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Serial-probe recognition in rhesus macaques: Effects of midazolam

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Abstract

A serial-probe recognition task was used to assess the effects of midazolam on visual attention and short-term memory in three rhesus monkeys. On each trial, six unique alphanumeric sample stimuli (list items) were presented sequentially followed by a choice period. Choosing the 'probe' stimulus was correct if the probe matched one of the list items; otherwise, choosing the 'default' stimulus (a white square) was correct. Behavior was examined under a range of doses of midazolam (0.065, 0.13, 0.26, and 0.52 mg/kg IM). Midazolam did not significantly reduce choice accuracy or change the shape of the serial position function and did not significantly reduce choice responding. However, choice reaction time was significantly increased by the two highest doses of midazolam. Responding directed at the sample stimuli was reduced at the two highest doses of midazolam. Furthermore, 0.52 mg/kg midazolam significantly increased sample-stimulus reaction time at all six serial positions. Overall, these data suggest that the two highest doses of midazolam tested increase reaction time, but do not directly impair short-term visual recognition memory. This is noteworthy because such doses appear capable of protecting against nerve agent-induced seizures.
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Keywords: Visual conditional discrimination; Short-term recognition memory; Choice latency; Sample-stimulus reaction time; Benzodiazepine anticonvulsant; Midazolam; Operant touch screen response; Rhesus monkeys

1. Introduction

Benzodiazepines, such as diazepam and midazolam, are often prescribed for the treatment of anxiety and insomnia. This class of drugs is also used for sedation, muscle relaxation, analgesia, and amnesia pre-operatively in hospital settings. Another important capability of these drugs is in the treatment of active seizures (i.e., stopping ongoing seizure activity), whether the origin of the seizure is organic (e.g., epilepsy) or chemically induced (e.g., pesticide or chemical warfare nerve agent exposure). For example, in the clinical treatment of status epilepticus in adult humans, diazepam is usually administered intravenously (IV) as a bolus of 10–20 mg or rectally at 10–30 mg and these doses can be repeated, with the typical anticonvulsant dose being approximately 0.3–0.6 mg/kg. For midazolam, a more potent compound, the dose range is 5–10 mg intramuscularly (IM), rectally, or IV and this dose can be repeated once after 15 min, with the typical anticonvulsant dose being approximately 0.15–0.30 mg/kg (Shorvon, 1994; Towne and DeLorenzo, 1999).

Rapid termination of chemically induced seizures is essential for preventing serious long-term neurological, behavioral, and cardiovascular deficits, thus the rapid onset of an anticonvulsant's effect is of critical importance (Castro et al., 1992; Lallement et al., 1999; McDonough et al., 1999; Murphy et al., 1993). Midazolam may offer several key advantages over diazepam in the rapid treatment of nerve agent-induced status epilepticus. Specifically, midazolam is much more water soluble than diazepam and is therefore more rapidly absorbed following IM injection (Gerecke, 1983). The ability to administer an anticonvulsant IM is also important because establishing venous access in actively convulsing patients is difficult, potentially delaying drug treatment and, thus, seizure termination (Fountain and Adams, 1999; Towne and DeLorenzo, 1999). Prompt administration of IM midazolam has been shown to stop seizures of various origins in both humans and nonhuman primates usually within 2–3 min and almost always within 10 min or less

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(Galdames et al., 1997; Hayward et al., 1990; Lahat et al., 1992; Mayhue, 1988; Wroblewski and Joseph, 1992).

The US military currently provides its forces with Convulsant Antidote for Nerve Agent (CANA) autoinjectors capable of delivering 10 mg of diazepam IM for use in a nerve agent exposure situation (equivalent to approximately 0.14 mg/kg for a 70-kg soldier). The CANA autoinjector is employed when a first responder needs to render aid (i.e., "buddy aid") to incapacitated comrades exhibiting convulsions. Current doctrine states that medics and unit lifesavers can administer up to two additional doses at 10-min intervals to a convulsing casualty (Sidell, 1997), for a total of 30 mg of diazepam IM (equivalent to approximately 0.43 mg/kg for a 70-kg soldier). Given the necessity of IM administration in such circumstances and the apparent superiority of midazolam over diazepam in rate of absorption through this route, several studies have directly compared the efficacy of these two benzodiazepines in eliminating nerve agent-induced seizures. These studies, guided by current US military doctrine, administer pyridostigmine bromide pretreatment, then challenge animals with a large dose of nerve agent (e.g., 2LD50 of tabun, sarin, cyclosarin, soman, or VX), followed within minutes by IM injections of atropine and pralidoxime chloride (2-PAM). One of several benzodiazepine test compounds is administered at a specified time following the onset of seizure activity (as evidenced by cortical EEG electrodes) to gauge its anticonvulsant effect.

Using the approach outlined, McDonough et al. (1999) administered either IM diazepam or midazolam to guinea pigs 5 or 40 min after nerve agent-induced seizure onset to compare efficacies. Midazolam was more potent and more capable of rapid seizure control than diazepam at both delays to treatment. Shih et al. (2003) extended these findings by using six different chemical warfare nerve agents (tabun, sarin, soman, cyclosarin, VR, and VX) at 2LD50 as well as a 5LD50 dose of soman to induce seizures, and found midazolam to be the more potent and rapidly acting benzodiazepine anticonvulsant overall.

Using similar procedures in rhesus monkeys, Hayward et al. (1990) found that diazepam and midazolam (each at 1 mg/kg IM), given immediately following atropine and 2-PAM injections, were equally effective in hastening recovery and return to consciousness and reducing convulsions and brain lesions in rhesus monkeys following soman exposure. More recently, McDonough et al. (2002) directly compared the anticonvulsant efficacy of midazolam and diazepam in rhesus monkeys following a 2LD50 soman challenge. They found that a midazolam dose of 0.13 mg/kg, given once upon seizure onset and again 10 min later (for a combined dose of 0.26 mg/kg IM), was capable of terminating nerve agent-induced seizures in a majority of subjects, usually within about 30 min. An IM bolus dose of 0.32 mg/kg midazolam was comparably effective. In contrast, diazepam (0.4 to 0.63 mg/kg IM) terminated seizures in proportionally fewer subjects and seizure termination typically occurred after 80 min. These results suggest that the current treatment regimen with diazepam may be insufficient to terminate nerve agent-induced seizures and that midazolam may offer more reliable and more rapid seizure control, even at relatively low doses (about 0.3 mg/kg IM).

Unfortunately, benzodiazepines are known to produce unwanted behavioral side effects, such as sedation and amnesia (Lister, 1985; O'Boyle, 1988; Zbinden and Randall, 1967). However, such effects are generally observed at doses higher than those required for seizure control. For example, Castro (1995) found that diazepam significantly reduced accuracy of serial-probe recognition (SPR) performance in rhesus monkeys at doses of 1.6 mg/kg IM and higher, whereas choice reaction time was increased only at the highest dose (3.2 mg/kg IM). Schulze et al. (1989) used a battery of behavioral tests to evaluate the acute effects of diazepam in rhesus monkeys at doses of 0.25–4.0 mg/kg IV. They found that diazepam generally reduced delayed match-to-sample accuracy at doses of 1.0 and 2.0 mg/kg but did not significantly reduce task completion or sample-stimulus response rate even at 4.0 mg/kg. Performances on a progressive-ratio schedule and a color-position discrimination task were unaffected at these same doses. In contrast, task completion, response rate, and accuracy were generally reduced on an incremental repeated-acquisition task, but a clear dose-dependent effect was not observed (i.e., the 4.0-mg/kg dose did not reduce accuracy whereas the 1.0- and 2.0-mg/kg doses did). Accuracy on a temporal response-differentiation task was reliably decreased at doses of 1.0 mg/kg and higher. Taken together, the results of Schulze et al. (1989) and Castro (1995) suggest that diazepam doses of approximately 1.0 mg/kg and higher may alter various aspects of neurobehavioral functioning with or without direct response suppression.

Additionally, Hudzik and Wenger (1993) utilized simultaneous and delayed match-to-sample procedures in squirrel monkeys to evaluate doses of diazepam ranging from 0.1–1.0 mg/kg IM. Accuracy on the delayed match-to-sample task was reduced significantly at 0.55 and 1.0 mg/kg whereas accuracy on the simultaneous match-to-sample task was reduced significantly only at 1.0 mg/kg. It is noteworthy that sample-stimulus response rate was reduced significantly at doses of 0.3 mg/kg and higher. Baron and Wenger (2001) used a fixed 3-s delayed match-to-sample procedure in squirrel monkeys and found that diazepam decreased sample-stimulus response rate at doses of 1.0 mg/kg IM and higher, but significantly decreased accuracy only at 1.8 mg/kg and higher. Although the results were quite comparable across both Wenger studies, the slightly higher doses required for disruption in the latter study may have been due to the shorter retention interval (3 s) or to the decreased pre-treatment interval (15 min versus 30 min in the earlier study).

The goal of the present study was to determine whether doses of midazolam capable of controlling nerve agent-induced seizures produce deficits in attention, memory, and reaction time in a SPR task, a task that has been used extensively to evaluate compounds of military significance in rhesus monkeys (Castro, 1995, 1997; Castro et al., 1992, 1994; Matzke et al., 1999; Myers et al., 2002).

2. Method

The experimental protocol was approved by the Animal Care and Use Committee at the Walter Reed Army Institute of

Research and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, Publication No. 85-23, 1996), and the Animal Welfare Act of 1966 (P.L. 89–544), as amended.

2.1. Subjects

Three adult (approximately 12 years old) male rhesus monkeys (*Macaca mulatta*) weighing between 8.5–12.1 kg served. All monkeys had extensive experience with the six-item SPR task and had previously participated in a study evaluating effects of scopolamine and biperiden on SPR performance (Myers et al., 2002) 2.5 years prior to the present study. After a 2-year period without performing the SPR task, the monkeys reacquired the task and completed a study that utilized percentile schedules of short, long, and non-differential choice reaction time reinforcement. Several months of baseline training with no percentile schedule preceded the onset of the present study. The monkeys were housed individually in stainless steel squeeze-back cages (61 cm $W \times 71$ cm $D \times 86$ cm H) with free access to tap water. Primate chow (Certified Primate Diet 5048, Purina Mills, Inc., St. Louis, MO) was provided no sooner than 30 min after the daily session to maintain desired body weights at approximately 90% of free-feeding weight (individualized free-feeding weights had been determined for each adult rhesus monkey during a 2-year period without behavioral testing in which AM and PM feedings were sufficiently large that each animal failed to consume the entire daily ration). Throughout the study, the diet was supplemented with fresh fruits and vegetables each week. Weekly weights were used to adjust the extra-experimental ration to maintain desired target weights. The colony was maintained on a 12-h light/dark cycle with no twilight (lights on at 0600 h) and at 20–22 °C with a relative humidity of 50% (±15%), using at least 10 complete air changes per hour of 100% conditioned fresh air. The sessions began at approximately 1030, 5 days per week (Monday–Friday).

2.2. Apparatus

The subjects were tested unrestrained in their home cages. A 35.6-cm (14-in.) capacitive touch screen monitor (GoldStar StudioWorks, model GLD 45I, Microtouch Systems, Inc., Methuen, MA) was attached to the front wall of each cage, with the center of the screen 38.9 cm above the chamber floor. Because screen touches are difficult to execute around the screen's perimeter, the effective area of the screen was reduced by 1.5 cm on all four sides. Banana-flavored food pellets (750 mg, Bio-Serv Inc., Frenchtown, NJ) were delivered by a pellet dispenser (BRS/LVE Model QNB-400 1) into a food cup ($7.9 \times 10.8 \times 7.6$ cm) positioned in the front of the test chamber, accessible through an aperture (7.6 cm $W \times 5.4$ cm H) centered 15.1 cm below the lower edge of the touch screen and 11.6 cm above the chamber floor. A computer, running a custom-written Visual Basic® 6.0 routine, was used to control experimental events and collect all data.

2.3. Behavioral procedure

Each daily session consisted of 240 trials. On each trial, six unique sample stimuli (list items) were presented sequentially, separated by a 1-s interstimulus interval (ISI) during which the screen was blank. Each list item was a compound stimulus comprised of two superimposed, randomly selected ASCII characters of different size and color. The individual characters ranged from about 0.3 to 2.7 cm in length and 0.3 to 2.7 cm in width. Because the same ASCII character could be selected for a particular sample-stimulus, one character was 15% smaller than the other and was offset slightly above and to the left of the other to avoid perfect overlap and to achieve a greater diversity of compound sample stimuli. The RGB color saturation of each ASCII character ranged from 0 to 255. To exclude extremely dark characters but not true colors, at least one of the three saturation levels had to exceed 79. Each list stimulus was displayed in the top–center portion of the screen, about 13.5 cm from the left edge of the screen and about 4 cm from the top of the screen to the center of the stimulus. Each list item was presented for 3 s or until it was touched, at which point it was terminated and the ISI was initiated. After presentation of the sixth sample-stimulus, the screen was blank throughout the 1-s probe delay (retention interval) that preceded the choice period. During the 15-s choice period a probe stimulus was displayed in the lower-left or lower-right portion of the screen, and a standard or default stimulus (a 6.6-cm white square) was presented in the other portion of the screen, with equal frequencies of presentation on both sides. The probe item was a compound stimulus that matched a list item on half of all trials (120). Across these "matching" trials, probe items matched list items at each of the six serial positions with equal frequency (20 at each serial position). On matching trials, touching the probe stimulus was considered correct. In contrast, on "non-matching" trials the probe stimulus was not among those listed (novel) and touching the default stimulus was considered correct. A correct choice response immediately produced a conditioned reinforcer (the entire screen turned white for 0.25 s) every time, but produced a food pellet only 33.3% of the time, determined randomly by the computer (this probabilistic reinforcement schedule was used to maintain high, consistent levels of responding and avoid possible satiation). Touching the opposite stimulus was considered incorrect. Choice periods that elapsed without a response ended after 15 s and were considered incorrect. A 4-s intertrial interval (or ITI, during which the screen was blank) separated each trial, regardless of whether a choice was correct or incorrect. A response during the ITI reset the interval, although few such responses occurred. Prior to initiating the midazolam assessment, all animals achieved a criterion of stability on accuracy and choice reaction time. The overall accuracy and median choice reaction time for each of 10 consecutive sessions did not deviate more than 15% from the grand mean of all 10 sessions.

2.4. Injection procedure

Midazolam hydrochloride (Versed®, 5 mg/ml, Roche Laboratories, Inc., Nutley, NJ) was administered in the

appropriate volume into the posterior thigh muscle 30 min prior to the beginning of the experimental session. This 30 min pre-treatment interval was chosen because, in adult humans, peak plasma levels following IM administration of midazolam are reached at about 25 (Shorvon, 1994) to 30 min (Murray, 2002) and peak sedation occurs 30 to 60 min following injection (Murray, 2002). Thus, the test sessions, which averaged approximately 60 min, were designed to capture midazolam's peak effects. The injection site (left vs. right leg) was alternated across injections. Sessions were conducted Monday through Friday. Tuesday and Friday were injection days. Monday, Wednesday, and Thursday served as non-injection maintenance days. Midazolam was presented at five doses: 0.000 (0.5-ml saline as a vehicle control, VEH), 0.065, 0.13, 0.26, and 0.52 mg/kg, expressed as the weight of the salt. Each drug dose was administered twice during the study in accordance with a Latin-square design, with each monkey receiving a different dose on a particular injection day.

2.5. Data analysis

A repeated-measures analysis of variance (ANOVA) was used to evaluate main effects and interactions (Statistica, version 7.1, StatSoft, Inc., Tulsa, OK). When necessary, Fisher's least significant difference test was used for post-hoc comparisons. A significance level of $p<.05$ was used for all tests. All analyses of responding during the choice period (choice accuracy and choice reaction time) excluded trials in which no choice response was made. Five dependent measures were analyzed: accuracy, trials completed, choice reaction time, number of sample-stimulus presses, and sample-stimulus reaction time. Accuracy, the number of sample-stimulus presses, and sample-stimulus reaction time were analyzed using separate Determination × Dose × Serial Position repeated-measures ANOVAs. Trials completed and choice reaction time data were analyzed using separate Determination × Dose repeated-measures ANOVAs.

3. Results

Fig. 1 shows mean overall accuracy and SEM as a function of serial position for each dose of midazolam. Serial Position "NM" indicates those choice trials in which the probe stimulus did not match any of the sample stimuli (i.e., non-matching trials). Serial Positions 1 through 6 correspond to those trials in which the probe stimulus matched the sample-stimulus at a given serial position (i.e., matching trials). Accuracy data are based only on trials in which a choice response was made. Under vehicle conditions, overall accuracy equaled .79, .81, and .94 for the three monkeys. A Determination × Dose × Serial Position repeated-measures ANOVA indicated only a main effect of Serial Position ($F_{6,12}=4.11$, $p<.02$). Post-hoc analysis using Fisher's least significant difference test indicated that accuracy at Serial Position NM was significantly greater than accuracy at Serial Positions 1 and 2. Also, accuracy at Serial Position 1 was significantly lower than accuracy at Serial Positions 3, 4, 5, and 6. Accuracy at Serial Position 2 was

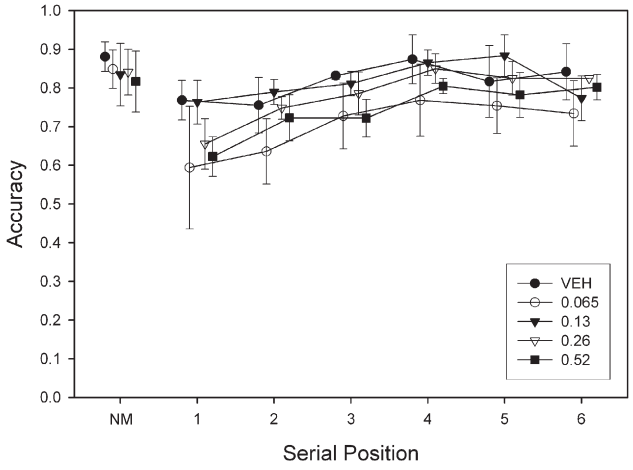

Fig. 1. Mean overall accuracy and the SEM as a function of serial position for each dose of midazolam. Serial Position NM indicates those choice trials in which the probe stimulus did not match any of the sample stimuli (i.e., non-matching trials). Serial positions 1 through 6 correspond to those trials in which the probe stimulus matched the sample-stimulus at a given serial position (i.e., matching trials). The data include only trials in which a choice response was made. Different line functions represent the different dose conditions (0.000 (VEH), 0.065, 0.13, 0.26, and 0.52 mg/kg midazolam), as indicated in the legend. For clarity, the data points corresponding to each serial position are offset slightly for each dose. No significant effect of dose was observed. See text for a full description of the significant effects of serial position.

significantly lower than that at Serial Position 4. Taken together, these results demonstrate a recency effect, that is, higher levels of accuracy at latter serial positions than at earlier serial positions. A primacy effect (i.e., higher accuracy at the first one or two serial positions) was not observed. Accuracy on non-matching trials (designated NM in Fig. 1) approximated the high levels observed at latter serial positions.

Recall that each choice period ended after 15 s if no response was made. Thus, the total number of trials completed for a given session equaled the total number of trials (240) minus the number of trials in which no choice response was made. The mean number of trials completed was 236, 238, 237, 236, and 228 for the 0.000, 0.065, 0.13, 0.26, and 0.52 mg/kg conditions, respectively. To determine whether midazolam reduced the number of trials completed, a Determination × Dose repeated-measures ANOVA was performed and showed no significant effects ($F_{4,8}=0.47$, $p=.75$). Thus, trial completion, broadly taken as an index of overall motivation, was not affected by midazolam.

Choice reaction time equaled the latency between onset of the choice period and a choice response. The median choice reaction time was determined for each session, and trials in which no choice response was made were excluded from calculations. The median was used as the measure of central tendency instead of the mean because the median is less affected by extreme scores and because reaction time distributions are often positively skewed. Fig. 2 shows the mean choice reaction time at each dose (and the SEM), plotted on semi-logarithmic coordinates. A Determination × Dose repeated-measures ANOVA indicated a significant main effect of dose ($F_{4,8}=9.23$, $p<.005$). Post-hoc tests indicated that choice

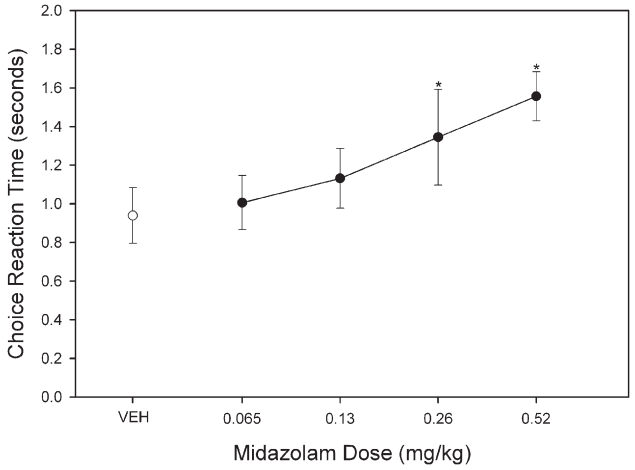

Fig. 2. Mean of the median choice reaction times (in seconds) and the SEM as a function of dose, plotted on semi-logarithmic coordinates. Asterisks denote a significant ($p<.05$) effect of dose relative to the saline vehicle (VEH).

reaction time was significantly longer at the two highest doses of midazolam than at Vehicle or 0.065 mg/kg midazolam. Choice reaction time was also significantly longer at the highest dose relative to 0.13 mg/kg midazolam. Thus, midazolam produced dose-dependent increases in choice reaction time.

So far, only responding during the choice period has been presented; however, responding during the sample-stimulus period (during which the list stimuli were presented) is also of interest. Fig. 3 shows the mean number of trials in which a press occurred to the sample stimuli at a given serial position, 1 through 6. The different line functions represent the different drug conditions, as indicated in the legend. At each serial position, the maximum number of presses possible equaled the number of trials, 240. A Determination × Dose × Serial Position repeated-measures ANOVA indicated a significant main effect

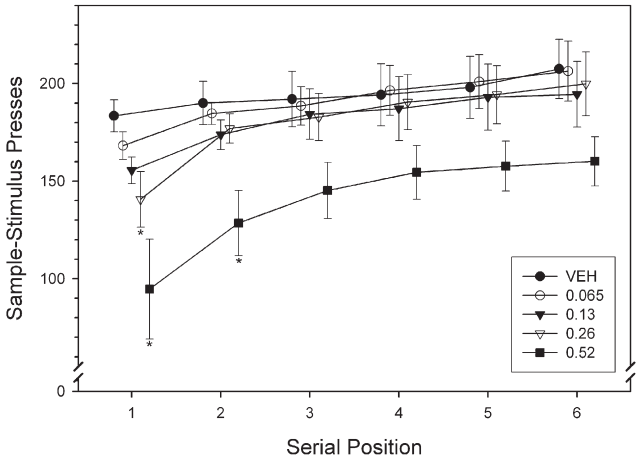

Fig. 3. Mean number of trials in which a press occurred to the sample stimuli at a given serial position, 1 through 6. Different line functions represent the different dose conditions (0.000 (VEH), 0.065, 0.13, 0.26, and 0.52 mg/kg midazolam), as indicated in the legend. At each serial position, the maximum number of presses possible equaled the number of trials, 240. Error bars represent the SEM. For clarity, the data points corresponding to each serial position are offset slightly for each dose. Asterisks denote a significant ($p<.05$) effect of dose relative to the saline vehicle (VEH).

of Serial Position ($F_{5,10}=4.44$, $p<.03$) and a significant Dose × Serial Position interaction ($F_{20,40}=6.32$, $p<.001$). To explore this interaction, a separate Determination × Dose repeated-measures ANOVA was conducted *within* each serial position. A main effect of dose was found only within Serial Positions 1 ($F_{4,8}=7.63$, $p<.01$) and 2 ($F_{4,8}=4.89$, $p<.03$). Follow-up tests indicated that, at Serial Position 1, the number of sample-stimulus presses was significantly lower than Vehicle at the two highest doses, 0.26 and 0.52 mg/kg. For both Serial Positions 1 and 2, the number of sample-stimulus presses at the 0.52-mg/kg dose was significantly lower than at all other doses. Thus, the highest doses of midazolam disrupted sample-stimulus pressing, but only at the earliest serial positions.

Despite some disruption by midazolam, sample-stimulus pressing occurred in all conditions and, for those trials in which a sample-stimulus press was made, the reaction time of the sample-stimulus responses allows further characterization of the effects of midazolam. Fig. 4 shows the mean sample-stimulus reaction time as a function of serial position for all doses of midazolam. (As above, each mean was calculated from the corresponding session medians and error bars represent the SEM.) A Determination × Dose × Serial Position repeated-measures ANOVA indicated significant main effects of Dose ($F_{4,8}=7.56$, $p<.01$) and Serial Position ($F_{5,10}=15.56$, $p<.001$), and significant Determination × Serial Position ($F_{5,10}=6.71$, $p<.01$) and Dose × Serial Position ($F_{20,40}=2.78$, $p<.005$) interactions. To explore these interactions, a separate Determination × Dose repeated-measures ANOVA was conducted *within* each serial position. For each of the six serial positions, no significant Determination main effect was found ($p>.08$ for Serial Position 1 and $p>.24$ for all other serial positions) nor was there a significant Determination × Dose interaction ($p>.30$ for each serial position). A significant main effect of Dose was

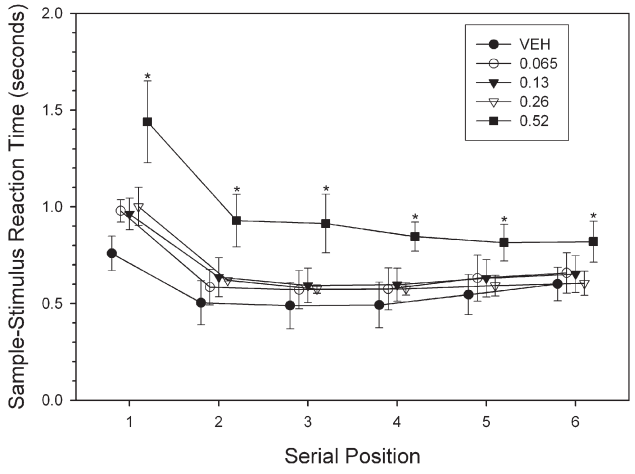

Fig. 4. Mean sample-stimulus reaction time (in seconds) as a function of serial position. Different line functions represent the different dose conditions (0.000 (VEH), 0.065, 0.13, 0.26, and 0.52 mg/kg midazolam), as indicated in the legend. Each mean was calculated from the corresponding session medians. Trials in which no sample-stimulus press was made at a given serial position were excluded from the analysis (compare to Fig. 3). Error bars represent the SEM. For clarity, the data points corresponding to each serial position are offset slightly for each dose. Asterisks denote a significant ($p<.05$) effect of dose relative to the saline vehicle (VEH).

observed for each of the six serial positions (Serial Position 1: $F_{4,8}=5.88$, $p<.02$; Serial Position 2: $F_{4,8}=5.21$, $p<.03$; Serial Position 3: $F_{4,8}=6.50$, $p<.02$; Serial Position 4: $F_{4,8}=8.05$, $p<.01$; Serial Position 5: $F_{4,8}=7.15$, $p<.01$; Serial Position 6: $F_{4,8}=9.27$, $p<.005$). Post-hoc tests indicated that sample-stimulus reaction time was significantly longer at 0.52 mg/kg than at all other doses at each of the six serial positions. Thus, only the highest dose of midazolam increased sample-stimulus reaction time, and it did so at all serial positions.

4. Discussion

At the doses studied, midazolam did not significantly reduce choice accuracy or change the shape of the serial position function. Midazolam did not significantly reduce the number of trials in which a choice response was made. Choice reaction time was significantly increased by the two highest doses of midazolam, 0.26 and 0.52 mg/kg. Similarly, responding directed at the sample stimuli was disrupted at the two highest doses of midazolam. Specifically, at Serial Position 1, the number of sample-stimulus presses was significantly reduced relative to vehicle at the two highest doses of midazolam, and at Serial Position 2 for the highest dose. Sample-stimulus reaction time was significantly longer at 0.52 mg/kg than at all other doses for all six serial positions.

The doses of midazolam used in the present study did not reduce choice responding (number of trials completed), suggesting that such doses do not reduce overall motivation of food-maintained behavior or produce motor incapacitation. Choice accuracy also was not reduced by midazolam in the present study. Although midazolam is known to produce anterograde amnesia in humans, the time frame over which recall or recognition memory is typically evaluated is often on the order of several minutes (Conner et al., 1978; Dundee and Wilson, 1980), or even days (c.f., Hennessy et al., 1991). In the present case, subjects were required to recognize a stimulus in the choice period presented no more than 21 s prior (i.e., assuming no sample-stimulus presses were made, a stimulus presented in the first serial position would be separated maximally from the choice period by the 1-s interstimulus interval plus the 3-s sample-stimulus presentation duration, equaling 4 s, multiplied by all 5 subsequent sample stimuli, 4 s × 5 = 20 s, plus the 1-s retention interval, totaling 21 s). Given this short time period, it is perhaps not surprising that short-term recognition memory was not significantly disrupted using the present SPR procedure. It is worth noting that the degree of amnesia may depend heavily on route of administration. A few studies have demonstrated mild amnesic effects of intramuscular midazolam in humans (McAteer et al., 1984; Reinhart et al., 1985; van Wijhe et al., 1985) but, in general, the intravenous route appears to produce more reliable and complete amnesia (for a review, see O'Boyle, 1988). These effects were shown with midazolam alone, without the co-administration of other drugs (e.g., fentanyl or ketamine) commonly used in anesthetic practice.

The 0.52-mg/kg dose of midazolam altered choice reaction time, sample-stimulus pressing, and sample-stimulus reaction time. The increases in choice reaction time produced by midazolam are difficult to interpret definitively. Choice reaction time is believed to reflect two components: cognitive processing time and simple reaction time. One could suggest that either component was impaired by midazolam. Moreover, either or both components could be said to reflect overall task motivation. However, other metrics of motivation (namely, trials completed and choice accuracy) suggest that overall motivation was not impaired by midazolam. While it is possible that midazolam slowed cognitive processing, a more parsimonious account might be a direct sedative motor effect of the drug. Thus, midazolam impaired the ability of subjects to respond promptly— and this is borne out by increased choice reaction time, increased sample-stimulus reaction time, and reduced sample-stimulus pressing.

While behavior directed at sample stimuli is used to index attention, such responses are not required to observe the sample stimuli in the present procedure (but responding to the sample stimuli had the additional advantages of hastening presentation of the next sample-stimulus, the choice period, potentially the food reinforcer, and the next trial). To perform accurately in the choice period subjects must attend to the list stimuli in the sample period, but need not touch them. Perhaps this is why choice accuracy was not reduced despite significant reductions in sample-stimulus pressing. Thus, one straightforward explanation of the various SPR performance measures is that midazolam slowed motor responding while preserving motivation, visual attention, and recognition memory.

These results are in accord with the limited data from monkeys using analogous procedures to study benzodiazepines. For example, Hudzik and Wenger (1993) found that diazepam reduced sample-stimulus response rate at doses of 0.3, 0.55, and 1.0 mg/kg but accuracy was impaired only at the highest dose for simultaneous matching-to-sample and at the two highest doses for delayed matching-to-sample in squirrel monkeys. Using a different delayed matching-to-sample procedure in squirrel monkeys, Baron and Wenger (2001) produced decreases in sample-stimulus response rate at doses of 1.0 mg/kg IM and higher, but accuracy was decreased only at 1.8 mg/kg and higher.

Hironaka et al. (1992) reported analogous effects of diazepam in rhesus monkeys and rats. For rats, doses of 0.5–2.0 mg/kg diazepam (delivered subcutaneously) increased choice reaction time without significantly decreasing accuracy on a delayed matching-to-sample task. For the rhesus monkeys, Hironaka and colleagues reported that sample-stimulus response rate increased dose-dependently with diazepam doses of 0.5, 1.0, and 4.0 mg/kg delivered intragastrically, but delayed match-to-sample accuracy was consistently reduced only at the 4.0-mg/kg dose. Also using rhesus monkeys, Sahgal and Iversen (1980) implemented a modified Konorski pair-comparison task with delays of 0 to 16 s and demonstrated that sample-stimulus and choice reaction times were increased with doses of chlordiazepoxide (10 to 80 mg/kg IP) that failed to reduce accuracy, presumably reflecting a sedative effect of the drug in the absence of memory disruption.

The primary purpose of the present study was to provide data regarding the behavioral incapacitation produced by midazolam at doses capable of providing seizure protection. Specifically, the

range of doses was selected to encompass the dose that might be provided in a battlefield environment. Taken together, the results of the present study suggest that levels of midazolam likely to afford seizure protection do not produce deficits in working memory or motivation, but may significantly increase reaction time, which may be of critical importance in a battlefield setting.

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