Pharmacology Biochemistry & Behavior, Vol. 16, pp. 605–609, 1982. Printed in the U.S.A.

Effects of Benactyzine on an Equilibrium and Multiple Response Task in Rhesus Monkeys[1]

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Received 12 March 1981

FARRER, D. N., M. G. YOCHMOWITZ, J. L. MATTSSON, N. E. LOF AND C. T. BENNETT. *Effects of benactyzine on an equilibrium and multiple response task in rhesus monkeys.* PHARMAC. BIOCHEM. BEHAV. **16**(4) 605–609, 1982.—Five *Macaca mulatta* were tested for performance changes following exposure to benactyzine HCl (intramuscular) at four doses ranging from 0.054 mg/kg to 1.7 mg/kg. Subjects simultaneously operated a primate equilibrium platform and responded to a multiple alternative reaction task. Performance on both the continuous and discrete tasks were progressively degraded as benactyzine dose increased. At the higher doses it appeared that they concentrated their efforts on one of the tasks while neglecting the other. Thus, performance on one task might be normal and at the same time performance on another task would be degraded.

Rhesus monkey Benactyzine Operant performance Behavior

ORGANOPHOSPHATE poisons are powerful psychoactive compounds that have a variety of central nervous system effects [7]. Exposure to organophosphate pesticides has been suggested as a major cause of aircraft accidents among crop duster pilots [8,11]; however, the anticholinergic drugs that are administered as therapy for organophosphate poisoning may also interfere with efficient flying performance [8]. Benactyzine, a peripherally and centrally anticholinergic, has been considered for treatment of anticholinesterase poisioning [10], but is known to cause subjective changes and significant performance decrement in humans [2, 4, 10]. Because of the importance of equilibrium in aviation, a literature search was conducted to determine the effects of benactyzine on equilibrium. No human or animal studies were found on this subject. In addition, no reports were found concerning the behavioral toxicity of benactyzine on any tasks performed by nonhuman primates.

Thus, there is a need to better define the behavioral properties of benactyzine, and to provide background information for future experiments involving anticholinesterases in nonhuman primates. More specifically, the purpose of this experiment was to identify benactyzine dose-response relationships, using performance end—points, in rhesus monkeys.

METHOD

Subjects

Five male rhesus monkeys (*Macaca mulatta*), weighing between 5.5 and 7.0 kg, were randomly selected and trained. Subjects were returned to their home cages between training or testing periods, where they were fed monkey biscuits twice daily and had free access to water during the daytime.

Apparatus

The Primate Equilibrium Platform (PEP), illustrated in Figure 1, was used as the primary apparatus in this experiment. The monkey was seated in the center of this "flight" simulator which was capable of rotations about two axes (pitch and roll). For this experiment, only the pitch axis was used. To simulate air turbulence, the platform was driven from horizontal by an externally generated input signal, in this case quasi-gaussian white noise with a band width of 0 to 0.3 Hz generated by a Hewlett-Packard Noise generator.

As in an aircraft, the subjects compensated for the turbulence by manipulating a control stick mounted directly in front of them. They reacted to changes in platform position with stick movements. By effectively tracking the input

[1]The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources—National Research Council. Selection and training of the monkeys was accomplished by D. J. Barnes and his technicians, and data collection was assisted by J. A. Bachman.

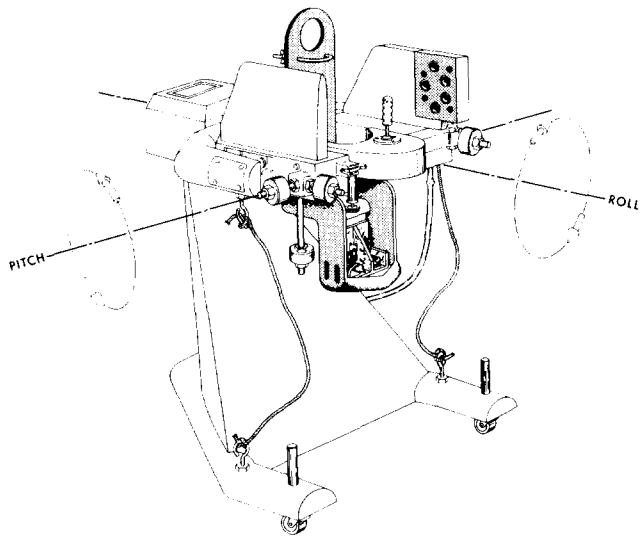

FIG. 1. The primate equilibrium platform with touch sensitive response time lights.

signal, subjects learned to maintain the platform in a relatively horizontal position.

A light touching task was also used in this study. The panel contained five stimulus-response lights located to the left of the control stick. These were arbitrarily designated as an alert light (yellow) and four fire lights (red). The yellow alert light simulated an engine-trouble warning light, and the four red fire lights represented switches that must be thrown to activate engine fire-suppression systems. Each red light was equidistant (7.3 cm) from the yellow light to assure comparable response times. The yellow light was illuminated (concurrent with a 1000-Hz tone) to initiate a trial sequence. The primate had to touch the yellow light within 5 seconds. Immediately upon making a correct response, this light was extinguished and one of the four red lights was illuminated (without an auditory cue) requiring the primate to make a second response within 4 seconds. The order of red light presentation was balanced, although the sequence of such presentation was randomized.

Primate responses to the PEP control stick were relatively continuous and defined by analysis of output voltages, whereas responses to the lights were discrete; the subject's light-touching performance therfore was best described by accuracy and response time parameters.

Procedure

Subjects were trained using standard operant techniques with mild electric shock as motivation. Training began with chair adaptation until the monkeys would sit quietly for one hour. The next step required shaping behavior by successive approximation so the monkeys would grab the control stick. Then the monkeys were taught to move the control stick in the correct direction, i.e., when the PEP tilted forward they were to pull the stick back, and backward tilt required a forward movement of the stick. During this phase the trainer manually tilted the PEP forward or backward and then manually returned it to horizontal when the monkey made the appropriate stick response. Forward tilt was taught first, then backward, and then alternately one or the other. Next, the control stick was made active making the monkey directly responsible for correcting PEP position, although the trainer still caused PEP displacement. When this behavior became reliable, an input voltage was connected to cause displacement of the PEP. Initially, the input voltage was a 0.1 Hz sine wave, which was gradually increased to 0.3 Hz. When control of the 0.3 Hz sine wave was accomplished, the input voltage was switched to white noise that was bandpass filtered at 0 to 0.1 Hz. As proficiency increased, the bandpass was widened to 0.3 Hz.

Whenever the PEP tilted beyond $\pm 15°$ from horizontal, a shock to the feet occurred every second until the PEP was returned to within the shock limits. Shock duration was 0.2 sec, with average and maximum intensities of 0.3 mA and 0.5 mA. The shock was terminated after 5 to 10 sec in the event of severe drug induced performance decrement, when a monkey could not escape the shock. Normally, a trained monkey was only shocked 0 to 4 times per work period.

After PEP performance was reasonably efficient, separate training sessions for light-touching began. As with PEP, this was by successive approximations using shock avoidance as the motivator. The monkeys were first trained to press the yellow alert light (5 sec allowable response time), which immediately extinguished and caused avoidance of shock. After the monkeys responded appropriately to the alert light, they were trained to press whichever red light came on with a 4 sec allowable response time. When light-touching performance was proficient, the tasks were combined. For a short period of time it was often necessary to simplify the PEP task by reducing the white noise input to 0.1 Hz. Soon, however, the monkeys would work out their own method of simultaneously operating the PEP and pressing the lighted buttons.

A performance period consisted of three hours of work per day divided into sixty 3-min intervals in which PEP output voltages were sampled by a PDP-12 to compute average platform position and its variability or wobble. Variability (ADJ RMS) was the root mean square of the output voltages adjusted for the average platform position, and is thoroughly described elsewhere [13,14]. In general, a small value for ADJ RMS indicated good PEP performance. Average response time and percent accuracy for the alert and fire lights were calculated concurrently on the PDP-12 computer. The PEP/light-touching system operated continuously for one 30-min session, was idle a minute or two while the animal subject was given an injection (control vehicle, or benactyzine at one of the prescribed dose levels), and then was restarted to run continuously for the remaining 2 1/2 hours of the period.

Data Analysis

Experimental design. Subjects were assigned to random to one of the four benactyzine HCl sequences (e.g., 0.17; 0.054; 1.7; 0.54 mg/kg) which were selected and administered in the counterbalanced design shown in Table 1. All doses of benactyzine HCl were prepared by the U.S. Army Biomedical Laboratory, Aberdeen Proving Grounds, MD (Lot Number 770930-B). In all cases, the injections were intramuscular in the anterolateral thigh. Subjects were tested with each subject run under control vehicle conditions at least one day immediately preceding each treatment day. This provided estimates of control performance against

TABLE 1

DOSE SCHEDULE IN mg/kg

Subject	Trial 1	2	3	4
576	A	B	C	D
888	B	C	D	A
584	C	D	A	B
900	D	A	B	C
572	A	B	C	D
896	B	C	D	A

Where A=0.54 mg/kg, B=0.17 mg/kg, C=0.054 mg/kg, and D=1.7 mg/kg.

Subject 572 became ill and died of bloat early in the experiment.

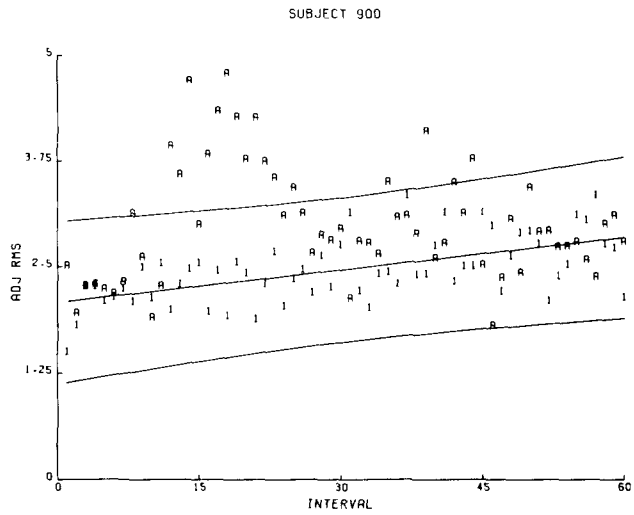

FIG. 2. Effects of 1.7 mg/kg benactyzine on equilibrium performance of monkey 900. ADJ RMS was the error or wobble of the platform around its average position, measured in degrees. A's were treatment intervals and 1's were control vehicle intervals. Injections were given between intervals 10 and 11, so that intervals 1 to 10 were preinjection. Interval duration was 3-min. Upper and lower bounds were 95% simultaneous tolerance limits.

which treatment changes could be measured. A minimum of one week occurred between benactyzine injections. The control vehicle which is used by the U.S. Army was designed to increase the long term stability of benactyzine. It consisted of 0.5 mg/ml methylparaben, 0.05 mg/ml propylparaben, U.S.P. water, acidified with HCl to pH 2.6 to 2.8. The lowest dose of benactyzine HCl was 0.054 mg/kg which represented the 4 mg contained in the Army self-injector for an "average" man. The higher three doses (0.17, 0.54, 1.7 mg/kg) were selected as one-half log increments above the lowest dose.

Incidence of change. Examination of performance effects was accomplished by fitting a least squares line to the sixty 3-min intervals from each control vehicle run that preceded each treatment run, and constructing a region of normal behavior around this line using simultaneous tolerance limits for P=0.95, α=0.05 [6]. These limits represent a conservative testing procedure because they contain at least 95% (i.e., 100 P%) of the population of all control vehicle intervals over the entire 60 interval period. In contrast, the "usual" 95% confidence limits (i.e., (1-α) 100%) are narrower because they represent the probability of a single 3-min interval lying within the test period as 95%. If one were to draw inferences about more than one interval, the probability of committing a type 1 error with the "usual" 95% confidence limits will exceed 5%. A performance change by the 95% simultaneous tolerance criterion was indicated when four or more of the 60, 3 min intervals on treatment days fell outside these tolerance limits. This technique has been applied in reaction time experiments [12] and in PEP studies [14].

RESULTS

Data from a typical test period is shown in Fig. 2 for monkey 900 at 1.7 mg/kg benactyzine. Fifteen intervals were out-of-bounds, mostly clustered 0 to 30-min postinjection (intervals 11 to 20), with fewer out-of-bounds intervals occurring as time progressed.

Table 2 contains a performance summary of the out-of-bounds intervals for each monkey, dose, and task. Subjects 584 and 896 were the only monkeys to have degraded performance (yellow light response time only) at 0.054 mg/kg. At 0.17 mg/kg, monkeys 596 (ADJ RMS) and 888 (yellow light response time) had degraded performance. At 0.54 and

TABLE 2

NUMBER OF 3 MIN INTERVALS OUT-OF-BOUNDS*

Task	Monkey	Benactzyine Dose (mg/kg) 0.054	0.17	0.54	1.7
ADJ RMS	596	0	7	6 (5)	25
	888	3	3 (1)	28	10
	584	0	3	3	5
	900	0 (8)	0 (2)	13	15
	896	0	0	2	17
Yellow Light	596	2	1	0	26
	888	1	11	13	1
	584	8	0	0	3
	900	0	1	6	39
	896	8	0	2	5
Red Light	596	2	2	1	24
	888	0	2	14	5
	584	0	0	10	7
	900	0	3	0	10
	896	4	0	3	4

*Each run contained 60 intervals and with P=0.95, α=0.05 simultaneous confidence limits. The existence of four or more out-of-bounds intervals indicates significant performance change. Two sided tests were conducted and the number in ( ) indicate those cases when performance improved in contrast to the majority of table entries indicating the number of times performance was degraded.

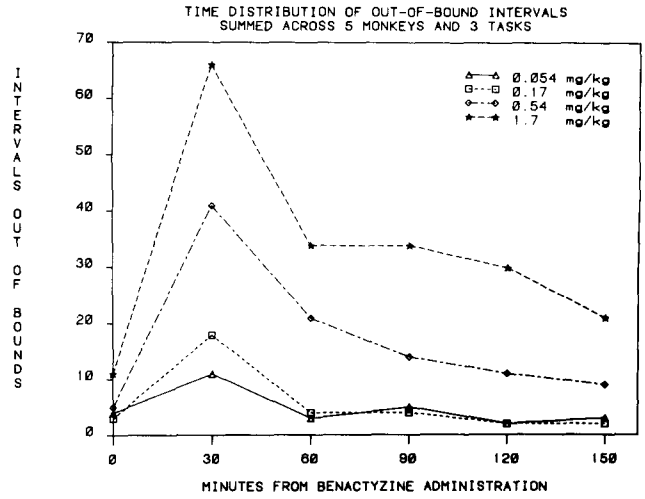

FIG. 3. Time distribution of out-of-bounds intervals, summed across five monkeys and three tasks. Total possible out-of-bounds intervals was 150 for any 30-min period.

1.7 mg/kg all monkeys had degraded performance, although it was common for performance on some tasks to be normal at the same time performance was degraded on other tasks.

Figure 3 distributes the out-of-bounds intervals over six, 30-min time periods by summing all out-of-bounds intervals indicating degraded performance for all monkeys for all tasks. With 10 intervals per individual 30-min period, and 5 monkeys with 3 tasks, there were 150 intervals at risk at each test. Although the most dramatic effects occurred at the 0 to 30-min postinjection period, progressively less severe degradation persisted throughout the remaining test time for the 0.54 and 1.7 mg/kg doses. Thus, the time of return to normal performance did not occur by the end of this study for the higher doses.

DISCUSSION

Equilibrium performance and multiple response time tasks were significantly degraded by benactyzine. The monkeys did not efficiently track the "turbulent air" at the higher doses, and they also responded more slowly to the yellow and the four red lights. Performance decrements were detected at the 0.17 mg/kg dose and above, but were questionable at the 0.054 mg/kg dose. Consistency of response among the tasks did not exist. Monkeys were normally able to operate the platform and push the lighted buttons, but as benactyzine dose increased, this multiple task capability diminished. At the highest dose, three monkeys had significant degradation of all three tasks, while the other two monkeys were still able to respond normally to the yellow alert light. The most severe decrements in all tasks were noted during the first 30 minutes, postinjection. There were mild lingering effects for the remaining test period. Since both equilibrium performance and discrete response times are important factors in aviation, it is apparent that the use of benactyzine in this environment will have to be approached with caution.

Information on the effects of benactyzine on human task performance is limited. Two studies where each person received 2 mg have shown a high incidence of subjective changes (e.g., feelings of heaviness in the limbs and the head), but neither suggested that the average person suffers a performance degradation at this dose [1,4]. In studies where each person received 5 to 10 mg, clear-cut changes in task performance have been noted [13,10] (e.g., increased reaction times and errors). Incapacitation has been reported at 12 mg/person. An interesting observation [3] was that during human performance of a buzzer versus bell choice reaction time task, benactyzine increased the normal tendency to concentrate on one stimulus while paying less attention to the second stimulus. In this fashion, response times and accuracies may remain normal for one stimulus (e.g., the buzzer), but performance may significantly degrade on the other (e.g., the bell). This may be analogous to our observations that, at higher doses of benactyzine, monkeys often performed one or two tasks well, but at the same time performed poorly on another.

It would be impossible to relate this human data to our monkey data without granting a great deal of latitude and interpretation and using a liberal amount of conservatism in reaching a conclusion. The 2 mg/person in humans produced side effects, but probably not enough to affect performance except in a few particularly susceptible people. Performance, however, was not measured at this dose. Two mg in a 75 kg person would be about 0.027 mg/kg. Five mg or so (approximately 0.067 mg/kg) causes a clear-cut, but not serious performance decrement; while 10 to 12 mg (ca. 0.15 mg/kg) causes a serious performance decrement or incapacitation.

In contrast to these human dose-response estimates, the rhesus monkeys demonstrated a detectible but mild change in performance at 0.17 mg/kg and moderate to severe decrements at 1.7 mg/kg. There were no instances of incapacitation in this study. A rough inspection of these human and monkey dose-response estimates suggests the human dose-response curve is much steeper than the monkeys, and that human beings are much more sensitive to performance degradation, on a mg/kg basis, than are monkeys. Because the dose-response curves do not seem to be parallel, no simple monkey-to-man extrapolative factor can be derived from this data.

Since neither the human literature nor our primate studies revealed tasks that were uniquely sensitive to benactyzine, it appears that human performance and clinical data may be generalized to the aviation environment. Therefore, in the absence of aviation-specific human benactyzine data, one might consider the 2 mg (0.027 mg/kg) dose from the human literature to be a good estimate of when problems start to appear to aviators.

In monkeys, it was concluded that 1.7 mg/kg is a debilitating dose of benactyzine, and the threshold dose was near the 0.054 mg/kg minimum dose examined in this report. These conclusions are presented based on this five monkey experiment, and it is acknowledged that large sample sizes are required to identify a precise threshold dose, and more definitive dose-response relationships.

ACKNOWLEDGEMENTS

The authors want to thank the referees for carefully reading this manuscript. Their comments are appreciated.

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