission.) When a lever was chosen, both levers were

retracted. Choice of the Immediate lever caused the immediate delivery of one pellet; choice of the Delayed lever caused the delivery of 4 pellets following a delay. In the Cue condition, the houselight was switched off at the moment of choice and a stimulus light above the chosen lever switched on for the duration of the delay. In the No Cue condition, the stimulus light was not switched on. In the Houselight condition, the houselight remained on instead. These three conditions are illustrated in Figure 68.

Following any delay, the stimulus light was switched off, the traylight was switched on and the reinforcer for that lever was delivered. Multiple pellets were delivered 0.5 s apart. If the rat collected the pellets before the next trial began, then the time from delivery of the first pellet until a nosepoke occurred was recorded as the collection latency. The traylight was switched off, and in the Houselight condition the houselight remained on for another 6 s (eating time). In other conditions there was no houselight illumination during this time. If the rat did not collect the food within 10 s of its delivery, the operant chamber entered the intertrial state, though collection latencies were still recorded up to the start of the next trial. The chamber was then in the intertrial state and remained so until the next trial. There was no mechanism to remove uneaten pellets, but failure to collect the reward was an extremely rare event (see *Results*).

The delay was varied systematically across the session. A session consisted of 5 blocks, each comprising two trials on which only one lever was presented (one trial for each lever, in randomized order) followed by ten free-choice trials. Delays for each block were 0, 10, 20, 40 and 60 s respectively. As trials began every 100 s, the total session length was 100 minutes; subjects received one session per day.

Pharmacological and behavioural manipulations

A stability criterion was defined as follows: after excluding single-lever trials, choice ratios (delayed lever responses ÷ total responses) were calculated for each rat using the summed responses for three consecutive sessions, and subjected to analysis of variance (ANOVA) with delay as a within-subjects factor. When the effect of delay was significant at the $\alpha = .01$ level, the rats were considered to have criterion performance from the first session of the three. The degree of sensitivity to the effects of the delay within each session was also assessed by calculating the slope of the linear regression of %choice of the large reinforcer against log(delay + 1 s) for each subject, though this measure did not form part of the criterion. Following attainment of the criterion, baseline assessments were performed on seven sessions immediately prior to the start of pharmacological and behavioural manipulations, which were conducted as listed in Table 18.

Table 18: Experiments performed.

Group	Houselight during delay and feeding period	Stimulus light during delay	Manipulations, in order
Houselight (n = 8)	On	Off	amphetamine 1.0, 0.3 and 1.6 mg / kg omission of delays addition of cues hungry versus sated (rapid) hungry versus sated (longer term) descending delays
No Cue (n = 8)	Off	Off	amphetamine 1.0, 0.3 and 1.6 mg / kg chlordiazepoxide 10.0, 1, 3.2 and 5.6 mg / kg α-flupenthixol 0.25, 0.125 and 0.5 mg / kg extinction
Cue (n = 8)	Off	On	amphetamine 1.0, 0.3 and 1.6 mg / kg chlordiazepoxide 10.0, 1, 3.2 and 5.6 mg / kg α-flupenthixol 0.25, 0.125 and 0.5 mg / kg omission of cues

Drugs. *d*-Amphetamine sulphate, α-flupenthixol dihydrochloride and chlordiazepoxide hydrochloride (Sigma, UK) were all dissolved in sterile 0.9% saline to give a final volume of 1 ml / kg and injected intraperitoneally 10 min before the start of the session (60 min for flupenthixol). Doses were calculated as the salt and are listed in Table 18.

Drug studies. Each dose was tested over six sessions, with each rat experiencing either DVDVDV or VDVDVD (D drug session, V vehicle), counterbalanced across rats. Responding under each dose was compared with responding during the vehicle sessions that alternated with that dose. This approach has the advantage of being able to compare each drug dose with vehicle data collected across the same time period, increasing the power to detect drug effects if the baseline shifts gradually; it also implies that any drug carry-over effects would reduce the power to detect effects. Collecting data for three drug and three vehicle sessions enabled accurate determination of choice by giving 30 choice trials at each delay / dose combination. Between each six-session dose study, at least two days elapsed on which no injections were given.

Omission of delays. Following testing with amphetamine, the Houselight group were not included in further pharmacological studies but were tested under a range of behavioural manipulations. To establish whether they were still sensitive to the delays, they were first tested on six sessions alternating between the normal task and a version in which all delays were zero. Half of the rats began with the Delay and half with the No Delay condition.

Introduction of a cue. The Houselight group were next tested with successive sessions alternating between Cue and No Cue conditions, both of which were initially unfamiliar, in the same fashion as the drug studies (ABABAB design). As these animals learned the response–reward contingency without the cue light, introduction of the cue was expected not to provide additional information about the reward; thus, according to theories of Pavlovian conditioning (see Dickinson, 1980), the cue should not have entered into association with the reward, and was therefore not predicted to affect choice.

Satiation. To exclude the interpretation that drug or delay effects were due to differences in primary motivation, the Houselight group were returned to their original signalling conditions, and were tested while alternating between hungry and sated states on consecutive days in the same manner as the drug / vehicle studies described above. Following a 'hungry' session, animals were placed on free food (lab chow) until the start of the next day's 'sated' session, at which time the food was again removed for the 'hungry' session to follow. The comparison is therefore between animals on ~22 h food deprivation versus the sated state.

To establish whether prolonged deprivation had an effect on choice, a further satiation experiment was performed on the same subjects: half were placed on free food for a week while half remained hungry. They then performed the task for three sessions, after which the deprivation state was reversed for a week and a further three sessions' data collected.

Descending delays. To demonstrate that the basic effect of delay did not depend on an ascending series of delays, the Houselight group were next trained under a descending series of delays (60, 40, 20, 10, 0 s) under their normal signalling conditions.

Omission of a cue. Following drug testing, the Cue group were tested with sessions that alternated between the Cue and No Cue conditions in an ABABAB design, and subsequently with an AAABBB design (three consecutive cue sessions followed or preceded by three no-cue sessions). The reason for this was as follows: It was expected that manipulations where the subjects were required to learn through their experience of the delays during the session (that is, manipulations that affected choice *retrospectively*) would be better detected by the AAABBB design, as this gives greater opportunity for expression of that learned behaviour under constant conditions. In contrast, this was not expected of manipulations that affected the subjects' preference for delays that were about to occur (prospective choice; this distinction follows Killeen & Fetterman, 1988). While drugs are in principle capable of affecting choice prospectively, without requiring new learning, the only possible way that omission of the cue could affect choice behaviour is retrospectively: the subjects must learn that the cue no longer follows choice of the Delayed lever. In the ABABAB design, such learning might be obscured by the rapidly alternating contingencies.

Extinction. Following drug testing, the No Cue group were alternated between their normal task and extinction sessions, in which no reinforcement was delivered, in order to assess whether choice was controlled by a temporal stimulus (the passage of time within a session) or only by the exemplar (forced-choice) trials.

Statistical analysis

General statistical techniques were described in Chapter 2.

For baseline data, measures were calculated for each subject using pooled responses from all sessions, because an analysis using session as a within-subjects factor would reduce the power to detect effects of between-subjects factors (Bradley & Russell, 1998). Similarly, measures were calculated across the three session pairs of each drug study or behavioural manipulation. Choice ratios were calculated as the percentage of responses in which the Delayed lever was chosen, for free-choice trials only.

RESULTS

1. Acquisition and baseline performance

Acquisition of sensitivity to delay

In all groups, the rats' behaviour became sensitive to the delay following a number of training sessions (Figure 69A shows data for the Houselight group). In the first session, preference for the Delayed lever declined as the delays were introduced (not shown), presumably reflecting a degree of extinction as the delay was introduced. After this, preference for the delayed lever increased again until it was favoured at all delays. Finally, delay sensitivity was seen. It can be seen from Figure 69B that individual rats varied considerably in their preferences, despite the regular sampling of both levers at the start of each block.

Effect of cues on speed of acquisition

The presence of a cue during the delay speeded the acquisition of delay sensitivity. Following identical training procedures, the Houselight group reached criterion from session 11 (i.e. analysis of data from sessions 11–13, but not before, showed a significant effect of delay at $\alpha = .01$); the No Cue group met the criterion from session 18 and the Cue group from session 8. To confirm this effect statistically, the linear regression slopes (see *Methods*) for the first 14 sessions were subjected to an ANOVA. These slopes are shown in Figure 69C; analysis by group × (session × S) revealed a significant effect of session ($F_{8.149,138.531} = 6.021$, $\tilde{\varepsilon} = .627$, $p < .001$), reflecting the acquisition of delay sensitivity, and a group × session interaction ($F_{16.298,138.531} = 2.507$, $\tilde{\varepsilon} = .627$, $p = .002$), indicating faster acquisition in the presence of a cue.

2. Baseline performance

Effect of cues on choice (between-subjects comparison)

All three groups reached a similar pattern of choice once they had satisfied the delay-sensitivity criterion (Figure 69D). There were no significant effects of the cue condition on choice (terms involving cue: Fs < 1, NS) though there was a significant effect of delay ($F_{1.905,40.002} = 38.489$, $\tilde{\varepsilon} = .476$, $p < .001$). Similarly, there was no effect of cue on the regression slope measure (one-way ANOVA, $F < 1$, NS), even for the last baseline day ($F_{2,21} = 1.42$, NS). Taken on its own, this result suggests that the cue helps subjects to learn the contingencies in operation, but once these have been learned the cue plays no role in choice.

Omissions and latencies

Subjects' performance was reliable. Analysis across all groups showed that total omissions (failures to initiate a trial or respond on a lever) increased with delay ($F_{2.042,46.975} = 10.689$, $\tilde{\varepsilon} = .511$, $p < .001$) and there was a significant but small tendency to slower initiation at long delays ($F_{2.283,47.937} = 8.632$, $\tilde{\varepsilon} = .571$, $p < .001$), plausibly due to a degree of satiation. However, even at the final delay, omissions were only ~10%, or one out of the 12 trials (Table 19), despite the potential for rats to eat >10 g of pellets per session. Overall, of the 8400 choice trials analysed in the baseline data, subjects failed to initiate 5.7% of trials, and failed to respond to only 0.05% of initiated trials. Forced-choice trials were also responded to consistently: of the 1680 forced-choice presentations, 4.5% were not initiated and of those that were initiated, only 1.7% were not responded to.

Table 19: Omissions at different delays.

Delay (s)	% omissions (all kinds), mean ± SEM
0	1.54 ± 0.49
10	2.53 ± 0.58
20	5.46 ± 1.76
40	8.68 ± 2.14
60	10.81 ± 2.54

Subjects responded faster on the lever producing the large reinforcer ($F_{1,15} = 17.829$, $p = .001$) but this was independent of the delay and group (Fs ≤ 1.203, NS). Food was collected within 10 s of delivery on 99.9% of rewarded trials. The latency to collect food was not affected by the delay, the cue condition, or the subject's preceding choice (Fs < 1.327, NS).

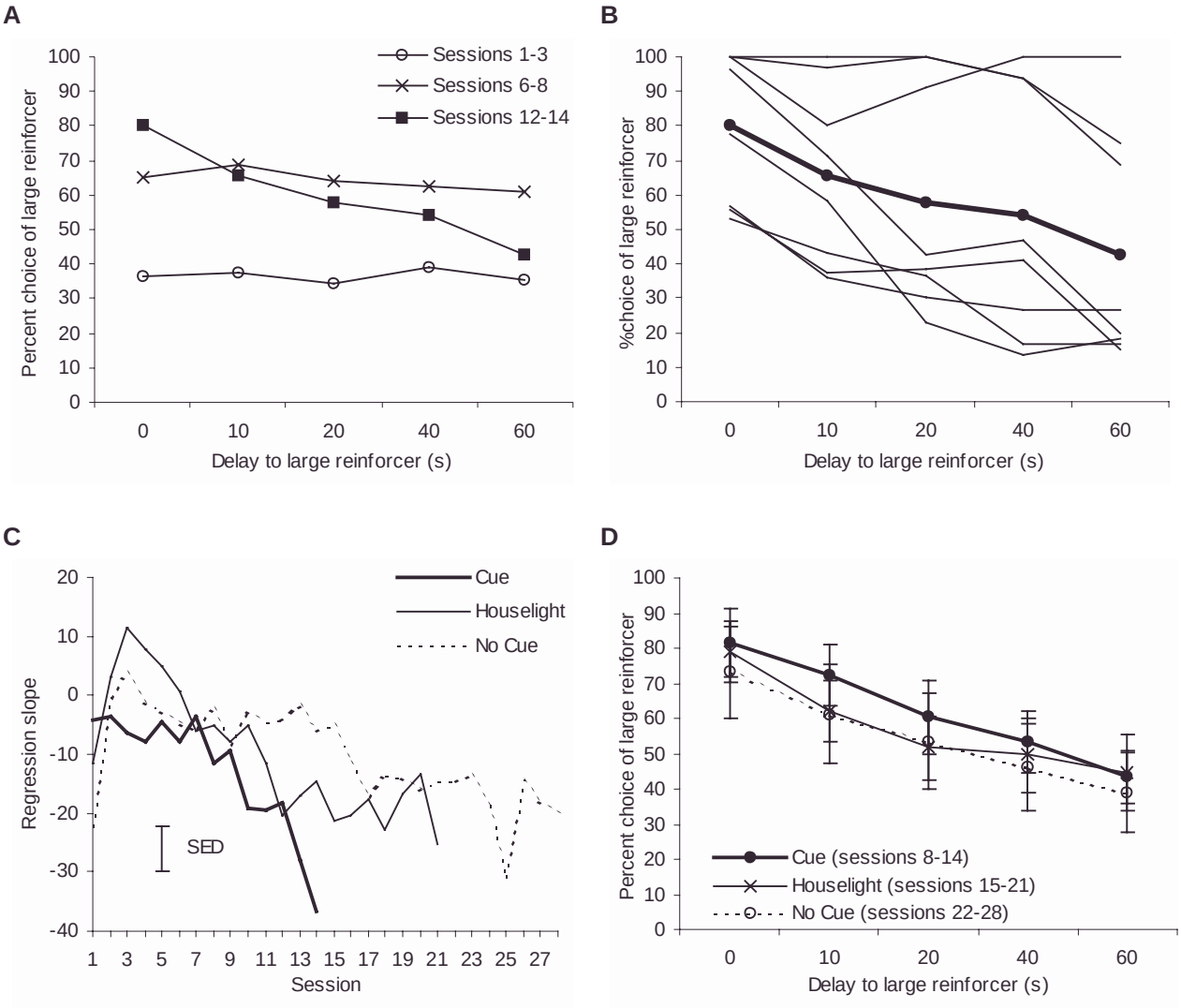

Figure 69. Task acquisition. **A:** Group means at different time points for the Houselight group. **B:** Individual records for the Houselight group, sessions 12–14, together with the group mean (*thick line*). **C:** Acquisition in different cue conditions as assessed by a regression slope measure (see text). *SED*, standard error of the difference between means for the group × session interaction. The SED is the appropriate index of variation for comparison between different mean values (see e.g. Howell, 1997 for derivation). **D:** Responding under different cue conditions immediately prior to drug testing.

3. Pharmacological manipulations

In all drug studies, choice was analysed using an ANOVA with the model (dose × delay × S), and the main effect of delay on choice remained highly significant throughout ($p < .003$). While there appeared to be a small tendency for the within-session shift in preference to be more pronounced with prolonged experience of the task, there were no between-group differences in responding under vehicle for any drug / dose study (choice ratios, all *F*s < 1; slope measures, maximum $F_{2,21} = 2.42$, NS); thus, drug effects at each dose can be interpreted relative to the same group baseline. The use of a within-subjects design allows small drug effects to be detected, but the individual variability discussed above allows a strong interpretation — for a drug effect to be found, that drug must have consistent effects despite subjects' starting from different individual baselines.

Effects of d-*amphetamine*

Choice. The effects of amphetamine depended on the cue condition (Figure 70; Figure 71). In the Houselight group, amphetamine did not affect choice at any dose (main effects, *F*s < 1; interactions with delay, *F*s < 2.08, NS). In the No Cue group, amphetamine *reduced* preference for the large reinforcer at 1.0 mg / kg (drug × delay interaction, $F_{4,28} = 3.336$, $p = .024$) and at 1.6 mg / kg (main effect of drug, $F_{1,7} = 6.834$, $p = .035$), but had no effect at 0.3 mg / kg (maximum $F_{1,7} = 3.30$, NS). In the Cue group, amphetamine *increased* preference for the large reinforcer at 0.3 mg / kg (main effect, $F_{1,7} = 12.393$, $p = .01$), and had no effect at other doses (*F*s < 2.25, NS). The increase in preference for the large reinforcer caused by this dose, calculated as an arithmetical difference between choice ratios in the drugged and vehicle conditions, was 8.4% when averaged over all delays (ranging from a 2% increase at 20 s delay to an increase of 17.3% at 10 s). The only dose that produced a significantly delay-dependent effect was 1.0 mg / kg in the No Cue group, which significantly reduced choice ratios at 40 s delay ($p = .018$ by one-way ANOVA) but not at other delays ($p = .088$ at 20 s and $p > .266$ otherwise).

The effect of amphetamine to increase preference in the Cue group was not due to altered responding in the zero-delay condition. Firstly, although the absence of a drug × delay interaction for 0.3 mg / kg strictly does not justify simple effects analyses, which also have lower power, such analyses showed that the effects at 10 s and 60 s (but not at 0 s) were significant in their own right. Secondly, elimination of the zero-delay condition from analysis did not alter the conclusion that 0.3 mg / kg caused a significant increase in choice ratios ($F_{1,7} = 9.801$, $p = .017$); the mean within-subject increase was 9.2% (as an arithmetical difference of %choice) in this analysis. Nonparametrically, six of eight rats showed an increase in preference for the delayed reinforcer calculated over all non-zero delays (Wilcoxon matched-pairs signed-ranks test, $p = .036$), and seven of eight rats showed an increase at the largest delay ($p = .025$). Nor did elimination of the zero-delay condition alter the conclusions about other doses.

The existence of a cue-dependent effect of amphetamine was confirmed statistically by testing data from the Cue and No Cue groups for a significant cue × drug or cue × drug × delay interaction; this was found for 0.3 and 1.0 mg / kg (cue × drug × delay, $F_{8,112} = 2.498$, $p = .016$). The highest dose, 1.6 mg / kg, had marked effects on omissions and consequently did not demonstrate such an interaction. The functional relevance of the cue-dependent effect was assessed directly from the total mass of food obtained on choice trials at non-zero delays: 1.0 mg / kg amphetamine reduced the amount of food obtained by animals in the No Cue group by 10.7% (mean within-subject change from saline), but this dose caused the Cue group to obtain 12.7% *more* food.

Omissions. Only the highest dose of amphetamine increased omissions. As there were few omissions, the percentage of trials on which an omission (of the initiation or choice type) occurred was calculated and analysed independently of the delay. There was a significant overall effect of dose ($F_{1.286,27.008} = 24.709$, $\tilde{\varepsilon} = .429$, $p < .001$), but no effect of cue (cue: $F_{2,21} = 2.465$, $p = .109$; cue × dose: $F_{2.572,27.008} = 2.401$, $\tilde{\varepsilon} = .429$, $p = .098$). Over all groups, the percentages of trials on which an omission occurred were 1.8 ± 0.3 (saline), 1.2 ± 0.6 (0.3 mg / kg), 1.9 ± 0.6 (1.0 mg / kg) and 15.7 ± 2.9 (1.6 mg / kg). Pairwise comparisons established that the 1.6 mg / kg dose differed from all other doses, which did not differ from each other.

Initiation latencies. Amphetamine slightly reduced initiation latencies at 0.3 mg / kg, and progressively increased them at higher doses. The mean initiation latencies in seconds (across all delays) were 1.207 ± 0.056 (saline), 1.057 ± 0.051 (0.3 mg / kg), 1.517 ± 0.108 (1.0 mg / kg), and 2.042 ± 0.139 (1.6 mg / kg). An analysis of data from all three groups revealed an effect of drug ($F_{1.969,45.289} = 32.905$, $\tilde{\varepsilon} = .656$, $p < .001$), of delay ($F_{2.967,68.248} = 2.895$, $\tilde{\varepsilon} = .742$, $p = .042$) and an interaction ($F_{4.513,103.805} = 4.38$,

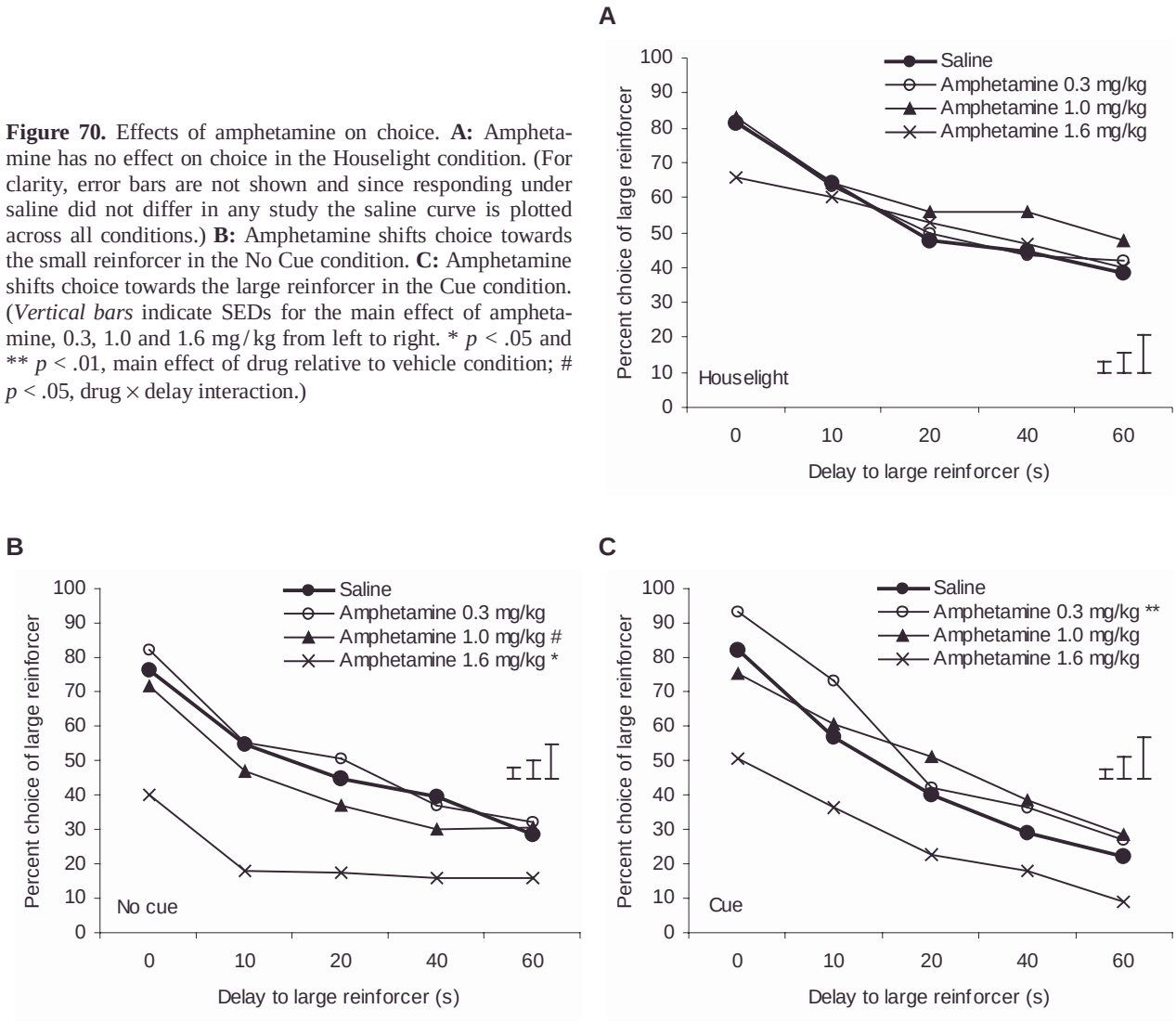

Figure 70. Effects of amphetamine on choice. **A:** Amphetamine has no effect on choice in the Houselight condition. (For clarity, error bars are not shown and since responding under saline did not differ in any study the saline curve is plotted across all conditions.) **B:** Amphetamine shifts choice towards the small reinforcer in the No Cue condition. **C:** Amphetamine shifts choice towards the large reinforcer in the Cue condition. (*Vertical bars* indicate SEDs for the main effect of amphetamine, 0.3, 1.0 and 1.6 mg/kg from left to right. * $p < .05$ and ** $p < .01$, main effect of drug relative to vehicle condition; # $p < .05$, drug × delay interaction.)

$\tilde{\varepsilon} = .376$, $p = .002$), though this interaction was attributable to the fact that 1.6 mg/kg had a greater effect early on in the session (other doses had effects independent of the delay: an analysis without the highest dose showed no such interaction; $F < 1$, NS). Pairwise comparisons of the main effect of drug with a Sidak correction showed that all doses differed from each other ($p \leq .001$).

Choice latencies. The two higher doses (1.0 and 1.6 mg/kg) increased choice latencies, especially early in the session. An analysis across the three groups using the design (drug × response × delay × S) revealed a significant drug × delay interaction ($F_{3.644,21.986} = 4.833$, $\tilde{\varepsilon} = .305$, $p = .007$). However, the effects of amphetamine did not depend on the response being made (drug × response: $F_{3,18} = 2.767$, $p = .072$).

Nosepoking during the delay. Amphetamine dose-dependently reduced the proportion of the delay spent nosepoking in the food alcove from 16% (saline, mean across all delays) to 8% (1.6 mg/kg) ($F_{3,18} = 12.062$, $p < .001$; nosepoking data were unavailable for the Houselight group). In addition, independently of the effects of amphetamine, the presence of the cue supported higher levels of nosepoking, particularly at long delays (cue × delay, $F_{3,18} = 4.519$, $p = .016$); the maximum effect occurred at 60 s delay, when the Cue group nosepoked for 16% of the delay (mean across all doses) and the No Cue group for 12%. This indicates that the cue had behavioural effects even in trained animals.

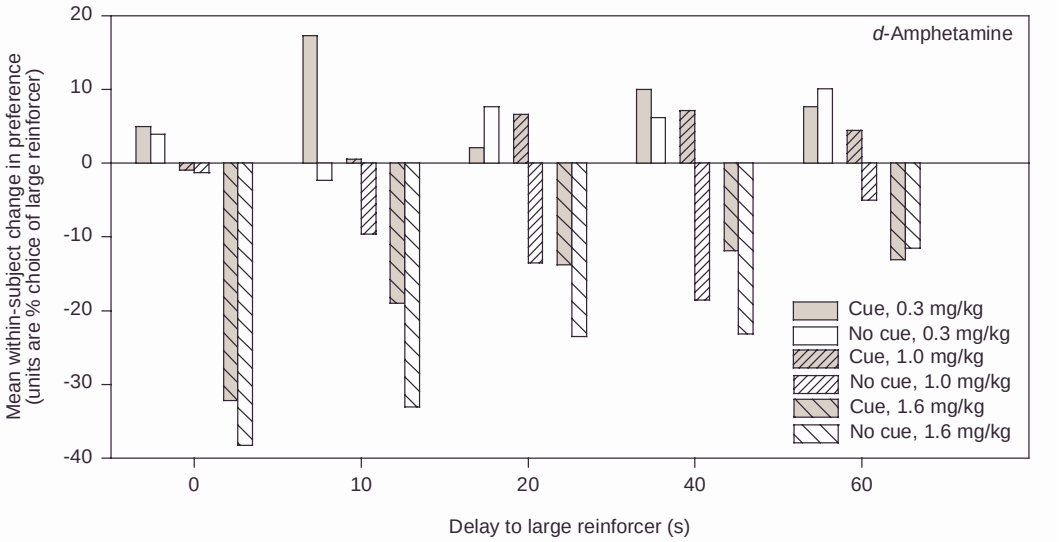

Figure 71. Effects of amphetamine on choice, replotted as mean within-subject changes for the Cue and No Cue groups. For each subject, the choice ratio under vehicle (as %choice of the large reinforcer) was subtracted from that under amphetamine, at each delay; the ordinate is the mean of these values. The effects of amphetamine on the Cue and No Cue groups differed significantly at 0.3 and 1.0 mg / kg (see text).

Food collection latencies. In this study, subjects collected the immediate reward faster than the delayed reward; neither amphetamine nor the delay had any influence on collection latency. An analysis across all groups using the model (drug × response × delay × S) revealed a main effect of response (response: $F_{1,6} = 10.53$, $p = .018$), but no other terms were significant (maximum $F_{1.139,6.835} = 3.351$, $\tilde{\varepsilon} = .38$, $p = .109$).

To summarize, at doses that did not grossly alter responding, the presence of a cue altered the effects of amphetamine on choice. Amphetamine had a cue-independent effect to reduce preference for the delayed reinforcer, and a cue-dependent effect to increase preference.

Effects of chlordiazepoxide

Choice. Chlordiazepoxide (CDP) generally promoted choice of the Immediate lever, and its effects did not alter in the presence of a cue (Figure 72). CDP had effects at all doses used except 1.0 mg / kg. Half a session's worth of data from one subject in the No Cue were lost from the 10 mg / kg study due to a malfunction.

In the No Cue group, chlordiazepoxide promoted choice of the smaller reinforcer, but only at 10 mg / kg ($F_{1,7} = 14.876$, $p = .006$), a dose that also increased the omission rate (see below); it had no effect on choice at other doses (closest to significance: main effect for 3.2 mg / kg, $F_{1,7} = 3.424$, $p = .107$). In the Cue group, the effects varied according to the dose of CDP and the delay. At 10 mg / kg the effect was similar to that for the No Cue group but not significant ($F_{1,6} = 5.729$, $p = .054$). However, 5.6 mg / kg caused a smaller but highly significant shift towards the small reinforcer (drug × delay interaction, $F_{4,28} = 2.871$, $p = .041$; main effect of drug: $F_{1,7} = 17.414$, $p = .004$), an effect that was significant at 10- to 40-s delays (simple effects, $p \leq .024$) but not for 0 or 60 s ($p \geq .058$). At 3.2 mg / kg, CDP had mixed effects (drug × delay interaction, $F_{4,28} = 2.843$, $p = .043$), promoting choice of the large reinforcer at 10 s ($F_{1,7} = 6.973$, $p = .033$) and of the small reinforcer at 40 s ($F_{1,7} = 6.831$, $p = .035$); effects at other delays were not significant ($p \geq .07$). The lowest dose, 1.0 mg / kg, had no effect in either group (maximum $F_{1,7} = 2.956$, $p = .129$).

Overall, no evidence for a cue-dependent effect of CDP was found. As before, data from the Cue and No Cue groups were tested for a cue × drug or cue × drug × delay interaction: no such terms were significant.

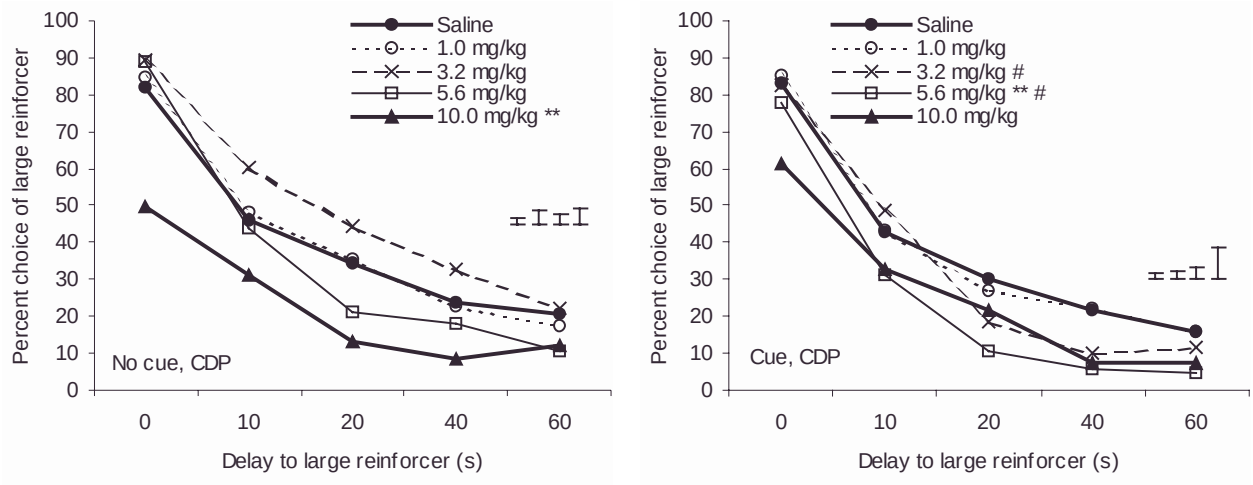

Figure 72. Effects of chlordiazepoxide on choice. As before, each line represents the mean of 8 subjects' choice ratios, calculated for three drugged sessions each, except the saline curve, which is calculated across all four dose studies (12 sessions) for simplicity of presentation as responding under saline did not differ in any dose study or between signalling conditions. (*Vertical bars* indicate SEDs for the main effect of chlordiazepoxide, 1.0, 3.2, 5.6 and 10.0 mg / kg from left to right. ** $p < .01$, main effect of drug relative to vehicle condition; # $p < .05$, drug × delay interaction.)

Omissions. Only the highest dose (10 mg / kg) markedly increased omissions ($F_{1.187,16.611} = 24.442$, $\tilde{\varepsilon} = .297$, $p < .001$). Indeed, this dose induced obvious somnolence in a number of subjects within minutes of administration. The percentages of trials on which an omission occurred were 1.4 ± 0.3 (saline), 1.2 ± 0.3 (1.0 mg / kg), 1.6 ± 0.4 (3.2 mg / kg), 4.1 ± 1.3 (5.6 mg / kg) and 33.2 ± 6.0 (10.0 mg / kg). Pairwise comparisons showed that 10.0 mg / kg differed from all other doses ($p \leq .004$ in all cases) but no other doses differed from each other ($p \geq .255$).

Initiation latencies. The highest dose (10 mg / kg) increased initiation latencies, particularly at the start of the session, but no other dose had an effect. Mean initiation latencies in seconds were 0.994 ± 0.093 (saline), 1.02 ± 0.088 (1.0 mg / kg), 0.883 ± 0.072 (3.2 mg / kg), 0.958 ± 0.06 (5.6 mg / kg), and 1.823 ± 0.149 (10 mg / kg). An analysis by cue × (drug × delay × S) revealed a main effect of drug ($F_{1.61,20.926} = 29.785$, $\tilde{\varepsilon} = .401$, $p < .001$) and a drug × delay interaction ($F_{7.803,101.442} = 3.12$, $\tilde{\varepsilon} = .488$, $p = .004$); no other terms were significant (drug × delay × cue: $F_{7.803,101.442} = 1.934$, $\tilde{\varepsilon} = .488$, $p = .064$; other terms: $F < 1$, NS). Pairwise comparisons showed that 10 mg / kg differed from all other doses ($p \leq .001$), which did not generally differ from each other (1 versus 3.2 mg / kg, $p = .049$; all other comparisons, $p \geq .171$). An analysis without the data for 10 mg / kg did not exhibit any delay-dependent drug effects (Fs < 1.118, NS).

Choice latencies. The pattern of results was identical to that for initiation latencies. There were insufficient data to analyse using a full model with cue and response as factors, so (drug × delay × S) was used. This revealed a main effect of drug ($F_{1.752,24.533} = 82.666$, $\tilde{\varepsilon} = .438$, $p < .001$) and a drug × delay interaction ($F_{3.213,44.978} = 11.472$, $\tilde{\varepsilon} = .201$, $p < .001$) but no effect of delay ($F < 1$, NS). Pairwise comparisons showed that 10.0 mg / kg differed from all other doses ($p < .001$), which did not differ from each other ($p \geq .068$).

Nosepokes during the delay. CDP did not have consistent effects on nosepoking. An ANOVA by cue × (dose × delay × S) revealed a complex pattern of results, there being a dose × delay × cue interaction ($F_{4.852,19.407}$ = 4.621, $\tilde{\varepsilon}$ = .404, p = .006). However, inspection of the data revealed that these results were entirely due to an aberrant increase in nosepoking at 40 s under 10 mg / kg in the Cue group; analysis without the 10 mg / kg data showed no significant effects of any term (p > .093).

Food collection latencies. CDP did not affect the latency to collect the reward. Again, there was insufficient data to use a full model, so (drug × delay × S) was used; no terms were significant (drug: $F_{1.264,17.693}$ = 1.636, $\tilde{\varepsilon}$ = .316, NS; delay: $F_{2.205,30.867}$ = 2.401, $\tilde{\varepsilon}$ = .551, NS; drug × delay: $F_{1.7,23.795}$ = 1.263, $\tilde{\varepsilon}$ = .106, NS).

Effects of α-flupenthixol

Choice. α-Flupenthixol had a weak effect to promote choice of the small reinforcer, irrespective of the cue condition (Figure 73). This effect reached significance for the No Cue group at 0.125 mg / kg (main effect, $F_{1,7}$ = 6.805, p = .035) and for the Cue group at 0.25 mg / kg ($F_{1,7}$ = 8.204, p = .024); though this effect was statistically independent of delay, it was numerically greatest at delays of 20–60 s. No other effects were significant, though there was a tendency for 0.125 mg / kg to promote choice of the small reinforcer in the Cue group as well ($F_{1,7}$ = 4.415, p = .074). The pattern of choice remained remarkably stable at high doses despite a large increase in omissions (see below).

α-Flupenthixol had a greater effect to decrease choice ratios in the Cue condition than in the No Cue condition at 0.125 mg / kg: in addition to a main effect of α-flupenthixol to decrease choice ratio scores ($F_{1,14}$ = 7.846, p = .014), there was a cue × drug × delay interaction ($F_{4,56}$ = 2.671, p = .041). Analysis of simple effects of drug at different delays showed that this interaction was due to a greater effect of 0.125 mg / kg to decrease choice ratios in the Cue than in the No Cue group at 40 s delay (simple cue × drug interaction, $F_{1,14}$ = 7.597, p = .015). However, this cue-dependent effect was small and there were no such effects at 0.25 and 0.5 mg / kg.

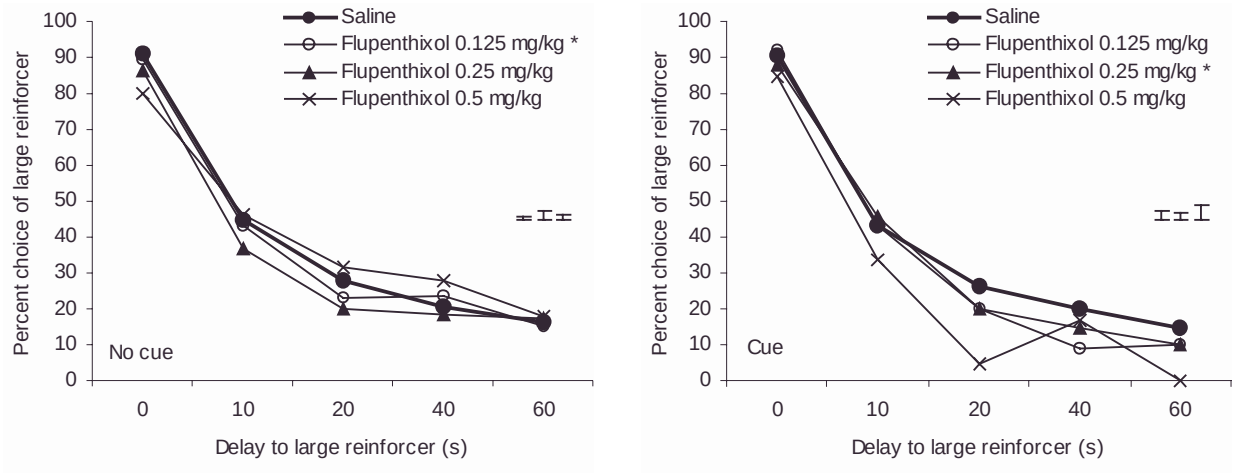

Figure 73. Effect of α-flupenthixol on choice. As before, each line represents the mean of 8 subjects' choice ratios, calculated for three drugged sessions each, except the saline curve, which is calculated across all four dose studies for simplicity of presentation as responding under saline did not differ in any dose study or between signalling conditions. (*Vertical bars* indicate SEDs for the main effect of α-flupenthixol, 0.125, 0.25 and 0.5 mg / kg from left to right. * p < .05, main effect of drug relative to vehicle condition.)

Omissions. The higher doses of α-flupenthixol increased omissions ($F_{1.405,19.676}$ = 73.813, $\tilde{\varepsilon}$ = .468, p < .001); this was independent of the cue (Fs < 1). The percentages of trials on which an omission occurred

were 2.3 ± 0.6 (saline), 3.0 ± 1.1 (0.125 mg / kg), 7.3 ± 1.8 (0.25 mg / kg) and 44.4 ± 4.6 (0.5 mg / kg). Pairwise comparisons showed that 0.5 mg / kg differed from all other doses ($p < .001$ in all cases); in addition, 0.25 mg / kg differed from saline ($p = .026$) but no other doses differed from each other ($p \geq .145$).

Initiation latencies. Flupenthixol dose-dependently increased initiation latencies, particularly at long delays, late in the session. An ANOVA by cue × (drug × delay × S) revealed main effects of drug ($F_{1.835,14.683} = 4.678$, $\tilde{\varepsilon} = .612$, $p = .029$) and delay ($F_{1.746,13.969} = 4.065$, $\tilde{\varepsilon} = .437$, $p = .045$) and a drug × delay interaction that escaped significance ($F_{2.664,21.308} = 3.091$, $\tilde{\varepsilon} = .222$, $p = .054$). No other terms were significant (Fs ≤ 1.221, NS).

Choice latencies. Flupenthixol's effects on choice latencies were similar to those on initiation latencies, with an increase in latency particularly at long delays. As there were insufficient data for a full model at all doses, the design cue × (drug × delay × S) was used. There were main effects of drug ($F_{2.405,19.242} = 7.854$, $\tilde{\varepsilon} = .802$, $p = .002$) and delay ($F_{4,32} = 11.205$, $p < .001$) and a significant drug × delay interaction ($F_{5.118,40.945} = 8.098$, $\tilde{\varepsilon} = .427$, $p < .001$). In addition, there was a cue × drug × delay interaction ($F_{5.118,40.945} = 2.486$, $\tilde{\varepsilon} = .427$, $p = .046$). No other terms were significant ($p \geq .094$). However, simple interaction analyses did not reveal a dose whose effects were demonstrably different in the Cue and No Cue groups (delay × cue interactions, $p > .128$).

Nosepokes during the delay. α-Flupenthixol dose-dependently blocked the ability of the cue to sustain higher rates of nosepoking (Figure 74). In this analysis, the number of omissions at 0.5 mg / kg was so high that it was necessary to omit these data for analysis of the other doses. This revealed a dose × cue interaction ($F_{1.343,10.748} = 9.573$, $\tilde{\varepsilon} = .672$, $p = .007$) in addition to main effects of dose ($F_{1.343,10.748} = 19.636$, $\tilde{\varepsilon} = .672$, $p = .001$) and cue ($F_{1,8} = 9.465$, $p = .015$), and a dose × delay interaction ($F_{6,48} = 5.645$, $p < .001$); no other terms were significant (Fs < 1.4, NS). Analysis of simple effects of the drug (across all delays) showed that in the No Cue group, subjects' nosepoking was unaffected by flupenthixol, while nosepoking was significantly reduced by the 0.25 mg / kg dose in the Cue group.

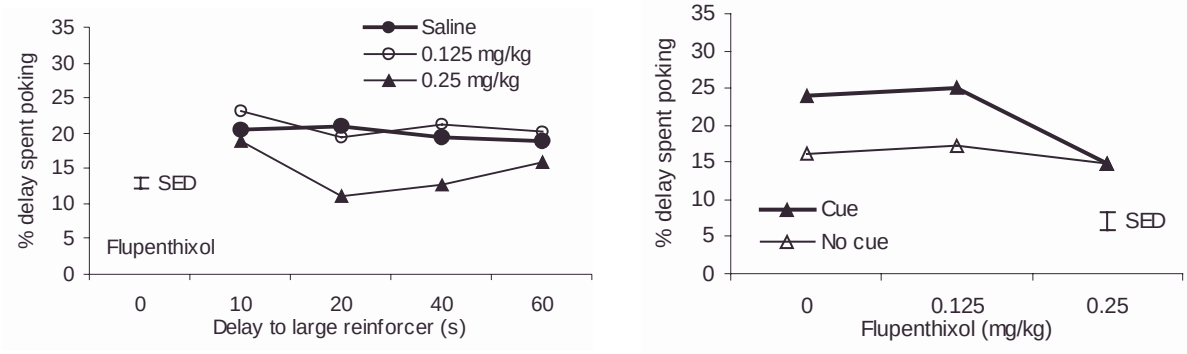

Figure 74. Effect of α-flupenthixol on nosepoking during the delay to reinforcement. The **left** panel shows data from both groups (*SED*, standard error of the difference for the main effect of α-flupenthixol; ** $p < .01$, difference from saline). The **right** panel, plotting data averaged across all delays, illustrates that the normal ability of the cue to sustain nosepoking was abolished by α-flupenthixol (*SED*, standard error of the difference for the dose × cue interaction; ## $p < .01$ for this interaction).

Food collection latencies. α-Flupenthixol did not affect the latency to collect reward. Data were analysed using the model cue × (drug × delay × S); aside from slightly longer collection latencies at long delays (main effect of delay: $F_{2.258,18.036} = 3.677$, $\tilde{\varepsilon} = .564$, $p = .041$), no terms were significant ($p \geq .141$).

4. Behavioural manipulations

Omission of delays

Omission of delays had clear effects to increase preference for the large reinforcer (Figure 75; Houselight group). There were significant effects of the Delay / No Delay factor ($F_{1,7}$ = 7.802, p = .027), trial block ($F_{2.023,14.159}$ = 17.005, $\tilde{\varepsilon}$ = .506, p < .001) and a significant interaction ($F_{1.589,11.121}$ = 8.094, $\tilde{\varepsilon}$ = .397, p = .009). The effect of omitting the delays was not complete, as subjects still altered their preference across the session in the absence of any delays (simple effect of trial block in the No Delay condition, $F_{4,28}$ = 6.736, p = .001).

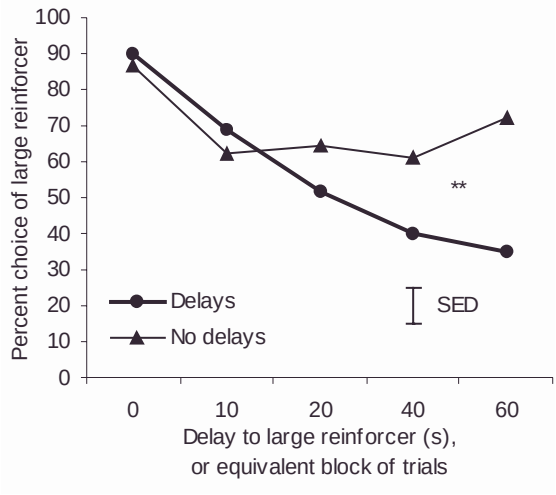

Figure 75. Effect of removing all delays on choice (Houselight group). (*SED*, standard error of the difference for the interaction term; ** p < .01 for this term.)

The development of a preference for the large reinforcer throughout a no-delay session was not immediate. Figure 76 shows the manner in which preferences changed across individual sessions (note that the data set is slightly different from that in Figure 75). Inspection of this figure suggests that the typical within-session shift towards the small reinforcer was present for the first no-delay session and was gradually eliminated. Indeed, analysis of the data from the three no-delay sessions using the model (session pair$_3$ × trial block$_5$ × S) demonstrated a change in the pattern of responding across the sessions (p = .013), with subjects exhibiting a within-session shift in preference on the first session of the three (p = .006) but not the third (p = .241). Subjects always exhibited always exhibited a within-session preference shift within delay sessions ($p \leq .003$), although it can be seen that the pattern of responding changed in these sessions too (p < .001). In particular, the alteration in the change in preference at from 0 s to 10 s delay during Delay sessions is interesting, as the 10-s block is first block during which the rat can determine whether it is experiencing a Delay or a No Delay session. If the rat were not successfully using the forced-choice trials as exemplars for the rest of the block, one would expect such 'contamination' of performance in delay sessions by experience of sessions without delays.

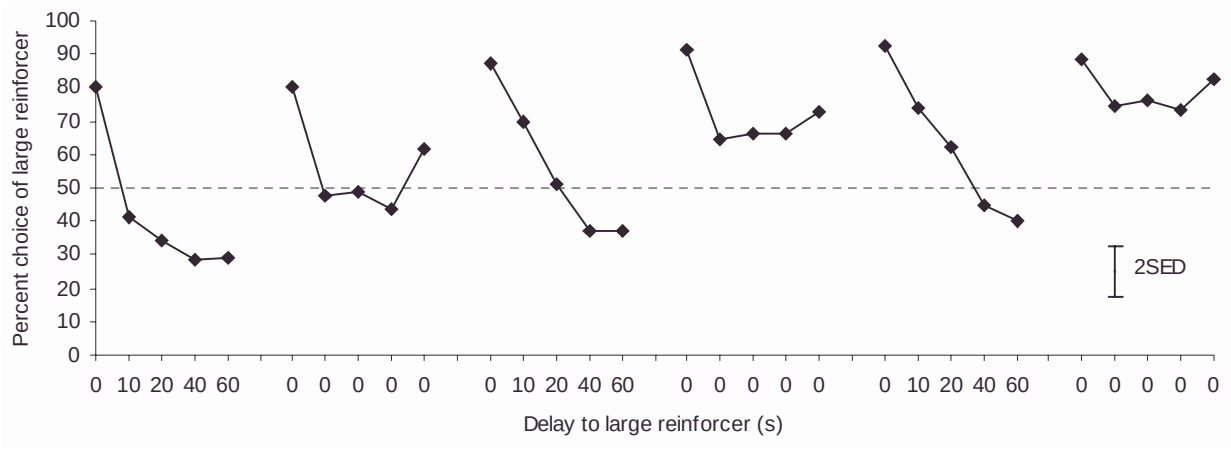

Figure 76. Group means for Houselight group (n = 8) when delays were omitted. The data are those shown in Figure 75, except that the session preceding the previous test is included, and the last session of the test omitted, for those rats who began the previous test with the No Delay condition. (*2SED*, twice the standard error of the difference for the session pair × delay / no-delay × trial block interaction; see text.)

Effect of cues on choice (within-subjects comparison)

While Figure 69D (p. 178) showed the effects of the cue condition on choice in a between-subjects comparison, a more sensitive test is a within-subjects comparison; not only does this have increased statistical power, but it reduces the potential for a learned adaptation to compensate for underlying cue effects on choice.

Introduction of a cue

The Houselight group were trained with successive sessions that alternated between Cue and No Cue conditions (both of which were initially unfamiliar) in the same fashion as the drug studies. As predicted, the cue had no effect on choice, even when the manipulation was extended to twelve sessions (Figure 77); analysis showed $F < 1$ (NS) for all terms involving cue.

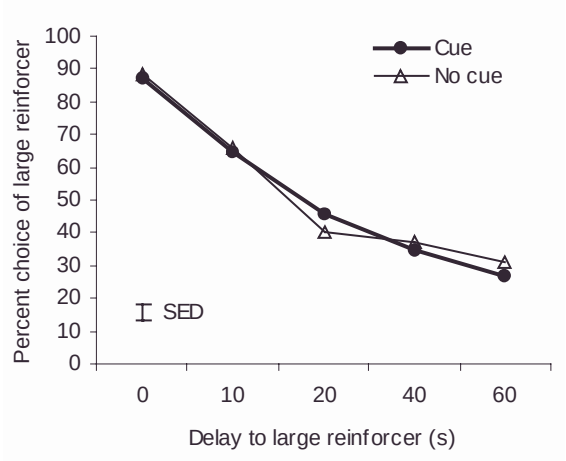

Figure 77. Lack of effect of introducing a cue for the Houselight group. The first six such test sessions are shown; no differences were observed in the second six sessions. (*SED*, standard error of the difference for the interaction term.)

Omission of a cue

Removing the cue from the Cue group reduced subjects' ability to choose the large, delayed reinforcer, although only when subjects experienced several consecutive sessions without the cue. Omitting the cue in alternate sessions (ABABAB design) did not affect choice ($F \leq 1.255$, NS for all terms involving cue).

However, when the Cue group experienced three consecutive cue sessions followed or preceded by three no-cue sessions (AAABBB design), an effect of cue emerged. The cue supported more frequent choice of the large reinforcer, particularly at long delays (Figure 78). An analysis of choice ratios as (cue × delay × S) showed a significant cue × delay interaction ($F_{2.636,18.451} = 3.564$, $\tilde{\varepsilon} = .659$, $p = .039$). Examination of individual subjects' performance showed that at every non-zero delay, six out of eight rats showed more frequent choice of the large reinforcer in the presence of the cue.

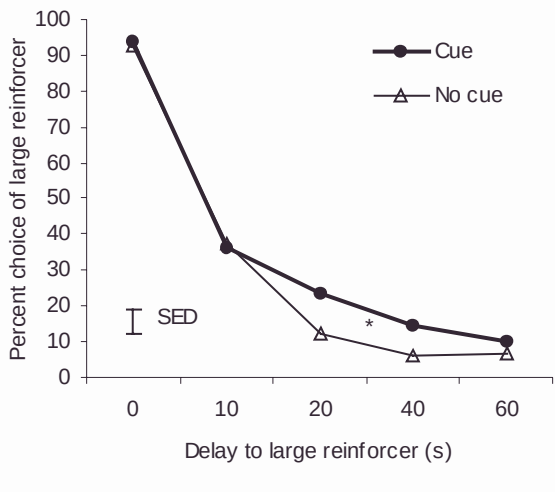

Figure 78. Effect of removing a cue from the Cue group: three consecutive cue sessions were given, followed by three consecutive no-cue sessions (or the reverse order, counterbalanced). (*SED*, standard error of the difference for the delay × cue / no cue interaction; * $p < .05$ for this term.)

Effects of prefeeding

Sating the subjects by giving 22 h free access to food had no effect on choice, despite progressively increasing initiation latencies through the session (Figure 79). Analysis of choice using the model (hunger × delay × S) showed no significant terms involving hunger ($F < 1$, NS). Every animal made more omissions when sated (heterogeneity of variance necessitated a non-parametric test: Wilcoxon matched-pairs signed-ranks test, $p = .012$). Initiation latencies were reliably increased by satiation: an analysis of variance using the model (hunger × delay × S) revealed a main effect of hunger ($F_{1,7} = 12.368$, $p = .01$) and hunger × delay ($F_{1.969,13.781} = 5.269$, $\tilde{\varepsilon} = .492$, $p = .02$), with no main effect of delay ($F_{1.474,10.321} = 2.378$, $\tilde{\varepsilon} = .369$, NS).

Prolonged satiation or deprivation had no effect on choice (Figure 79D). Maintenance on a more severe food deprivation regimen for a week reduced body mass to 86.1% of that following a week's free access to food (mean within-subject change), yet the effect of deprivation on choice was not significant (Fs ≤ 1.38, NS).

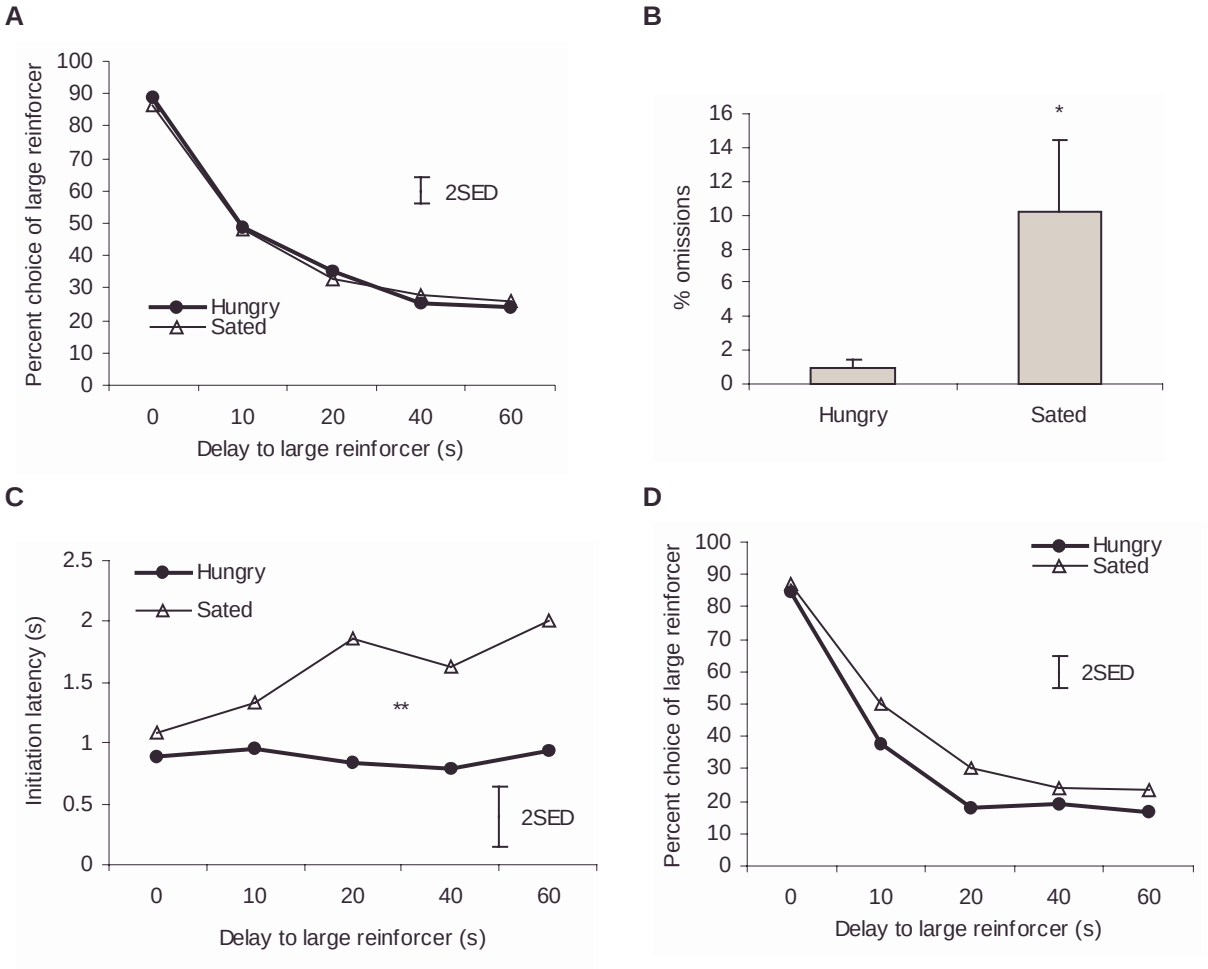

Figure 79. Effects of prefeeding. **A:** Satiation had no effect on choice. **B:** Satiation increased the number of omissions made. **C:** Satiation increased initiation latencies as the session progressed. **D:** Choice was not affected even when subjects were maintained in each deprivation condition for a week prior to testing. (*2SED*, twice the standard error of the difference for the hunger × delay term; * $p \leq .05$, ** $p \leq .01$ for the effect of hunger.)

Descending delays

Changing from an ascending to a descending series of delays reversed the direction of the subjects' preference shift within the session (Figure 80); the preference shift does not therefore depend on the use of an ascending series of delays. After the change, the group took 11 sessions to re-satisfy the stability criterion, suggesting that trained animals adjust their responding to a new pattern of delays at a similar speed to naïve subjects.

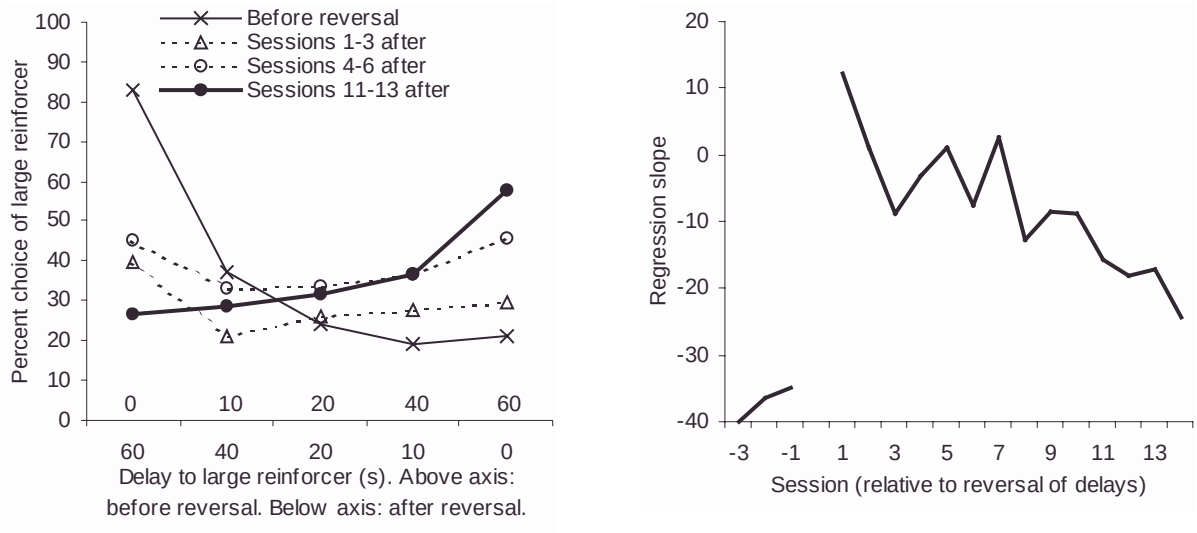

Figure 80. Reversal of delays. **Left panel** shows the effect of delay reversal on choice (Houselight group). Each line represents data from three consecutive sessions. The curve labelled 'before reversal' was part of the long-term satiation experiment, and met the stability criterion (effect of delay significant, $p < .01$). The first three sessions also met this criterion, but in the (now) inappropriate direction. After this, no set of sessions met the stability criterion until post-reversal sessions 11–13, and subsequently. **Right panel** shows mean regression slopes calculated for each session individually (see Methods). The reversal renders the subjects' preference 'incorrect' (shift from appropriate negative slope to inappropriate positive slope) and this slope declines gradually back towards the previous level.

Extinction

Extinction increased the number of omissions (from 4.8 ± 2.7 to 33.0 ± 8.3 per session; $F_{1,7} = 16.7$, $p = .005$). Extinction also affected choice in that preference tended towards indifference (50% ratio; Figure 81). However, an effect of delay remained in extinction: preference for the large reinforcer still declined throughout the session. Thus, extinction caused the animals to respond infrequently and randomly, but their tendency to choose the lever formerly associated with large reinforcement persisted for the first block despite the forced-choice trials preceding it. An analysis of choice ratios by (extinction × delay × S) showed effects of extinction ($F_{1,7} = 6.83$, $p = .035$), delay ($F_{4,28} = 36.5$, $p < .001$) and extinction × delay ($F_{4,28} = 6.98$, $p < .001$). There was also a simple effect of delay in the Extinction condition ($F_{3,18} = 7.20$, $p = .003$), in which responding differed significantly from 50% choice in the first block (one-sample t test: $t_7 = 5.13$, $p = .001$) but during no other block ($|t| < 1.01$, NS).

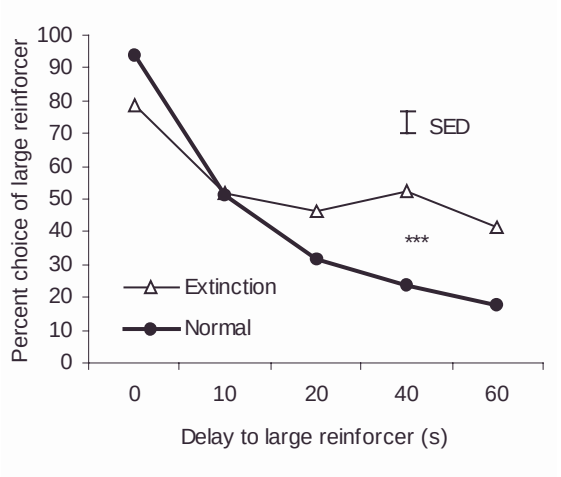

Figure 81. Choice in extinction. (*SED*, standard error of the difference for the interaction term; *** $p < .001$ for this term.)

DISCUSSION

The effects of amphetamine on impulsive choice depended strikingly upon whether the delayed reward was signalled, with amphetamine increasing impulsivity in the unsignalled condition and decreasing impulsivity when the delay was bridged by a signal. The dopamine receptor antagonist α-flupenthixol had opposite, although less marked effects in the cued condition than amphetamine. In contrast, effects of chlordiazepoxide on choice were not influenced by signalling the delayed reward. In order to interpret these results, the factors controlling baseline performance will first be considered.

Task validation

This work replicates and extends the findings of Evenden and Ryan (1996) concerning performance on this task. Subjects' choice behaviour gradually came under the control of the programmed delay during training, even though the overall rate of reinforcement on each lever never varied and the optimal strategy was always to choose the Delayed lever. Rats remained sensitive to the delays even after prolonged training. The within-session shift in preference was not due to satiation or fatigue: not only did animals reliably collect food even at the end of the session, but prefeeding and prolonged changes in deprivation state failed to affect choice behaviour (in agreement with Richards (1997b), though not with Bradshaw and Szabadi (1992) or Ho *et al.* (1997)). Removing the delays dramatically increased rats' preference for the large reinforcer, compared to the same time point in a normal session. Finally, when subjects were switched from an ascending to a descending series of delays, their preference came to shift in the opposite direction.

Some rats were far from 100% choice of the large reinforcer at zero delay; this differs from typical findings with discrete-trial and ratio schedules, where maximization is the norm (see Mackintosh, 1974, pp. 190–195). The departure from 100% was also greater than that found by Evenden and Ryan (1996); these authors always exposed rats to the differences in reinforcer magnitude before delays were introduced, whereas in the present study both were introduced simultaneously. The training procedure of Evenden & Ryan (1996) allows better establishment of the contingency between the 'large' lever and its reward, given that acquisition of this contingency may be impaired by the delay (Dickinson *et al.*, 1992), but establishes a bias for the large lever by the time the delays are introduced. In addition, Evenden and Ryan (1996) used a greater difference in reinforcer magnitudes between the two levers.

Some subjects were initially biased towards the Immediate lever and some towards the Delayed lever. Casual observation suggested that subjects tended to be biased towards the right-hand lever in each case, which was the lever trained second in the initial FR schedule. The training procedure was for a criterion number of responses in a fixed *time*, so subjects accumulated more responses on the second lever trained as the instrumental contingency was already established and generalized from the first lever. This probably encourages the development of habitual responding on the second lever to be trained; such habits depend on the number of reinforcers obtained (Dickinson *et al.*, 1995), and the effect might be avoidable by training to a criterion total number of reinforcers per lever. It is of some interest that Tomie *et al.* (1998) used exactly the same training procedure as the present study, and effectively the No Cue condition (the group slowest to acquire delay sensitivity in this experiment), and found a number of rats with extreme positional bias ('delay-insensitive'). However, this effect can only decrease the power of tests to find effects of neuropharmacological manipulations on choice.

The rats' persistence in shifting their responding from the Delayed to the Immediate lever during sessions when all delays were zero, and during extinction sessions, implies that they failed fully to use the forced-choice trials as exemplars for the subsequent block of choice trials (thus, performance on the task

cannot be accurately characterized as 'fully-informed choice'). It suggests strongly that the passage of time or trials acted as a discriminative stimulus that came to control responding, because when all delays are zero, no other stimulus is likely to cause a shift in choice from large to small reward. Subjects may take many sessions to acquire the characteristic within-session shift in choice, and to reacquire criterion performance when the delay sequence is reversed, in part because they must learn a session-wide temporal discrimination.

Role of signals present during the delay

The acquisition of delay sensitivity was facilitated by the presence of a discrete cue signalling the delayed reward, presumably by promoting discrimination between early and late trials and by speeding learning of the instrumental contingencies. This cue had no gross effects on stable choice performance, although it supported a higher rate of nosepoking in the food alcove during the delay. However, removing the cue demonstrated that it promoted or supported choice of the large reinforcer in animals that learned the task in its presence, despite extensive experience with the task (these subjects were nearing their hundredth session) and with no differences in primary reinforcement. The effects of cue omission were manifest only when animals had the opportunity to learn over several sessions that the cue was no longer contingent upon responding, as observed for other schedules controlled by response-contingent stimuli predicting reward (Everitt *et al.*, 1989; Arroyo *et al.*, 1998).

The effects of the stimulus light cue are notable, because there are several environmental stimuli that could provide information to the subjects about the impending food reward. The absence of the small reinforcer following choice is an unambiguous signal for all subjects that the large reward is imminent, but an even more obvious cue is having just responded on the Delayed lever (see also Garrud *et al*., 1981). In the Houselight condition, the houselight was paired in an overlapping fashion with both the large and the small reward, and was also present at the start of the trial. Unambiguous interpretation of this group's results is therefore difficult. However, the Cue and No Cue conditions differed in only one respect: the presence or absence of a stimulus light preceding the large, delayed reward.

The results were entirely consistent with the cue being a conditioned reinforcer. Confirmation of this would require demonstration that the effect of the stimulus was due to its association with primary reinforcement, that the effect on behaviour was a consequence of the response–stimulus contingency, and that the response had never produced primary reinforcement (Mackintosh, 1974, p. 234). Such a demonstration would also be required to be certain that the cue did not become aversive by virtue of the long delay to reward. However, the conditions existed for a positive Pavlovian association to form between the predictive cue and the large reinforcer: the reward was delivered at a range of times after the onset of the stimulus, and never in its absence. The absence of an effect of introducing a cue light to the Houselight group, for whom it provided no extra information, argues against a simple 'stimulus-seeking' explanation of the cue's effects in undrugged animals. Faster acquisition of delay sensitivity in the presence of conditioned reinforcement is to be expected if such sensitivity is a consequence of discrimination learning (Grice, 1948), as suggested above. Finally, the absence of the cue at the moment of choice precludes its role as a discriminative stimulus in the usual sense; whether the cue acts as a conditioned reinforcer by acquiring some properties of the reinforcer or by providing information about its availability is a separate question (see Mackintosh, 1974, pp. 250–259).

Effects of *d*-amphetamine

As predicted, amphetamine had a dual effect on choice of delayed reinforcement, comprising a cue-independent effect to reduce preference for the large, delayed reward, and a cue-dependent effect to increase this preference. Relative to vehicle conditions, the magnitude of the amphetamine increase in the Cue condition was moderate, altering an average of 9% of subjects' choices from 'immediate' to 'delayed' reward decisions at non-zero delays. However, comparing the Cue and No Cue groups showed that the cue made a large difference to the effects of 1.0 mg / kg amphetamine on responding, altering an average of 16% of decisions from 'immediate' to 'delayed' choices. The cue-dependent effect of amphetamine to increase preference for the delayed reinforcer was consistent across subjects and resulted in a substantial increase in the amount of food earned by the Cue group.

These effects of amphetamine are consistent with previous work on impulsive choice, and may explain certain discrepancies in the literature: Evenden and Ryan (1996) used a task equivalent to the No Cue condition in the present study and found that amphetamine reduced preference for the large, delayed reward. The opposite result has been obtained using the adjusting-amount procedure (Richards *et al.*, 1997b), in which subjects make repeated choices between an immediate, variable amount of water and a delayed large reinforcer. Richards *et al.* sounded a tone for the duration of the delay, analogous to the Cue condition here, and have shown that amphetamine and the amphetamine analogue methamphetamine increase preference for the larger, delayed reward (Richards *et al.*, 1997a; 1999; Wade *et al.*, 2000). It is therefore clear that signals during the delay must be taken into account in future research on delayed reinforcement.

It is suggested that the cue-dependent effect of amphetamine reflects the potentiation of conditioned reinforcing properties of the cue, which predicts the arrival of a large reward. The efficacy of conditioned reinforcers is selectively increased by amphetamine and related compounds (Hill, 1970; Robbins, 1976; Robbins *et al.*, 1983) and this effect depends on a predictive relationship between the conditioned stimulus (CS) and the primary reinforcer (Robbins, 1976; Robbins & Koob, 1978). In the present study, the cue supported choice of the large reinforcer in animals trained in its presence, and amphetamine potentiated this effect; it is conceivable that the impulsivity-reducing effects of amphetamine in this task were entirely due to its actions to increase the efficacy of conditioned reinforcement. The neural locus for the impulsivity-reducing effects of amphetamine remains to be established, though this hypothesis predicts that it would be the nucleus accumbens shell as this is the critical site for the potentiation of conditioned reinforcement by amphetamine (Taylor & Robbins, 1984; Parkinson *et al.*, 1999b), a drug whose systemic effects in this respect are relatively weak (Robbins *et al.*, 1983). The finding that amphetamine's effects depended on the training history of the subjects is also analogous to that of Terrace (1963), who suggested that drug effects on S+ / S– discrimination depended upon whether the training procedure established the S– as aversive; in the present study, the cue-dependent effects of amphetamine are hypothesized to depend on training that establishes the cue as an appetitive stimulus.

It is unlikely that this result simply represents another instance of the phenomenon that behaviour controlled by external stimuli is less susceptible to disruption by amphetamine (Laties & Weiss, 1966; Carey & Kritkausky, 1972; Laties, 1972). Firstly, it should be noted that amphetamine might fail to disrupt behaviour controlled by external stimuli because it potentiates the effects of conditioned reinforcers, rather than because it improves discriminative stimulus control (Laties *et al.*, 1981), and there is little evidence to suggest that amphetamine facilitates control by purely discriminative (noncontingent) stimuli (e.g. Moerschbaecher *et al.*, 1979) or promotes responding for informative stimuli that are not themselves paired with reward (Branch, 1975). Secondly, the fact that amphetamine *increased* preference for the

large reinforcer in the presence of the cue implies that the cue does more than ameliorate an amphetamine-induced deficit.

One other interpretation deserves consideration. At the point when drug testing began, all groups had attained the same degree of control of behaviour by the delays. Nevertheless, as the cue affected the speed of task acquisition, the effects of each dose were assessed at different time points relative to the start of training in each group (earliest in the cued group). These slight temporal differences might thus account for the observed differences in the effects of amphetamine between the cued and uncued groups. However, this seems unlikely, as direct comparison of the vehicle data for each dose studied revealed no differences whatsoever in responding between the groups.

The cue-independent effect of amphetamine might reflect some specific psychological process. For example, amphetamine has been suggested to increase the speed of an 'internal clock' (Meck, 1983; Gibbon *et al*., 1997); this might have affected choice prospectively (i.e. the subject perceives itself to be at a later time-point in the session than it actually is, hastening the within-session shift towards the Immediate lever), or it may have affected retrospective choice (i.e. in the drugged state, the subject experiences a given delay as longer than it remembered, causing a decrease in its preference for the Delayed lever). However, all drugs tested tended to shift preference towards the smaller reinforcer at high doses that significantly increased initiation latencies and omissions; thus this preference for the immediate reinforcer might be a non-specific drug effect. For example, a disinhibiting effect on operant behaviour, an impairment of stimulus control or an impairment of memory for the instrumental contingency resulting in delayed reward might all favour the response producing an immediate reinforcer, although it cannot be known which, if any, of these putative mechanisms were operating. Nevertheless, this general tendency makes the cue-dependent effect of amphetamine the more striking.

Effects of chlordiazepoxide

CDP was used as a positive control for possible non-specific drug effects on performance, because it does not affect the control over behaviour by conditioned reinforcers (Robbins *et al.*, 1983). As predicted by this account, it did not interact with the cue condition in determining choice of the two reinforcers. At the highest dose used, CDP reduced preference for the delayed reinforcer (increased impulsivity); this was true of high doses of all drugs used and may represent a non-specific drug effect (see above). At doses that did not severely disrupt responding (as assessed by the omission rate), an increase in impulsive responding was also observed, and at one dose CDP shifted preferences in both directions within the session (3.2 mg / kg, Cue group), being the only occasion when it caused a decrease in impulsivity. CDP had no consistent effects on latencies (other than at the highest dose) or on nosepoking during the delay.

The finding that CDP generally reduced tolerance of delayed reward is in contrast to the demonstration by Evenden and Ryan (1996) that another benzodiazepine, diazepam, increased preference for the delayed reward in this task. However, the present finding is in accord with the effect of CDP and other benzodiazepines to promote an 'impulsive' strategy in a T-maze task (Thiébot *et al*., 1985). The action of benzodiazepines to increase impulsivity has been suggested to depend on a decrease in serotonin neurotransmission (Bizot & Thiébot, 1996; Bizot *et al.*, 1999); indeed, one benzodiazepine subgroup, the triazolobenzodiazepines, can *increase* 5-HT release and has been shown to reduce impulsive choice in the T-maze (Bizot *et al.*, 1999). While CDP blocks serotonin release *in vivo* (Soubrié *et al*., 1983), and serotonin depletion has been shown to increase impulsivity in some tasks (Wogar *et al.*, 1993b; Richards & Seiden, 1995) with serotonin reuptake inhibitors having the opposite effect (Thiébot, 1986), this does not readily explain the discrepancy: diazepam and chlordiazepoxide have similar effects *in vitro* on midbrain

serotonin neurons (Thiébot *et al.*, 1982), and Evenden and Ryan (1996) found that the mixed serotonin receptor antagonist metergoline decreased impulsivity in the present task. While the effects of benzodiazepines on impulsive behaviour and the basis of these effects remain uncertain, the present results suggest that signals during a delay to reinforcement do not contribute to their action.

Effects of alpha-flupenthixol

In general, doses of α-flupenthixol that did not severely disrupt responding had small effects to reduce preference for the large, delayed reinforcer (i.e. to reduce tolerance of delay or promote impulsive choice). Its effects in the Cue condition were therefore opposite to those of amphetamine, as was predicted from its action as a dopamine receptor antagonist. Although interactions with the cue were not marked, those interactions were in the predicted direction: α-flupenthixol had a greater capacity to reduce tolerance of delay when the cue was present. As dopamine receptor antagonists, including α-flupenthixol, tend to impair the control over behaviour by conditioned reinforcers and its potentiation by amphetamine (Robbins *et al.*, 1983; Cador *et al.*, 1991; Wolterink *et al.*, 1993; Killcross *et al.*, 1997a), these results are consistent with the conditioned reinforcement hypothesis. Not only was α-flupenthixol able to impair the cue's effects to support choice of the large reinforcer, but it dose-dependently abolished the ability of the cue to sustain nosepoking in the food magazine during the delay (a form of conditioned approach behaviour). Taken together with the amphetamine result, this suggests that dopamine-dependent mechanisms contribute to the capability to choose a delayed reward by contributing to the effectiveness of conditioned reinforcers. However, α-flupenthixol also promoted impulsive choice in the absence of the cue; as this was an effect common to all three drugs tested, this may represent a non-specific disinhibiting effect or lack of stimulus control, such as has been observed for other neuroleptic drugs (Canon, 1979; Szostak & Tombaugh, 1981).

Conclusions

One function of conditioned reinforcement is to bridge temporal gaps between an animal's actions and primary reinforcement. This capacity can assist animals in learning discriminations based on delayed reinforcement (Grice, 1948), but can also contribute to performance of well-learned tasks. In artificial situations, conditioned reinforcers can even control behaviour to the detriment of performance (Williams & Dunn, 1991). The present study has demonstrated that stimuli present during a delay to reinforcement, probably by acting as conditioned reinforcers, can influence the effects of psychomotor stimulants. This has implications for the understanding and treatment of disorders of impulsive choice in humans, including ADHD; in particular, it suggests that the maximum benefit of psychostimulant treatment in this disorder will be obtained when behaviour is highly controlled by conditioned reinforcers, and when the availability of delayed reward is clearly signalled (see also Sagvolden *et al.*, 1998). In addition, it supports the idea that 'delay discounting' of the efficacy of future rewards is not a unitary process (Ainslie, 1975), but rather that the observed phenomenon of discounting arises from several underlying processes, of which conditioned reinforcement is one.