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Effects of Anticholinergic Drugs on DRL Performance of Rhesus Monkeys[1]

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McDONOUGH, J. H., JR. *Effects of anticholinergic drugs on DRL performance of rhesus monkeys.* PHARMAC. BIOCHEM. BEHAV. 17(1) 85–90, 1982.—Four adult rhesus monkeys were trained to stable performance baselines on a differential reinforcement of low rates (DRL) 28 sec schedule for food pellet presentation. The effects of graded doses of atropine SO_4, benactyzine HCl, and scopolamine HBr on performance were studied. All three anticholinergic compounds produced dose-related decreases in the number of food pellets earned. The number of responses was decreased only by the highest scopolamine dose. The unimodal peak of interresponse times (IRTs) was flattened under drug conditions such that there were roughly equal frequencies of responding in all IRT intervals. Drug potencies for producing these effects were: scopolamine > atropine > benactyzine.

DRL schedule Schedule-controlled behavior Atropine Benactyzine Scopolamine Anticholinergic Rhesus monkey

A CONSISTENTLY reported psychopharmacological effect of anticholinergic drugs in human subjects is alteration in the perception of passage of time [13, 15, 18]. Differential reinforcement of low rates (DRL) schedule-controlled performance has commonly been used as an operant conditioning analog to study this drug effect in animal subjects. In rats, atropine and scopolamine increase overall DRL response rates and decrease the number of rewards earned [4, 6, 14, 20, 21]. In squirrel monkeys, moderate doses of scopolamine increased unreinforced responding but did not change the number of reinforced DRL responses, while higher drug doses decreased both responding and rewards [25]. These drug effects obtained in a nonhuman primate are not entirely consistent with those reported for rats. Specifically, both the rodent and nonhuman primate studies reported that anticholinergics elevate DRL response rates, yet in squirrel monkeys this increase in responding did not affect the number of reinforcements earned, while in rats quantitatively similar increases in responding consistently decreased rewards. Additionally, all of these studies employed either a single anticholinergic drug and/or a limited range of drug doses. The present experiment investigated the effects of three anticholinergic compounds, atropine, benactyzine, and scopolamine, on well-trained DRL performance by rhesus monkeys.

Atropine, benactyzine, and scopolamine present relatively different antimuscarinic pharmacological profiles. In terms of central anticholinergic activity, benactyzine has been reported to be more potent than atropine in the ability to suppress the EEG α-rhythm [8, 18, 30]. Human psychopharmacological studies show scopolamine is the most potent of the three compounds, with a relatively fast onset of effects ($\simeq$ 30 min) and a duration of action of 4–5 hr following IM injection [15]. Benactyzine acts very rapidly ($\leq$15 min), but has a relatively brief time course (1–2 hr) when administered IM [9, 10, 13]. Atropine has a slow onset following IM injection (45–60 min), yet exerts its central effects for up to 7 hr [15]. The greatest difference between these three compounds is in their peripheral antimuscarinic activity. Scopolamine has a stronger action on the iris, ciliary body, and certain secretory (salivary, bronchial, and sweat) glands, while atropine is more potent on heart, intestine, and bronchial muscle, and has a more prolonged action [12]. In contrast to either atropine or scopolamine, benactyzine possesses relatively little peripheral antimuscarinic activity, having one-hundredth to one-third the potency of atropine depending on the measure employed [13]. The use of these three antimuscarinics was intended primarily to test whether each drug produced similar behavioral disruption of DRL schedule-controlled performance or whether behavioral changes were drug-specific.

METHOD

Subjects

The subjects were four adult rhesus monkeys (Macaca mulatta), two males and two females. They ranged in weight from 4–9 kg. The animals were individually housed in stain-

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less steel primate cages (60 cm wide × 68 cm deep × 76 cm high) with ad lib access to water. Food, with the exception of the rewards earned in the behavioral task, was restricted to a single supplemental feeding, including a slice of fruit, given at least 2 hr following the experimental session. These subjects had experience on this task for approximately 2.5 yr, and previously had some experience with the test compounds, but had not received any drug for at least 3 months prior to this study.

Apparatus

During the experimental sessions the subjects were chaired in standard Plexiglas primate restraint chairs which were placed inside a sound attenuating test booth located in a small room. The booth was equipped with an exhaust fan for frequent air exchange, a house light, and a speaker to present a masking noise. Inside the booth the animal sat facing two response levers, of which only the left one was active. Positioned to the right and front of the chaired subject was an open-faced square box. On the rear wall of this box was a 28V jewel-capped cue light. From the top of this box a pipe ran through the roof of the cubicle to a PPD-040 BRS-LVE pellet dispenser. Programming and data recording were accomplished with solid-state logic modules, electromechanical counters, and a Gerbrands cumulative recorder which were located in a room separate from the test chamber.

Procedure

The subjects were tested 60 min each day, at the same time of day, usually five days per week. The running order of subjects was kept constant. The start of a session was cued by the illumination of the house light and onset of =60 Db masking noise, both of which remained on throughout the session. A trial began by the illumination of the cue light which remained on until a response was made. The DRL interval was 28 sec. Responses prior to the 28-sec limit turned off the cue light for 2 sec (time out, TO), no reward was delivered, and the next trial began after the 2-sec TO. A response during the 2-sec TO initiated a further 2-sec TO and was recorded in the first interresponse time (IRT) bin. Responses later than the 28-sec limit also turned off the cue lamp for 2 sec, and during this time a 300-mg banana flavored food pellet (Noyes) was delivered. Data recorded were: total responses, total food pellet rewards earned, and the distribution of IRT's divided into 4-sec bins with the exception of the first bin which also included any TO responses.

All drugs were administered IM in the calf. Benactyzine and scopolamine were administered 15 min prior to testing while atropine was given 45 min prior to testing. The vehicle was distilled water for injection, USP, with 0.5% methyl paraben and 0.05% propyl paraben (W/V) added for stabilization, and adjusted to a pH 2.6 with 0.1 N hydrochloric acid. All drug concentrations were adjusted for a volume of 0.1 ml/kg. The drugs were prepared in lots and stored under refrigeration between drug tests. All doses are expressed as salts.

Four doses of each anticholinergic drug were used: atropine SO_4=0.014, 0.044, 0.14, 0.44 mg/kg; benactyzine HCl=0.054, 0.17, 0.54, 1.7 mg/kg; scopolamine HBr=0.010, 0.018, 0.032, 0.056 mg/kg. All subjects received all drug doses tested. For each drug, a balanced 4×4 Latin square was used to control for order effects of dosage, and the order of drug testing was randomized between subjects. The two sessions which preceded each drug test were considered as controls and vehicle injections were always administered before one of these two sessions. Thus, there were 4 vehicle and 4 no injection control sessions for each subject for each drug. In all cases, at least one week and four testing sessions without drug separated drug tests.

RESULTS

Preliminary analysis compared performance on days of vehicle injections with that on the control days when no injection was given. This revealed no reliable effects of vehicle injection on number of responses or number of food pellets earned. Therefore, the two sessions prior to each drug dosage tested were regarded as controls. The data of these eight control sessions were then averaged for each subject, and this average was regarded as a subject's control performance for that drug. The total number of responses, the total number of food pellets earned, and the IRT distributions were treated in this fashion. The total number of responses and the total number of food pellets earned were analyzed separately for each drug using single-factor repeated measures analysis of variance [31]. These data are shown in Fig. 1. Reliable effects ($p<0.05$) were further evaluated using Dunnett and Newman-Keuls tests.

Responses

Only scopolamine had a reliable effect on total number of responses (scopolamine: $F(4,12)=5.34$, $p<0.05$; atropine: $F(4,12)=0.35$; benactyzine: $F(4,12)=0.14$). This result was due entirely to the low number of responses emitted during the high dose sessions compared to control or the two low doses of scopolamine. The increase in responding observed after 0.01 mg/kg of scopolamine was not reliably different from control performance. As suggested by the relatively large standard errors, there was considerable between-subject variability in the way the three drugs affected responding.

Rewards

The subjects consumed all food pellets earned under each drug condition suggesting there were no nonspecific drug effects on food consumption. The analysis of the number of food pellets earned revealed that all three anticholinergic compounds had reliable effects on this measure of DRL performance (atropine: $F(4,12)=48.3$, $p<0.001$; benactyzine: $F(4,12)=10.51$, $p<0.01$; scopolamine: $F(4,12)=17.28$, $p<0.01$). All three drugs decreased the mean number of food pellets earned as a function of increasing drug dosage.

To compare the potencies of the three anticholinergic drugs, least squares linear regressions were performed using log drug dosage as the independent variable and earned rewards as the dependent variable. The results of all four of the benactyzine doses and all four of the scopolamine doses were used in computing the regressions. Only the three highest doses of atropine were used since inspection of the data indicated that the lowest atropine dose (0.014 mg/kg) did not contribute in any appreciable fashion to the obtained drug effect. The respective slopes (in log dose-effect units) and intercepts obtained from these regressions were: atropine = −53.21, 5.47; benactyzine = −27.02, 34.74; scopolamine = −43.83, −45.64. Tests of parallelism between all three slopes were then performed [28]. The results indicated only the slopes of atropine and benactyzine could not be considered parallel, $F(1,24)=6.45$, $p<0.05$. Therefore both atropine and benactyzine produced effects on this measure of perform-

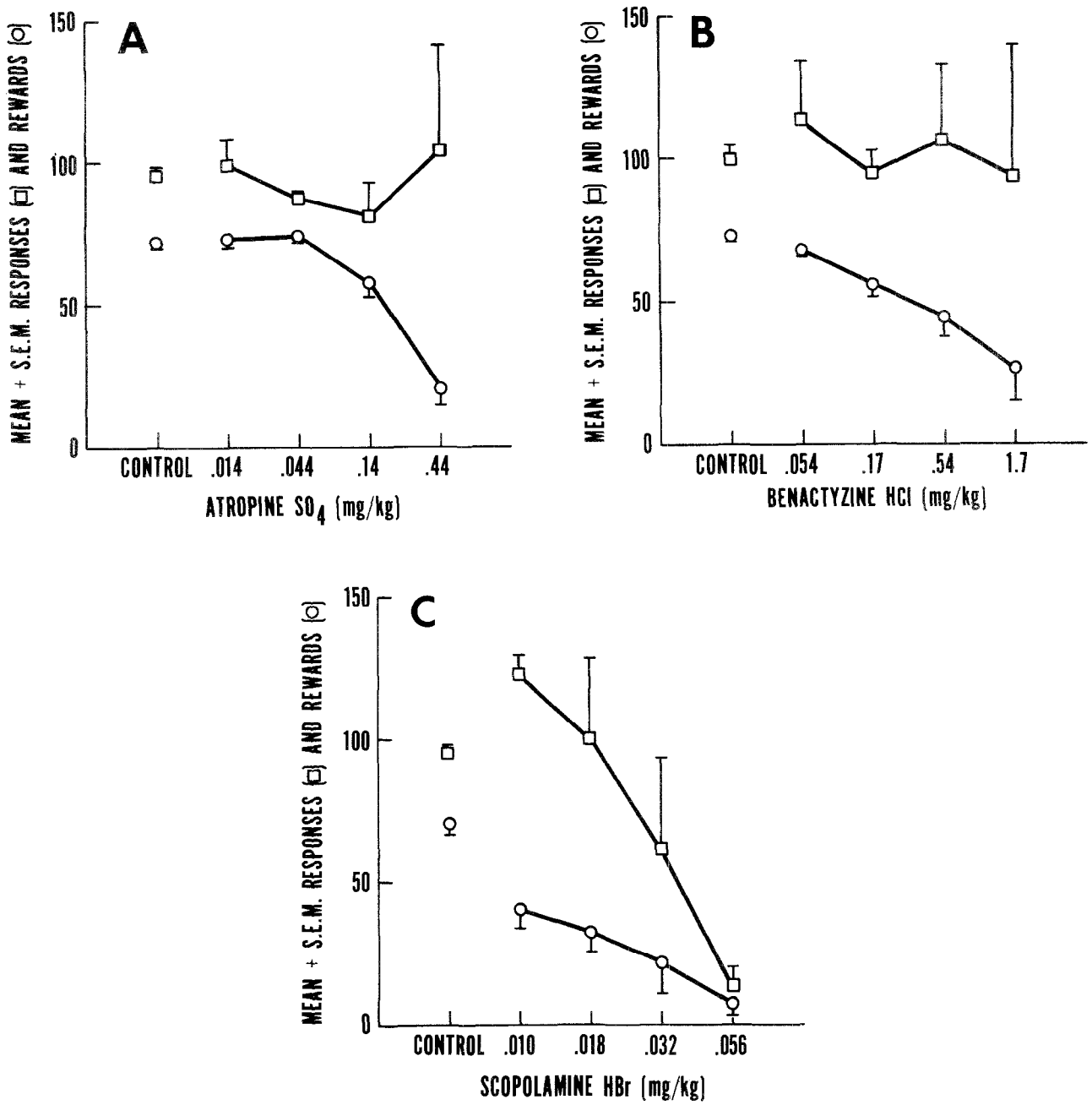

FIG. 1. Mean number of DRL responses (squares) and mean number of earned rewards (circles) with corresponding standard errors (vertical lines) for all control and all drug conditions (panel A=atropine; panel B=benactyzine; panel C=scopolamine).

ance which were similar to the effect produced by scopolamine but were different from one another. For each drug the 50% value of the mean number of food pellets earned under control conditions was entered into the respective regression and the equation solved for log dose. These doses should thus be equipotent for producing a 50% decrement on this measure of DRL performance. These doses and their potency relative to scopolamine are presented in Table 1. In terms of potency the three drugs can be ranked: scopolamine > atropine > benactyzine.

IRT

The IRT data were expressed as frequency distributions and are displayed in Fig. 2 for all drug dosages. It can be seen that under control conditions a stable temporal discrimination had been established as indicated by the sharp unimodal peak of responding which occurs around the point of the DRL interval limit (28 sec). All three anticholinergic drugs produced relatively similar effects on IRTs, although at different doses. There is a flattening of the sharp unimodal frequency distribution that is dose dependent. This effect is

TABLE 1

RELATIVE POTENCIES OF ANTICHOLINERGIC DRUGS FOR DECREASING REINFORCEMENTS ON THE DRL SCHEDULE

Drug	ED50* (mg/kg)	Potency Relative to Scopolamine
Atropine SO_4	0.270	0.052
Benactyzine HCl	0.870	0.016
Scopolamine HBr	0.014	1.0

*Dose resulting in 50% decrease in reinforcements are estimated by linear regression.

primarily due to the increased frequency of responding in the intervals shorter than 28 sec (i.e., nonreinforced responses), and a corresponding decrease in responding at intervals of 28 sec or longer. There is no consistent evidence in these data for a selective increase in very short IRTs or burst responses related to increasing doses of any of the three compounds. Additionally, there was a large increase in the number of responses with IRTs of ≥60 sec which is most apparent at the higher doses of all three drugs.

DISCUSSION

The major finding of the present study was that all three anticholinergic drugs produced decrements in DRL perform-

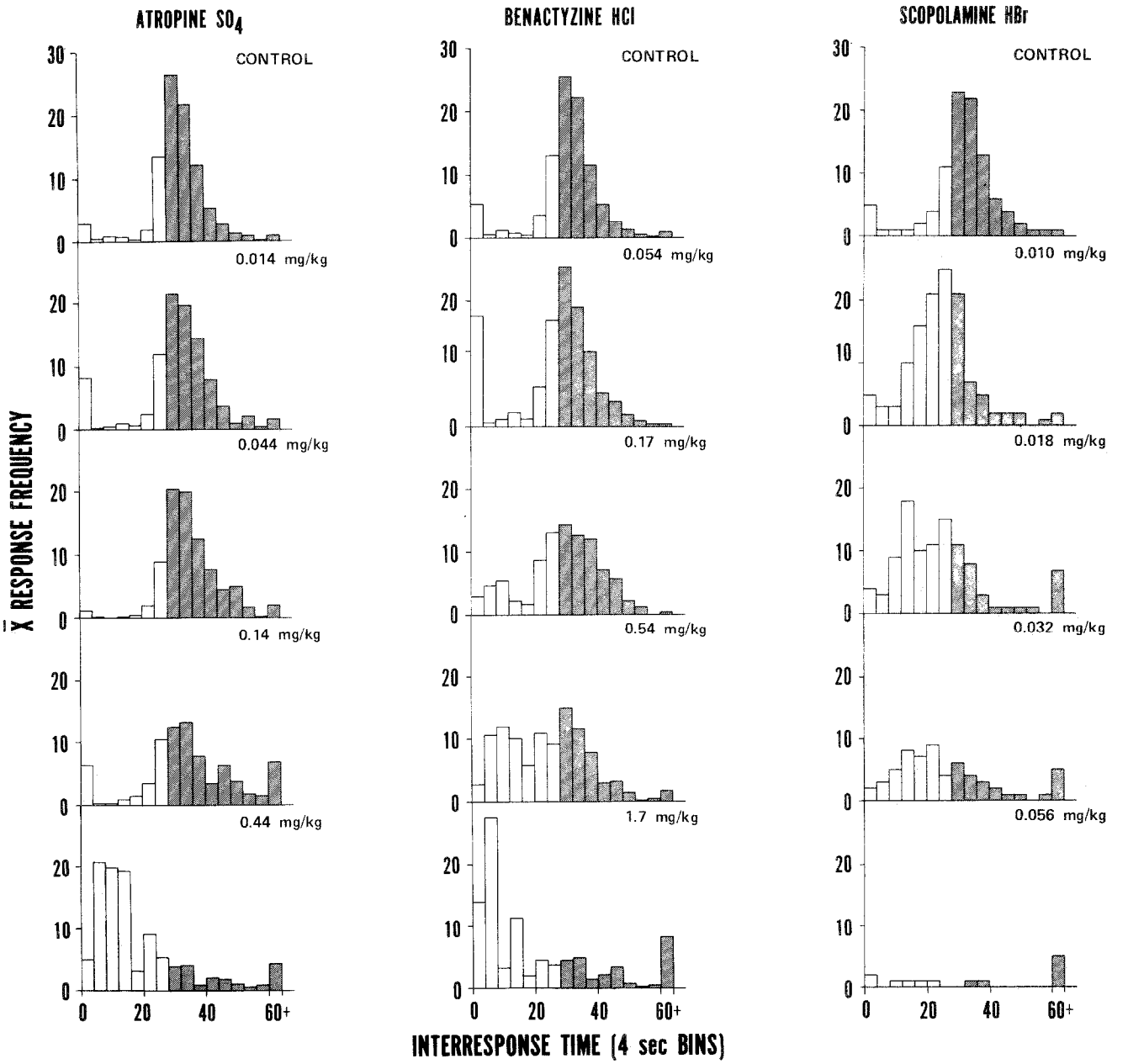

FIG. 2. Mean response frequencies of IRTs for all drug conditions and their respective controls. Shaded bars represent reinforced responses (≥28 sec).

ance at doses that did not materially alter overall response rates. Drug-induced changes in rates of responding showed a high degree of intersubject variability, an effect also observed in rats performing under a DRL schedule after administration of 0.5 mg/kg scopolamine [21]. By contrast, other studies have reported that atropine and scopolamine elevate DRL response rates in rats, which in turn leads to a loss in rewards [4, 6, 14, 20, 21]. Although the total number of responses became quite variable under drug conditions, the IRT data show a definite change in the patterning of responses. At moderate doses all three compounds disrupted the precise unimodal response distribution and there was a relative increase in "early" responses. Although the frequency of short IRTs was increased this did not elevate overall response rates due to decreases in reinforced responses and increases in excessive pausing (IRT $\geq$60 sec). Unlike a previous report [25], this relative increase in early responding was always accompanied by a loss of rewards. Although cholinolytics may increase DRL response rates in rats, the effects observed on IRT distributions [6,20] are the same as demonstrated in this study and distinguish the action of these drugs on DRL performance from other classes of compounds. The major effect of anticholinergics is to produce approximately equal response frequencies in all intervals, thus flattening the IRT distribution. This effect would indicate a complete loss of the temporal discrimination controlling performance [16]. The drugs nicotine, d-amphetamine, and delta-9-tetrahydrocannibinol shift the peaks of DRL IRT distributions to lower class intervals [19,26] which demonstrates a more selective drug effect on time estimation accuracy. In contrast, benzodiazepines and barbiturates disrupt DRL performance by selectively enhancing responses with very short IRTs [3,27], an effect which suggests these compounds are affecting some other aspect of behavior controlling performance than passage of time.

Only one drug dose tested in this study (0.056 mg/kg scopolamine) produced any reliable change in responding. The suppressant effects of high doses of scopolamine ($\geq$0.075 mg/kg) on nonhuman primate performance has been observed on both food and shock motivated operant tasks [2,25] and tasks requiring self-initiated trials [1]. By contrast, neither atropine nor benactyzine altered DRL response rates over a fairly wide range of doses. Doses as high as 3.2 mg/kg of atropine are required to depress variable-interval responding in monkeys to similar low levels as observed here with scopolamine [5]. The lack of agreement between studies as to how anticholinergics affect DRL response rates may possibly be explained by the nature of the DRL schedule itself. The disruptive effects of anticholinergic drugs on performance are most apparent on those aspects of a task under weak stimulus control [7]. The DRL task can also be considered to be one maintained by weak stimulus control since there are no exteroceptive stimuli associated with the procedure (except for the onset of the cue light marking the start of a trial) which the subject can use to gauge the accuracy of time estimation. In this respect a number of authors have noted that both rats [17] and nonhuman primates [11] trained on DRL schedules develop overt collateral behavior, and the amount of these activities may serve as a discriminative stimulus to guide performance. Each subject may develop idiosyncratic collateral behaviors, the different types or frequency of which may be more or less susceptible to modification by anticholinergics. This would explain why DRL response rate becomes highly variable between subjects while the ability to earn rewards is consistently degraded.

The regression analysis indicated significant differences in the potency of the three antimuscarinic compounds in producing effects on DRL performance. Scopolamine has consistently been reported to be the most potent antimuscarinic (of the compounds tested) for disrupting schedule-controlled behavior of both rats and nonhuman primates. The present results are also in agreement with findings that atropine is more potent than benactyzine in elevating the response rates of rats performing a nondiscriminated avoidance task [29], a procedure which also contains a timing component somewhat similar to that required in a DRL task. However, other studies with rats have found benactyzine to be more potent than atropine in ability to suppress spontaneous alternation [24] or to serve as the discriminative stimulus in drug discrimination experiments [23]. The doses of all three anticholinergics which were effective in disrupting DRL performance of rhesus monkeys in this study approximate those doses on a mg/kg basis which produce impairments of human cognitive function [9, 10, 13, 15, 18, 22]. In terms of general sensitivity and responsiveness to cholinolytic compounds of varying potency, non-human primates appear to closely model the human psychopharmacological response to these drugs.

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