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Shuttlebox Sidman Avoidance in Rhesus Monkeys: Day of Week and Amphetamine Effects

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RUSH, D. K. *Shuttlebox Sidman avoidance in rhesus monkeys: Day of week and amphetamine effects.* PHARMACOL BIOCHEM BEHAV 25(1) 145–148, 1986.—Due to its stability and sensitivity, the Sidman avoidance schedule has been often used to characterize the psychotropic effects of drugs. In the present study, the effects of d-amphetamine (0.125, 0.25, and 0.5 mg/kg IM) on shuttlebox Sidman avoidance by rhesus monkeys were investigated. Amphetamine resulted in increased avoidance rates as shown by both bin and mean inter-response time (IRT) analyses. These results demonstrate the potential usefulness of this combination of task, species, and apparatus for investigating the effects of psychotropic substances. In addition, analysis of baseline data indicated a small but significant day of week effect with more efficient performance at the end of the week. The implications of this finding for conducting drug studies involving repeated measurements (i.e., cross-over designs) are discussed.

Sidman avoidance Shuttlebox Amphetamine Bin analysis Day of week effect Rhesus monkey

SINCE its initial description, the Sidman schedule [11], due to its stability and sensitivity, has been widely used to study the effects of drugs [1]. The effects of amphetamine, for example, have been studied in rats, squirrel monkeys, and pigeons employing leverpress or keypeck responses [2, 3, 5]. In all studies, an increase in avoidance responding was found. The present experiment examined the generality of this finding by testing the effects of d-amphetamine on Sidman avoidance responding by rhesus monkeys in a shuttlebox, a combination of species, apparatus, task, and drug not previously employed.

METHOD

Subjects

Four male rhesus monkeys *(Macaca mulatta)*, ranging in age from 3 years 9 months to 4 years 3 months and in weight from 4–5 kg at the beginning of drug administration, served as subjects. They were housed in groups of 3–4 with other monkeys not included in this study in standard colony rooms (12 hr light cycle from 0700–1900 plus partial natural lighting; ad lib food and water). All four subjects had served in peer separation studies in which alcohol, amphetamine, and imipramine were administered. These studies terminated at least 6 months prior to the present investigation.

Apparatus

Avoidance training was conducted in a two-way shuttlebox constructed of stainless steel and Plexiglas (140×70×70 cm) designed and built for use with primates [10]. The two halves were separated by a barrier 30 cm high and a guillotine door which when raised permitted shuttle responses through a 23 cm high opening. Subject's position was detected by depression of spring mounted grid floors on each side. The entire apparatus was housed in an experimental room where 65 dB white noise masked extraneous sounds. Shock could be independently delivered to each side of the shuttlebox (Lehigh Valley generator/scrambler, 3 mA constant current DC shock). Control of contingencies and recording of responses were accomplished by relay equipment in a separate room.

Behavioral Procedure

Subjects were tested in a random order 5–6 days each week between 1200 and 1600. Following an initial 5 min adaptation period in one side of the shuttlebox, opening of the guillotine door signalled the start of the 50 min session. Subjects were required to respond from one side of the shuttlebox to the other according to a Sidman schedule [11] with a response-shock (R-S) interval of 30 sec, a shock-shock (S-S) interval of 2 sec, and a shock duration of 1 sec; that is, a response every 30 sec prevented the occurrence of a series of 1 sec shocks administered every 2 sec until the subject made a response over the barrier. At the end of the 50 min session, the guillotine door closed and the subject was removed from the apparatus.

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TABLE 1

PARAMETERS OF AVOIDANCE PERFORMANCE DURING THE THREE WEEK BASELINE PERIOD, ON NON-DRUG DAYS, AND AFTER SALINE

Parameter	Baseline	Non-Drug	Saline
5 min IRT	10.0 (1.8)	9.4 (1.2)	10.1 (1.2)
45 min IRT	12.6 (1.9)	12.1 (1.4)	12.4 (1.5)
Shock Trains	2.3 (1.1)	1.4 (0.5)	1.5 (0.3)
Shocks	2.9 (1.2)	1.9 (1.0)	2.1 (0.8)

The data shown are means ± SEM (in parentheses).
The 5 min IRT and 45 min IRT values are expressed in sec.

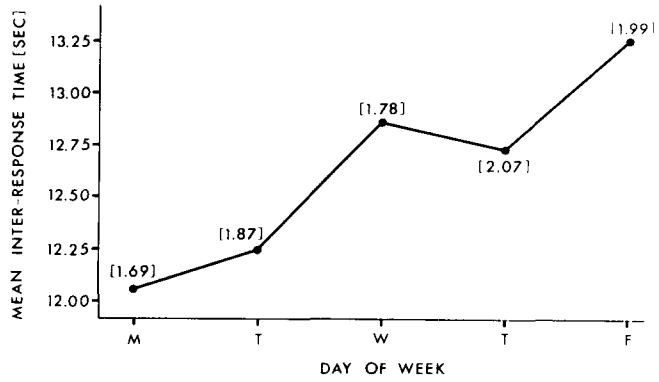

FIG. 1. Mean (±SEM in brackets) inter-response time (IRT) on each of the days of the week on which subjects were tested (Monday–Friday) during the 3 week baseline period prior to drug administration.

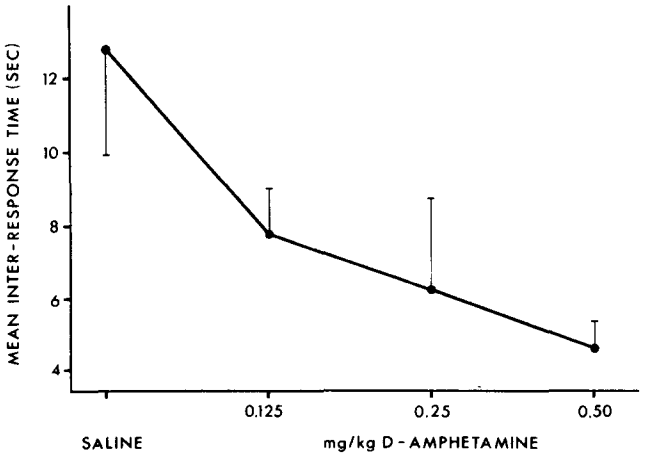

FIG. 2. Mean (±SEM) inter-response time (IRT) following the administration of saline and 3 doses of d-amphetamine.

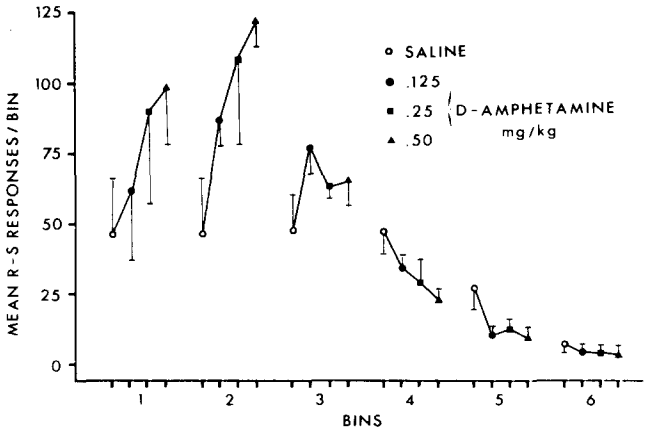

FIG. 3. Mean (±SEM) number of responses during each of the six 5-sec bins of the 30 sec R-S interval following the administration of saline and 3 doses of d-amphetamine.

Drug Administration

Following acquisition of stable Sidman responding, drug administration began. Subjects first received 3 saline injections followed by 2 replications of 3 doses of d-amphetamine sulfate (0.125, 0.25, and 0.5 mg/kg based on the salt weight). The order of dosing was determined randomly for each subject with the restriction that within each replication each subject received a different order. All injections were made IM (0.1 ml/kg) 30 minutes prior to testing and occurred at least 3 days apart, with two restrictions: (1) at least 1 of the 3 days was a test day and (2) subject's 45 min mean IRT (described below) on the day before injection fell within the range of values shown during a 3 week baseline period prior to the start of treatments.

Data Analysis

The mean inter-response time (IRT) in the first 5 min (the warm-up period) and the last 45 min of the 50 min session were calculated from frequency histograms of the number of responses in each of six 5-sec bins making up the 30 sec R-S interval. If a subject responded often, most responses were recorded in early bins and a low IRT value resulted; subjects that responded less often had more responses in later bins resulting in a high IRT value. In addition, the number of times that the R-S interval timed-out (the number of shock trains) and the total number of shocks (for 3 of 4 subjects the first shock of a shock train induced a response) were recorded over the 50 min session.

Baseline behavior analyses of warm-up and day of week effects were conducted on data collected in the last 3 weeks of the 14 week acquisition period prior to drug treatment. Means per subject for the various measures for the 3 week baseline period, non-drug days (the 9 days before saline or drug administration), and the drug treatment replications were analyzed with correlated t-test, 1- and 2-way repeated measures analysis of variance (ANOVA), and Duncan's multiple comparison procedure. $p<0.05$ was required for significance.

RESULTS

Baseline, Non-Drug, and Saline Sidman Avoidance Behavior

Table 1 presents the mean values of the various param-

eters collected during the 3 week baseline period, on non-drug days, and on days on which saline was administered. During the baseline period subjects were quite efficient at avoiding shock, waiting an average of 12.6 sec of the 30 sec allowed (during the 45 min main session) before making a response. The number of times the R-S interval timed out (shock trains) and the number of shocks were very low.

Subjects showed a warm-up effect and became more efficient at avoidance responding; they responded more frequently (the mean IRT was smaller) in the 5 min warm-up period than during the main 45 min session during the 3 week baseline period (correlated $t(4)=7.46$).

A 1-way ANOVA of the 45 min IRT data indicated a significant day of week effect in the 3 weeks prior to drug administration, $F(4,12)=4.98$. The linear trend over days was also significant, $F(1,3)=10.36$, indicating consistently changing IRT values over the course of the week (Fig. 1). Subsequent Duncan multiple comparisons indicated that the mean 45 min IRT was smaller on Monday than on Wednesday and Friday and smaller on Tuesday than on Friday, i.e., avoidance became more efficient as the week progressed.

A comparison of each of the parameters of avoidance responding, 5 min IRT, 45 min IRT, number of shock trains, and number of shocks during the 3 week baseline period, on the non-drug, and on the saline days revealed no effects (all F's$(2.6)<1.1$, all p's>0.40); that is, avoidance performance remained stable prior to and during the period of amphetamine administration. Some animals showed an increased avoidance rate on the days after injection of the highest dose of amphetamine, but insufficient data were available (not all subjects were tested on the days after the highest dose) to systematically investigate this phenomenon.

Effect of Amphetamine

The administration of d-amphetamine enhanced Sidman avoidance; the mean IRT (Fig. 2) decreased significantly, $F(3,9)=6.24$. Multiple comparisons revealed that the response to saline differed from the response to each of the three doses of d-amphetamine, which did not differ from each other. A significant linear trend, $F(1,3)=11.9$, suggested a dose related effect, however.

As demonstrated by Kuribara [5], analysis of the distribution of responses in the bins constituting the R-S interval may be a more sensitive method of assessing changes resulting from the administration of drugs. A 2-way (Drug × Bin) repeated measures ANOVA indicated that both main effects, drug treatment, $F(3,15)=5,2$, and bins, $F(5,15)=8.6$, as well as their interaction, $F(15,45)=3.1$, were significant. Inspection of group means in Fig. 3 revealed that the number of responses increased in the early bins and decreased in the later ones with increasing dose of d-amphetamine. As the interaction was significant, multiple comparisons were conducted within each level of each of the two main factors utilizing mean square error terms calculated separately for each set of comparisons [4]. For Bin 1, subjects made fewer responses when injected with saline than when injected with 0.5 mg/kg d-amphetamine. For Bin 2, the number of responses after saline was significantly less than after 0.25 and 0.5 mg/kg d-amphetamine. For Bins 4, 5, and 6 administration of d-amphetamine resulted in fewer responses in comparison to saline; for Bin 4 this was true for the two highest doses, and for Bins 5 and 6 for all three doses of d-amphetamine.

Comparisons across bins within each dose indicated that differences between bins increased with dose of d-amphetamine. The number of responses in the six bins of the 30 sec R-S interval did not differ when subjects were injected with saline. At the lowest d-amphetamine dose, the number of responses was greater in Bin 1 than in Bins 5 and 6 and in Bins 2 and 3 than in Bins 4, 5, and 6. Following a dose of 0.25 mg/kg, the number of responses was greater in Bins 1 and 2 than in Bins 4, 5, and 6. A dose of 0.5 mg/kg resulted in more responses in each of the first 3 bins than in each of the last 3 bins; in addition, the number of responses in Bin 2 was larger than in Bin 3.

DISCUSSION

Results of analyses of both bin and 45 min IRT measures corroborate previous findings [2, 3, 5] that the administration of d-amphetamine increases Sidman avoidance responding. The results of the present experiment extend these previous findings to include rhesus monkeys performing Sidman avoidance in a shuttlebox, a combination of species, task, and apparatus not previously investigated.

The increase in avoidance following d-amphetamine administration is often considered to be the result of a hyperactivity induced by this drug [8]. A variety of evidence suggests, however, that amphetamine does not always result in an increase in behavior. The administration of amphetamine has been consistently found to diminish social behavior in both primates (e.g. [7]) and rodents (e.g. [6]). Reductions in spontaneous motor activity in nonhuman primates [7], hyperactive boys, and both normal boys and adults [9] have also been reported. This variety of evidence suggests that amphetamine does not increase the rate of all kinds of emitted behavior and thus casts doubt on an explanation of increased avoidance as simply the result of a drug induced increase in activity. Although the results of the present study do not provide a basis for resolving this issue, they do demonstrate a method for its further examination.

The repeated measures design employed in the present study is both popular and useful for studying the effects of different drugs or different doses of a single drug on the same subjects. Unfortunately, data collected with this type of design can be strongly influenced by sequence or order effects (see [12] for a discussion of statistical aspects of these effects). When feasible, the order of administration of doses should be counterbalanced. The number of subjects increases factorially, however, with the number of doses administered. Although 3 doses requires only 6 subjects, 4 doses requires 24 subjects for a completely balanced design. If the number of subjects makes complete counterbalancing impracticable, care should be taken to ensure that assignment of drug doses controls as well as possible for known causes of variability, such as tolerance or sensitization, which can occur as a result of the order of dosing.

The present data indicate that day of week is also a variable to be encountered in designs involving repeated measurement of behavior. Failure or inability to counterbalance could result in systematic measurement of a particular dose or dose range during a particular period of the week. For example, drugs are often administered on Tuesdays and Fridays. If a drug is administered in increasing dose, a sawtooth dose-response curve may result from the combination of day of week and increasing (or decreasing) drug effect. At best, this would result in a nonlinear dose response curve, at worse in non-meaningful data due to the measurement error generated by the extraneous day of week variable.

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