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Multiple Behavioral Effects of Diazepam in Rhesus Monkeys

GENE E SCHULZE,[1] WILLIAM SLIKKER, JR AND MERLE G PAULE

Pharmacodynamics Branch, Division of Reproductive and Developmental Toxicology
National Center for Toxicological Research, Jefferson, AR 72079
and Department of Pharmacology and Interdisciplinary Toxicology
University of Arkansas for Medical Sciences, Little Rock, AR 72205

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SCHULZE, G E , W SLIKKER, JR AND M G PAULE *Multiple behavioral effects of diazepam in rhesus monkeys* PHARMACOL BIOCHEM BEHAV **34**(1) 29–35, 1989 —The acute effects of diazepam (Valium) were assessed using a battery of complex food-reinforced operant tasks that included responding in delayed matching to sample (DMTS, n = 5), conditioned position response (CPR, n = 7) progressive ratio (PR, n = 8), temporal response differentiation (TRD, n = 4), and incremental repeated acquisition (IRA, n = 9) tests Diazepam (0 25–4 0 mg/kg IV) produced significant dose-dependent decreases in the number of reinforcers obtained in the TRD and IRA tasks only TRD accuracy was significantly decreased at doses of 0 25, 1 0, 2 0, and 4 0 mg/kg when compared to vehicle injections Significant decreases in IRA accuracy generally did not occur at doses below 1 0 mg/kg DMTS accuracy was decreased at 0 5 mg/kg for some time delays but showed no clear dose-delay interaction Performance in the CPR and PR tests showed no significant effects of diazepam exposure over the dose range tested These results indicate that diazepam selectively disrupts performance of operant tasks in monkeys designed to model human correlates of time perception, learning ability and visual attention/short-term memory while not affecting tasks designed to model motivation and position/color discrimination

Monkeys Operant behavior Test battery Learning Memory Diazepam Drug effects
Delayed matching to sample Temporal response differentiation Progressive ratio
Incremental repeated acquisition Conditioned position responding

DIAZEPAM (Valium) is a widely used psychoactive agent prescribed routinely for its anxiolytic, sedative/hypnotic, anticonvulsant and muscle relaxing properties Diazepam produces these effects by facilitating gamma amino butyric acid (GABA) neurotransmission within the CNS (7) The acute behavioral effects reported for diazepam are numerous and varied Such CNS functions as short-term memory (4, 8, 10, 19), motor performance (2, 13, 14) and the ability to acquire new behaviors (learning) (1, 9, 11, 12), are reported to be affected by acute diazepam administration in animals and humans Many such investigations have focused upon diazepam's impairment of a single complex CNS function rather than upon its effects on several such functions simultaneously These reports seem to indicate that acute diazepam administration impairs a variety of behaviors in humans and animals

We have used a complex operant test battery approach in evaluating the neurobehavioral effects of marijuana and its psychoactive constituents (21,22) This study is one in a series conducted in order to validate the use of this test battery as an assessment tool in neurobehavioral toxicology Our approach to assessing the validity of the test battery has been to use relatively well characterized, reversibly acting reference compounds Neurobehavioral profiles (i e , selective behavioral effects) can then be generated for these prototypic compounds, compared to their known effects in humans and other species, and eventually used to compare with the effects produced by compounds with uncertain mechanisms of action (5) A similar classification scheme for categorizing chemically-induced behavioral effects in humans has been outlined elsewhere (26) The present experiments were specifically designed to measure the effects of intravenously administered diazepam on a variety of complex operant behaviors in rhesus monkeys Diazepam doses (0 25–4 00 mg/kg IV) were chosen for study based on the criteria that the highest doses grossly affected most behavioral endpoints and the lowest doses were without significant effects The behavioral tests contained in the battery include delayed matching to sample (DMTS), conditioned position responding (CPR), progressive ratio (PR), temporal response differentiation (TRD), and incremental repeated acquisition (IRA) These tests were included in the battery because reports suggesting that diazepam (as well as other drugs and toxicants) significantly affect(s) performance of similar tasks in humans and experimental animals (4, 14, 16) Diazepam was chosen for study here because of its well characterized mechanism of action (7) allowing it to serve as a reversibly acting prototypic benzodiazepine agonist.

[1]Requests for reprints should be addressed to Gene E Schulze, Ph D , Department of Toxicology, Hazleton Laboratories America, Inc , 9200 Leesburg Turnpike, Vienna, VA 22182

METHOD

Subjects

Nine male rhesus monkeys (*Macaca mulatta*) between three and six years of age (10–20% of maximal achievable lifespan) and weighing from four to nine kilograms at the beginning of the study served as subjects All animals had been previously trained under the schedules in the test battery for approximately two years and had been used in previous studies of acute marijuana smoke and acute THC administration (21,22) At the time of testing for this study, all nine animals exhibited stable (less than 15% variability over one month) preexposure baselines for the IRA schedule, eight animals for the PR schedule, seven for the CPR schedule, five for the DMTS schedule and four for the TRD schedule Only animals exhibiting stable baseline (nondrug) performance were used in the data analysis and presentation Animal housing, feeding, etc , were as described elsewhere (21)

Apparatus

The apparatus have been described in detail elsewhere (21) and consisted of portable restraint chairs, sound-attenuated behavioral chambers, operant panels and computer consoles The operant panel was equipped with three press plates that had to be pushed to effect a switch closure and four retractable levers that operated a switch when depressed The press plates and levers were aligned horizontally with the press plates above the levers A trough for reinforcer (banana-flavored pellet) delivery was located below the levers

Operant Schedules

The use and description of the operant tests contained in the primate behavioral test battery have been described in detail elsewhere (21) A brief description of each test follows

Incremental repeated acquisition (IRA) This task required subjects, using all four response levers extended, to press the levers in a specified order for food reinforcement The task began with the presentation of a one-lever response sequence (IRA1) Each response on the correct lever resulted in reinforcer delivery and after 20 correct sequences (criterion performance), a one-minute time-out period was followed by the presentation of an 'incremented' two-lever sequence (IRA2), such that a response on a different lever was required before a response on the original lever produced food After the 20th errorless two-lever sequence (i e , no errors were made between the first and last correct lever presses of the required sequence), the task was incremented to a three-lever sequence and so on, up to maximum of six-lever sequences

Conditioned-position responding (CPR) In the CPR test only the press-plates were used At the start of the test, only the center plate was illuminated with either a red, yellow, blue, or green color Subjects made observing responses to the center plate, after which it was extinguished and the two side plates were immediately illuminated white If the center plate color had been either blue or green, responding to the right plate resulted in reinforcer delivery If the center press plate was illuminated red or yellow, responding to the left plate resulted in reinforcer delivery Responding at the wrong position initiated a 10-second time-out period followed by initiation of another trial The sequence of color presentation was yellow, blue, green, red, however, the initial color for a session was presented randomly

Progressive ratio (PR) Animals were required to increase the amount of work (number of lever presses) required for each reinforcer Only the far right retractable lever (extended) was used in this schedule Initially, one or two lever presses (depending upon the individual subject) resulted in reinforcer delivery After each reinforcer was delivered, the response requirement was increased by the initial number of lever presses required for the first reinforcer Thus, if the initial requirement were two lever presses, the second reinforcer was obtained after four lever presses, the third after six lever presses, the fourth after eight, etc

Delayed matching to sample (DMTS) For this test, only the press-plate manipulanda were used At the start of each trial, one of seven white on black geometric symbols, the sample, was projected onto the center plate (side plates were dark) After an observing response was made to the center plate, it was extinguished for one of six time delays presented pseudorandomly (with a maximum of 20 reinforcers/delay) Of the five animals showing stable performance for this schedule, two were presented time delays of either 1, 2, 4, 8, 16 and 32 seconds while the remainder were presented delays of 2, 4, 8, 16, 32 and 48 seconds After the various time delays, all three plates were illuminated, each with a different geometric symbol, only one of which matched the sample A response to the "match" then resulted in reinforcer delivery, whereas nonmatching responses were followed by a 10-second time-out period (all plates darkened) and then initiation of another trial with either the same or a different sample (pseudorandomly presented)

Temporal response differentiation (TRD) For this test, only the far left retractable lever (extended) was used, and the subject was required to hold the lever in the depressed position for a minimum of 10 seconds but no loner than 14 seconds The animal thus was required to hit a specified "window" of time in order to obtain a reinforcer Releasing the lever too early or to late started another trial

Procedure

Behavioral sessions were conducted daily, Monday through Friday, and lasted approximately 50 min Subjects were rotated through a maximum of 12 behavior chambers such that no monkey was placed in the same chamber for two consecutive test days in order to avoid disruption of ongoing large-scale chronic behavioral studies and to randomize chamber effects Behavioral tests alternated daily For example, incremental repeated acquisition (IRA 35-min), conditioned position responding (CPR 5-min) and progressive ratio (PR 10-min) tests were presented on one day, the delayed matching to sample (DMTS 30-min) and temporal response differentiation (TRD 20-min) tests were presented the next test day

Drugs and Dosing Procedure

Diazepam (Sigma Chemical Co , St Louis, MO) was placed into a vehicle such that the final injection solution consisted of 10% ethanol (World Ethanol Co., Texas City, TX), 40% propylene glycol (Sigma Chemical Co , St Louis, MO) and 50% sterile bacteriostatic (0 9% benzyl alcohol) saline (Elkins-Sinn Inc , Cherry Hill, NJ) The purity of the diazepam was determined to be 99 5% by in-house HPLC analysis using a UV detector set at 230 nm Doses of diazepam (0 25, 0 50, 1 00, 2,00 and 4 00 mg/kg, IV) were administered using a minimum number of injections and given in a randomized order to avoid confounding tolerance development Injection volumes were 0 4 ml/kg Generally, diazepam was given on Tuesdays and Fridays while vehicle injections were given on Thursdays, thus allowing sufficient time for elimination and avoiding possible carryover effects Due to the daily alternation of behavioral tests, all doses were given twice to provide dose-response data for each set of operant tests A total of

five vehicle injections per operant test were administered Approximately 15 min following injections, subjects were placed into operant chambers and behavioral sessions began about one min later

Data Analysis

The endpoints measured in each task have been described in detail elsewhere (21,22) Three fundamental measures are monitored for each test and included percent task completed, response rate or latency, and response accuracy The percent task completed data are measures of a predetermined criteria of performance (usually earning 60 or 120 reinforcers, representing performance maximums) and are functions of both response rate and response accuracy The percent task completed endpoint shows intraanimal stability, is a convenient comprehensive measure useful for comparing drug effects on performance across tests (18,21) For the TRD test, additional endpoints are measured including mean duration and temporal distribution of lever holds and for the PR test the breakpoint (the last ratio completed for which the animal earned a reinforcer) was also measured

Statistical Analysis

The overall effect of drug treatments on performance for the various tests was determined using a one-way repeated measures analysis of variance (ANOVA) (24) Analysis of the performance of a large group of monkeys (>60) in this test battery has shown that performance in one task is independent of performance in another (unpublished observations) indicating the appropriateness of this type of statistical analysis If overall significance was evident ($p<0.05$), then performance at each dose was compared to vehicle control performance by Fisher's least significant difference (LSD) multiple *t*-tests (12) For DMTS group accuracy data, significance was assigned to those group means falling outside the ninety-five percent confidence intervals constructed from vehicle control observations at each time delay This type of analysis is routinely used to visually identify delay-dependent drug-induced alterations in matching accuracy and is not confounded by "missing data" produced by those animals who fail to make the response criteria for a given time delay at a given dose (18)

RESULTS

Overall Effect of Diazepam Vehicle

Overall, the diazepam vehicle produced no statistically significant group effects on performance in any of the behavioral tests contained in the test battery although some increase in variability was evident

Incremental Repeated Acquisition (IRA)

Percent task completed. Diazepam administration produced dose-dependent decreases in IRA percent task completed as illustrated in Fig 1A Compared to vehicle control performance, diazepam injections significantly decreased the percent task completed at the 0 5, 2 0 and 4 0 mg/kg doses

Mean response rate. The effect of diazepam on IRA response rates is illustrated for IRA2 (a two lever sequence) in Fig 1b Dose-dependent decreases in mean response rates for this component and for the IRA1 and IRA3 components (data not shown) were evident As for the percent task completed measure, significant decreases in response rates occurred at the 0 5, 2 0 and 4 0 mg/kg doses

Response accuracy The effect of diazepam on response accuracy is illustrated for IRA2 in Fig 1C Diazepam significantly

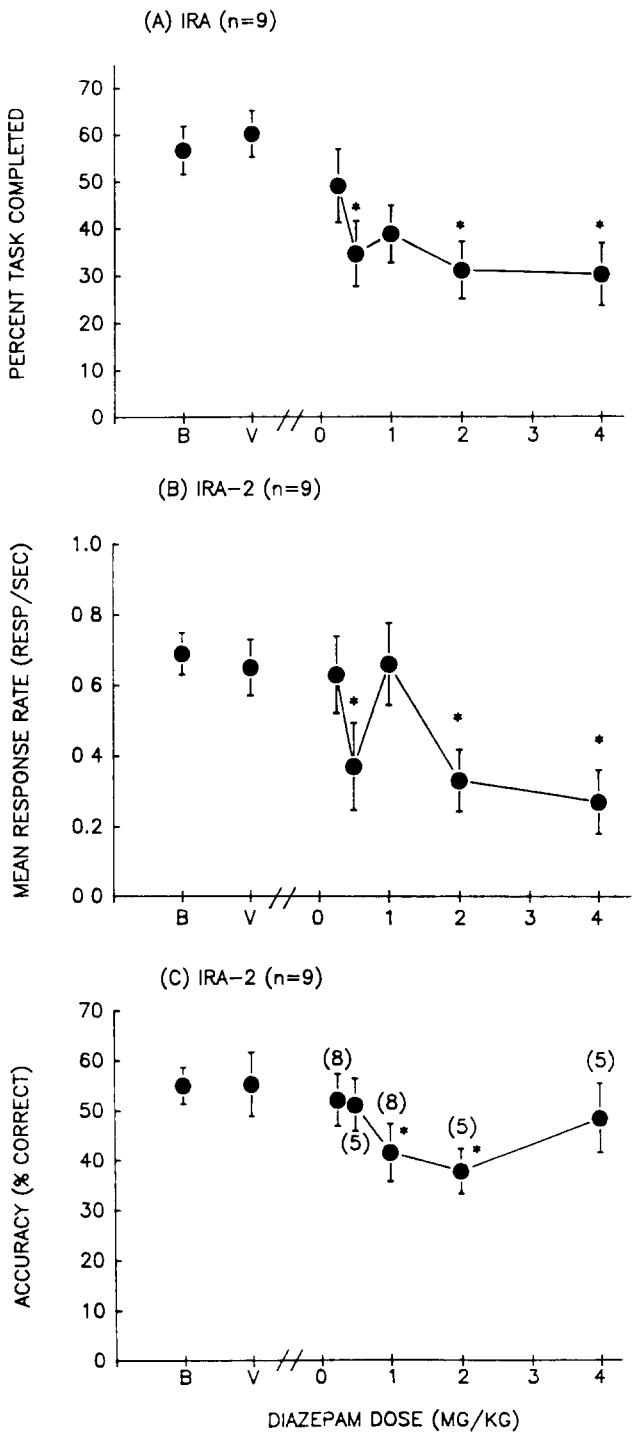

FIG 1 Effects of diazepam on IRA percent task completed, response rate and response accuracy for IRA2, n = 9 unless indicated otherwise Each point represents the mean ± SE Panel A depicts the effects of diazepam on percent task completed Panel B depicts the effects of diazepam on response rate for IRA2 Panel C depicts the effects of diazepam on response accuracy for IRA2 On the abscissa, the letter B represents the preexposure baseline of performance and the letter V represents vehicle control performance determined for five observations Asterisks represent significant difference from vehicle controls as determined by Fisher's (LSD) *t*-test ($p<0$ 05)

decreased response accuracy only following doses of 1 0 and 2 0 mg/kg

Conditioned Position Responding (CPR)

Percent task completed Diazepam administration had no statistically significant effect on CPR percent task completed (Fig 2A)

Mean observing response latencies Diazepam administration produced no statistically significant effect on mean observing response latencies (Fig 2B) In some animals increases in observing response latencies occurred following either the 0 5 or 1 0 mg/kg diazepam doses, leading to increases in mean observing response latencies However, these increases were evident in only one or two animals, thus the rather large standard errors associated with the group means

Response accuracy The accuracy of responding in the CPR test was not significantly affected by diazepam administration (Fig 2C)

Progressive Ratio (PR)

Diazepam had no statistically significant effect on overall response rates or breakpoint for this test (data not shown)

Delayed Matching To Sample (DMTS)

Percent task completed Diazepam administration produced no statistically significant effects on DMTS percent task completed (Fig 3A)

Mean observing response latencies Similar to the CPR task, diazepam administration produced no significant increases in mean observing response latencies (Fig 3B) In some animals (as noted in the CPR test) increases in mean observing response latencies occurred at the 0 25, 1 0 and 4 0 mg/kg doses leading to elevated group means and larger standard errors

Response accuracy Constructing 95% confidence intervals around vehicle control data (Fig 4), indicated that diazepam produced significant decreases in accuracy at most time delays at the 1 0 and 2 0 mg/kg doses Accuracy of DMTS responding after the 0 5 mg/kg dose was significantly depressed only at the 4-, 8- and 16-sec time delays The 1 0 mg/kg dose produced a delay-dependent decrease in accuracy with accuracy decreases becoming more pronounced at the longer time delays The 2 0 mg/kg dose, however, did not produce a delay-dependent decrease in matching accuracy Some variability in performance was observed between individual animals as well as between time delays within animals

Temporal Response Differentiation (TRD)

Percent task completed Diazepam vehicle alone produced a large increase in the variability of TRD responding (Fig 5A) Similar to performance noted in the DMTS and CPR tests, percent task completed under the TRD schedule was not significantly effected by diazepam administration (Fig 5A)

Response rate Overall, diazepam administration had no statistically significant effect on mean response rate in the TRD test Diazepam administration produced a biphasic dose-response curve for mean TRD response rates as illustrated in Fig 5B Lower doses of diazepam (0 25 and 0 5 mg/kg) did not effect response rates, the moderate dose of 1 0 mg/kg increased response rates while higher doses (2 0 and 4 0 mg/kg) resulted in response rates similar to those produced by the lower doses However, these changes were not statistically significant

Response accuracy Significant group decreases in TRD accuracy were evident at diazepam doses of 0 25, 1 00, 2 00, and 4 00 mg/kg and (Fig 5C) The decreased accuracy observed in some

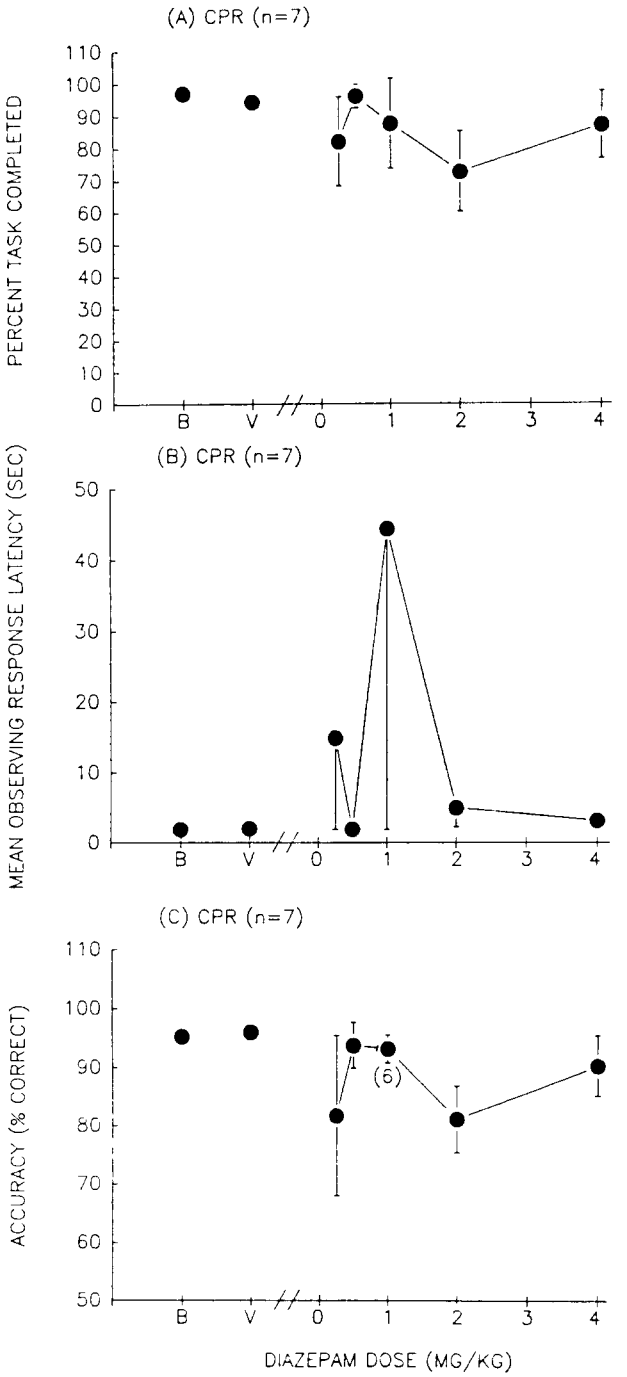

FIG 2 Effects of diazepam on CPR percent task completed, mean observing response latency and response accuracy, n = 7 unless indicated otherwise Data presented as described in Fig 1 Panel B depicts the effects of diazepam on CPR mean observing response latency Panel C depicts the effects of diazepam on CPR response accuracy Abscissa labeled as in Fig 1B

animals at diazepam doses of 1 00 and 2 00 mg/kg were not due to an absence of responding as evidenced by the data in Fig 5B, but appeared to be due to a shift in lever hold durations toward time intervals shorter than 10 sec

Additional endpoints Similar to the data for TRD response

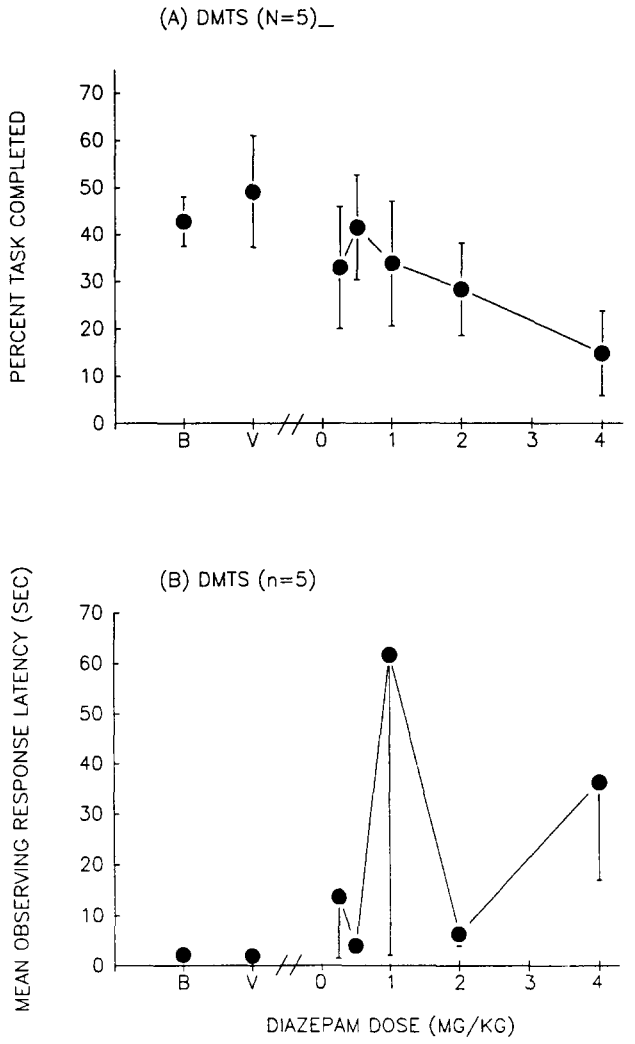

FIG 3 Effects of diazepam on DMTS percent task completed and mean observing response latency, n = 5 Panel A as described in Fig 1A Panel B depicts the effects of diazepam on DMTS mean observing response latency Abscissa labeled as in Fig 2B

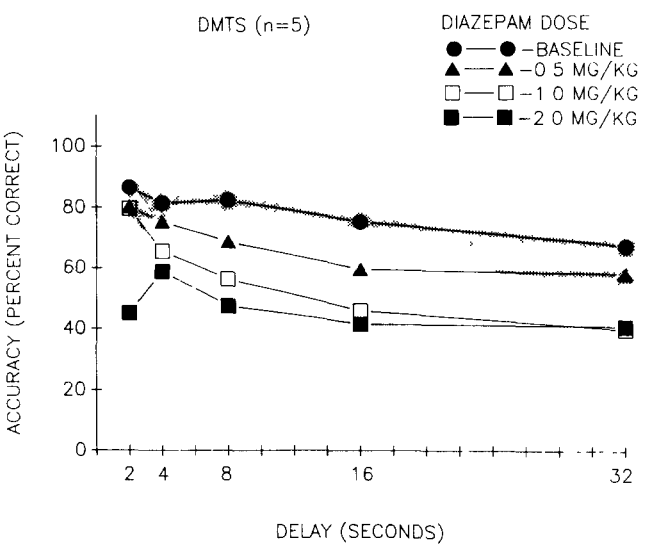

FIG. 4 Effects of diazepam on DMTS response accuracy after time delays of 2, 4, 8, 16 and 32 sec The shaded area represents the ninety-five percent confidence interval constructed from data from five vehicle control sessions Each point represents the mean accuracy of five animals Doses which abolished responding in some animals (0 25 and 4 0 mg/kg) were omitted

rate and percent task completed measures, the mean duration (in sec) that the lever was held in the depressed position was also not significantly affected by diazepam administration. The most striking and consistent effect of diazepam was to increase the frequency of lever-holds that were less than one second in duration (doses of 1 0 mg/kg and higher)

DISCUSSION

Diazepam administration produced selective disruption of performance in the operant tasks contained in this primate behavioral test battery TRD accuracy was decreased significantly at a dose of 0 25 mg/kg, IRA percent task completed at doses of 0 5 mg/kg and above, and DMTS accuracy was decreased most consistently at doses of 1 0 mg/kg and 2 0 mg/kg. Neither PR nor CPR performance was significantly affected by diazepam at the highest doses tested In comparison, the effects of delta-9-tetrahydrocannabinol (THC) in these same animals performing in the same operant test battery were quite different (21) Unlike diazepam, THC administration produced no consistently significant decrements in response accuracy in any performance except for that in the TRD test. The observation that these two different compounds produce clearly different behavioral effects in the same test system suggest that they affect complex operant performance through different CNS mechanisms (5)

Diazepam disruption of TRD performance resulted primarily from a drug-induced increase in lever-holds of very short (≤one sec) duration This effect appears to reflect a general loss of schedule control resulting in markedly increased rates of responding accompanied by a marked decrease in the number of lever holds of appropriate duration. Others have reported similar effects of diazepam in pigeons performing in spaced responding paradigms (15) and in rats performing under differential reinforcement of low-rate-responding schedules (20)

Disruption of IRA performance by diazepam resulted in a general decrease in percent task completed Both response rate and accuracy decreases contributed to this effect, and these findings are in good agreement with another report of diazepam effects on IRA performance in rats (18) Similar effects of diazepam have also been reported for repeated acquisition performance in pigeons (3,12) and humans (11) The monkey, however, appears to be less sensitive (on a mg/kg basis) to diazepam than humans. For example, the accuracy of IRA responding in monkeys was decreased significantly only at the 1 0 and 2 0 mg/kg doses where human response accuracy is reported to be affected 1 hour following doses as low as 0.3 mg/kg PO (11) At any rate, the qualitative effects of diazepam observed here on IRA performance appear consistent with those effects reported for other species including humans, thus strengthening the validity of this test for use in detecting toxicological or pharmacological effects

Diazepam is known to produce anterograde amnesia in humans (25) For this reason it has been suggested that diazepam does not affect short-term memory per se, but rather impairs the consolidation of short-term memory to long-term memory (8,19) In the present study, we found no reliable dose-delay interaction for diazepam-induced decreases in DMTS accuracy, although the 1 0

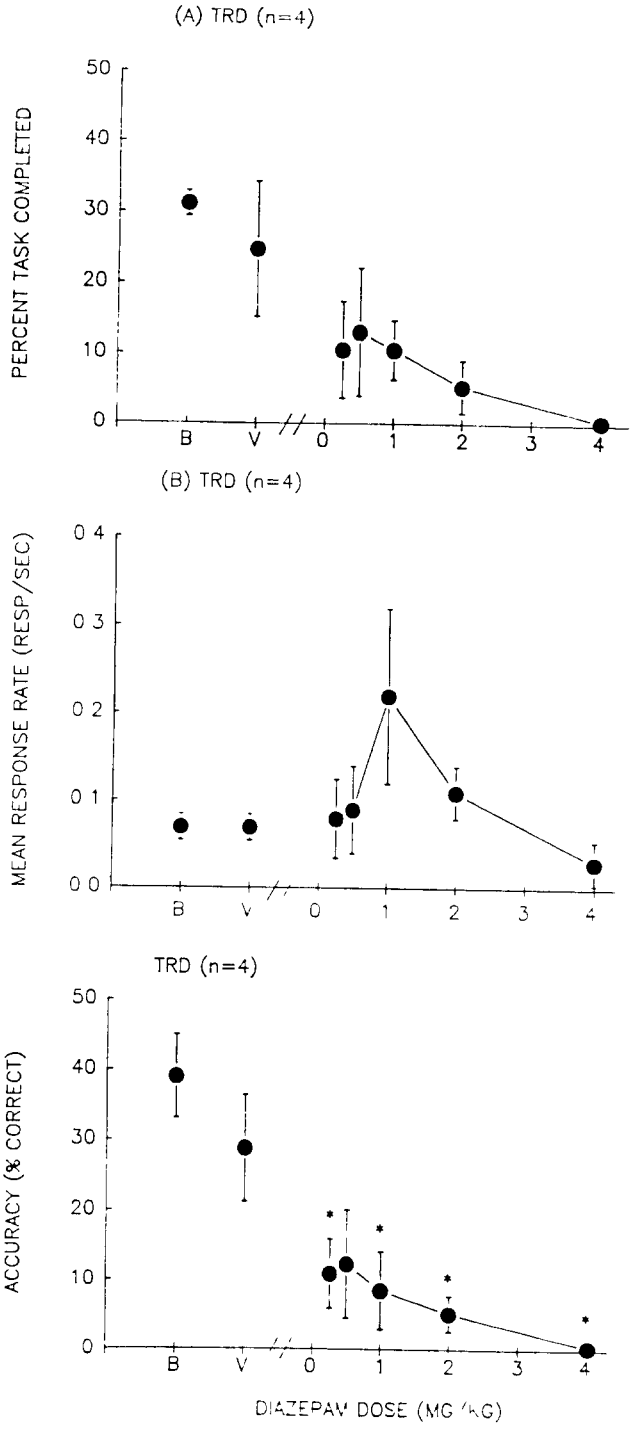

FIG 5 Effects of diazepam on TRD percent task completed, mean response rate and response accuracy (data presented as described in Fig 1A), n=4

mg/kg dose manifested a slight delay-dependent trend of accuracy decreases However, at the other doses, the effect of diazepam on this measure was approximately the same across time delays This observation argues against the hypothesis that diazepam specifically affects short-term memory processes and is consistent with the impairment of memory consolidation hypothesis The diazepam-induced trend of decreases in matching accuracy reported here parallels a report indicating that diazepam decreased matching accuracy in pigeons (15) at a single short time delay, probably reflecting a general effect of diazepam on attention and/or stimulus control (23)

Diazepam, at doses that significantly decreased matching accuracy, did not significantly affect observing response latencies (also a rate measure) in the DMTS test suggesting that motor function was not adversely affected for this task This is consistent with reports in humans which indicate that diazepam impairs memory consolidation at doses lower than are required to effect motor performance (9) This finding contrasts with that for performance in the IRA test where the accuracy-decreasing effects of diazepam appeared at doses that decreased response rates These observations may reflect general differences in response topographies (IRA = levers, DMTS = press plates) or in baseline differences in response rates between the two performances The observation that response rates in the other lever tasks (PR and TRD) were not decreased (and in some cases were increased) by diazepam weakens the argument that differences in response topography are responsible for the differences in drug sensitivity and strengthens the hypothesis that this probably represents the effect of different baseline rates of responding between the lever tasks (6)

In conclusion, the present data indicate that the acute effects of diazepam on performance in a battery of complex operant tasks are markedly different (consistent decreases in IRA and DMTS accuracy) than the acute effects of THC (response rate changes only) when given to the same animals performing the same tasks This observation suggests that in monkeys, THC and diazepam affect complex brain function (as measured by their operant performance) through different CNS mechanisms The accuracy decreasing effect of diazepam on performance thought to reflect some aspects of learning (IRA) and the lack of a delay-dependent decrement in DMTS accuracy (indicating no effect on short-term memory) compare favorably to reports on the effects of diazepam on similar performance in humans The primate operant test battery has proven to be a useful tool for studying the effects of psychoactive compounds on a series of complex, presumably central nervous system functions Additionally, the ability of compounds such as diazepam and THC to affect performance of one type and not another at the same dose suggests that performance in different components of the NCTR operant test battery are subserved, to a great degree, by different brain processes which can be selectively affected by drugs

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