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Linopirdine Does Not Improve Matching Performance in the Titrating Matching-to-Sample Paradigm

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NORDHOLM, A. F., E. MOORE AND G. R. WENGER. *Linopirdine does not improve matching performance in the titrating matching-to-sample paradigm.* PHARMACOL BIOCHEM BEHAV **52**(1) 205–210, 1995.—Linopirdine (DUP 996), a proposed cognitive enhancing agent, was studied in four squirrel monkeys (*Saimiri sciureus*) and six White Carneau pigeons responding under a titrating matching-to-sample paradigm (TMTS). Briefly, under this titration schedule, each trial began with the presentation of a sample stimulus on the center key of a three-key pigeon or squirrel monkey chamber. Completion of a fixed-ratio on the center key resulted in the termination of the stimulus presentation and the initiation of a delay period. The length of the delay changed as a function of the subject's performance. During the first five trials of each session, the delay was fixed at 3 s in length. On the sixth and all subsequent trials, the length of the delay increased, did not change, or decreased such that accuracy was maintained at approximately 80%. Following the delay, two of the three response keys were transilluminated with different colored lights. A single response on the key transilluminated with the same stimulus as the sample stimulus resulted in the presentation of food. A response on the key transilluminated with the stimulus that did not match the sample stimulus resulted in a timeout. Linopridine was administered in the pigeon (0.001–5.6 mg/kg) and squirrel monkey (0.01–1.0 mg/kg) 15 min before testing. Matching performance was not affected as measured by changes in mean delay values or percent accuracy even at doses that decreased rate of responding. These results suggest that the enhancement in cognitive function previously reported after administration of linopiridine may be limited to specific situations.

Squirrel monkey Pigeon Titrating matching-to-sample Linopirdine Cognitive enhancer

COGNITIVE enhancement as a result of drug administration has been difficult to study, and many models have been proposed. One such model, proposed for the study of short-term (working) memory, matching-to-sample, has several problems that have reduced its value as an effective paradigm for the study of the effects of drugs on memory function. For example, subjects are typically trained until stable performance is achieved. This stable performance is frequently associated with a high degree of accuracy, as measured by percent correct, that does not allow for improvement following drug administration, the "ceiling effect." Furthermore, drug-induced decrements in performance can only be reduced to chance levels (e.g., when given a choice between two stimuli the chance performance is 50%), typically called a "floor effect."

A second problem with the matching-to-sample paradigm has to do with response bias. When decreases in accuracy are observed, it may be the result of a decrease in memory function or of the responding being partially controlled by either position or color biases. A study done by Cumming and Berryman (5) showed that as the length of the delay increased, pigeons developed a greater tendency to respond on one key independent of the stimulus displayed on the key. Conversely, at short delays very little response bias occurred. Other investigators have demonstrated similar position or color biases (2,10). Thus, it is clear that there are a number of problems associated with the traditional matching-to-sample paradigm that make interpretation of data difficult.

It has been previously shown (13) that pigeons responding under a matching-to-sample baseline show decrements in accuracy as a function of delay lengths. Thus, accuracy can be

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changed as a function of the delay value. This also means that accuracy can be maintained at any given value by changing the length of the delay. Our laboratory has attempted to use this concept to solve the "ceiling" and "floor" effects associated with the matching-to-sample paradigm. In our procedure each experimental session is started at a standard delay value (e.g., 3 s). This delay either increments up, stays the same, or decreases as a function of the accuracy on previous trials [titrating matching-to-sample (TMTS)]. Changes in the delay value (mean and maximum) can be analyzed and animals can show either improvement or decrement as a result of drug administration. In addition, the random presentation of two stimuli on the three response keys following the delay has been shown (20) to markedly decrease the development of position biases in responding commonly observed when the two stimuli are only presented on the two side keys.

Linopirdine (Dup 996), a proposed cognitive enhancing agent, has been shown in vitro to increase acetylcholine, dopamine, and serotonin release from rat hippocampus, striatum, and cortical slices (7,17). In vivo linopirdine enhances stimulation-induced acetylcholine (ACh) release without increasing baseline levels of ACh release (11). It has also been demonstrated that linopirdine binds to a novel receptor in the rat brain (17) and that binding to this site is specific, reversible, saturable, and potent (K_d = 19 nM).

Behaviorally, this compound has been shown to increase retention of avoidance training and improve the performance of septal-lesioned animals in a water-maze task (3). It has also been shown to enhance the acquisition of active avoidance responding in rats and mice and increase the acquisition of a lever-pressing response for food in rats (4,6). Clinical trials with healthy human subjects given linopiridine have shown significant changes in electroencephalograph (EEG) activity consistent with an increase in vigilance (14).

Although drug-induced decrements in matching performance have been previously reported in pigeons (18–20) and squirrel monkey (9) responding under a TMTS paradigm, no drugs have been shown to consistently increase matching performance. Because linopirdine has been reported to enhance cognition, it was interesting to determine whether it would increase matching performance under this titrating procedure. Thus, the purpose of the present study was to determine whether cognitive enhancement, as evidenced by an increase in the delay value (mean or maximum), or percent accuracy would be demonstrated in animals (squirrel monkeys and pigeons) given linopirdine and responding under a TMTS paradigm.

METHOD

Subjects

Two groups of animals were used for this study. One group of animals consisted of four adult male squirrel monkeys (*Saimiri sciureus*), and the other, six male White Carneau pigeons. The squirrel monkeys were maintained at 80% of their free feeding weight by postsession feeding of Purina Monkey Chow biscuits (Purina Mills, St. Louis, MO) supplemented with access to fresh fruit or vitamin supplements 4 days/week. The pigeons were also maintained at 80% of their free feeding weight by postsession feeding with Pigeon checkers (Purina Mills). All animals were maintained on ad lib water in their home cages. A 12 L : 12 D cycle (lights on from 0700–1900 h) was used for both species, and all experimental sessions occurred during the light cycle. All animals had a prior history of responding under the TMTS procedure and all had received acute administration of a variety of drugs before the start of this experiment.

Apparatus

The apparatus for the squirrel monkeys has been described elsewhere (8). Briefly, it consisted of a Plexiglas chair that loosely restrained monkeys around the waist. On the chair panel in front of each monkey were three response keys (Model no. G-6315; Gerbrands Corp. Arlington, MA), each located 14 cm above the Plexiglas plate that surrounded the waist of the animal. Response keys were 10 cm apart and could be transilluminated with either a constant white light or a flashing blue light. Depression of the response keys with a force >0.15 N opened an electrical contact defining a response. Beneath the center key was a pellet cup into which could be delivered 97-mg banana-flavored pellets (P.J. Noyes Co., Lancaster, NH). The pellet cup could be illuminated with a 28-V DC green stimulus light located within the housing of the cup. Mounted on top of the panel was a 28-V DC yellow stimulus light (house light). The chair assembly was enclosed in a ventilated, sound- and light-attenuating enclosure, and the room and chamber were continuously flooded with ambient white noise.

Pigeons were trained and tested in standard pigeon chambers (Model G7313; Ralph Gerbrands Co. Arlington, MA) containing three response keys, each of which could be transilluminated with red or green light. The chamber was housed in a ventilated, sound- and light-attenuating enclosure. A feeder trough was located below the center key of the operant chamber. Opening of the key contacts defined the response and operated a relay mounted inside the enclosure, producing auditory feedback upon a response. A force >0.15 N was required to open the key contacts. The operant chamber was illuminated with two 28-V DC lightbulbs (house light) that remained on during the session except during food presentation and timeout periods. All events and data collection for both the pigeons and monkeys were controlled by microcomputers and Med Associates Inc. (St. Albans, VT) interfaces located in an adjacent room.

Procedure

The TMTS procedure for monkeys was initiated by illumination of the house light and the center key being transluminated with a white or blinking blue light . After 20 responses on the center key (FR 20), the center key was extinguished (observation phase). Following a delay of at least 3 s during which responses had no scheduled consequences, two of the three keys were transluminated. A correct response was defined as a single depression on the key that matched the sample stimulus presented on the center key during the observation phase (before the delay). Following a correct response the lights on the stimulus panel were turned off, a food pellet was delivered to the food well, and the food-well light was turned on for 3 s. If a response was made on the incorrect key (nonmatch), all illumination in the chamber was turned off for a period of 10 s. The next trial was initiated immediately following the 3-s period during which the food well was illuminated or following the 10-s timeout period following an incorrect response. Auditory feedback upon a response was provided by a relay mounted in the chamber.

During the first five trials of each session, the delay value was maintained at a constant 3 s. On the sixth and all subsequent trials, the length of the delay was determined by the subjects' performance on the previous five trials. If on each of

the five previous trials the correct matching response was made, the delay value increased by 3 s. If on four of the previous five trials a correct match was made, the delay value was maintained at its current level. If a correct match was made in three or fewer of the previous five trials, the delay value was decreased by 3 s, to a minimum of 3 s. The session terminated after 60 trials or 3600 s.

The training of pigeons under the TMTS procedure has been described elsewhere (18-20) and is similar to that already described for the squirrel monkey. Briefly, each trial began with the illumination of the house light and center key in a standard three-key operant chamber, which was randomly assigned either a red or green color (observation phase). The 15th response on this center key (FR 15) turned off the center key and initiated a delay period during which all stimulus-key lights were extinguished. After the delay of at least 3 s, two of the three keys were transilluminated, one with red light and one with green. Which two of three keys were illuminated on a given trial varied randomly among the left, center, and right response keys. A single response on the key transilluminated with the same color as presented on the center-response key during the observation phase was defined as a correct response (matching response) and resulted in a 5-s access period to Purina pigeon checkers. A response on the key transilluminated with the stimulus color that was not presented during the observation phase was considered an incorrect response and resulted in a 5-s timeout period during which all lights in the experimental chamber were extinguished. A response on a darkened key was counted but had no consequences. Following either the 5-s access to food or the timeout, the center key was again transilluminated with either red or green lights and the next trial was initiated. The length of the delay following the observation phase was determined in exactly the same manner as previously described for the squirrel monkey. The session terminated after 60 trials or 3600 s.

All animals in this experiment were tested Monday through Friday, between the hours of 0730 and 1600 h. Drug was administered on Tuesdays and Fridays, and saline was administered on Thursday. Both pigeons and monkeys were placed into the experimental chamber 15 min before the start of the test session.

Drug Administration

Linopirdine (DuPont Merck Pharmaceutical Company, Wilmington, DE) was dissolved in a vehicle consisting of 40% propylene glycol, 10% ethanol, and 50% saline. Doses ranging from 0.001-5.6 mg/kg were tested in the pigeons and 0.01-1.0 mg/kg in the squirrel monkeys. All animals were injected intramuscularly, 15 min before the start of the test session in a volume equivalent to 1 ml/kg body wt. Drugs were typically administered on Tuesdays and Fridays with saline given on Thursdays. All doses were calculated and are expressed as the free base. Doses of drug were administered to both pigeons and monkeys in a mixed sequence such that each pigeon or each monkey received a different dose on a given day.

Data Analysis

In both species the following data were collected for each session: mean and maximum delay values that were achieved during the session, percentage of trials in which a correct matching response was made (accuracy), and number of completed trials and overall response rate on the center key during the observation phase. For all means, SE are shown. The determination of the mean control values were based on five sessions per animal. The SE of the control mean was derived by dividing the total standard deviation ($n - 1$) by the square root of the number of animals participating in the studies (monkeys = 4, pigeons = 6). If an animal failed to complete at least 10 trials during the session, the data for the session were not included in the calculation of the group mean for the mean and maximum delay values or the percent accuracy. Statistical significance was determined by using an analysis of variance (ANOVA) with a posthoc Bonferroni's method ($p < 0.05$). When the data failed either the test for normality or equal variance, the nonparametric Kruskal-Wallis ANOVA on ranks was used followed by a posthoc Dunn's test ($p < 0.05$).

RESULTS

Figure 1 shows the results of linopirdine administration in the pigeon. Under control conditions the performance of the pigeons was characterized by mean and maximum delay values of 12.4 ± 0.7 and 27.7 ± 1.4 s, respectively, 79.1 ± 0.4% accuracy and 1.1 ± 0.05 responses/s. Statistical analysis using a Kruskal-Wallis ANOVA on ranks demonstrated that there was no effect of linopridine on mean delay [$H(9) = 2.11$, $p = 0.98$, NS], maximum delay [$H(9) = 1.71$, $p = 0.99$, NS], or percent accuracy [$H(9) = 10.6$, $p = 0.30$, NS]. There was a significant difference in overall center key rate [$H(9) = 20.7$, $p < 0.01$]. Posthoc analysis using Dunn's method [$p < 0.05$] revealed a significant difference in response rate between saline and the highest dose of linopirdine (5.6 mg/kg). All animals completed 60 trials during the session under control and drug conditions.

Figure 2 shows the results of linopirdine administration in the squirrel monkey. Under control conditions for the squirrel monkeys, the mean and maximum delay values were 7.77 ± 0. 1 and 19.7 ± 1.5 s. Similarly, the percent accuracy and responses per second were 72.9 ± 1.0 and 1.72 ± 0.1, respectively. A Kruskal-Wallis ANOVA revealed no effect on mean delay value [$H(5) = 0.71$, $p = 0.98$, NS], maximum delay value [$H(5) = 3.44$, $p = 0.63$, NS], percent accuracy [$H(5) = 5.47$, $p = 0.36$, NS], or overall center key response rate [$H(5) = 6.52$, $p = 0.26$, NS]. Although a decrease was observed in the mean responses per second at the highest dose of linopiridine tested (1 mg/kg), this decrease did not reach statistical significance. All animals completed 60 trials during the session under control and drug conditions.

DISCUSSION

It is widely accepted that degeneration of cholinergic neurons occurs in the nucleus basilis of Meynert in patients suffering from Alzheimers's disease (AD). This has been speculated to be partly responsible for the cognitive decline seen in the AD patient. To this end, drugs such as linopirdine that increase neurotransmitter release, primarily from cholinergic neurons, have provided initial promise in the search for treatment of AD.

Our results show that in both pigeons and squirrel monkeys responding under a TMTS schedule, linopirdine did not increase accuracy, mean, or maximum delay values at doses that decreased response rate. A reason for this could be that this paradigm is not sensitive to improvements in cognition. To date many drugs have been tested under the TMTS paradigm, and none have been shown to increase cognition (9,18-20), as measured by increasing mean or maximum delay values or percent accuracy. Although no drugs have been shown to increase the delay values or percent correct, diazepam, pento-

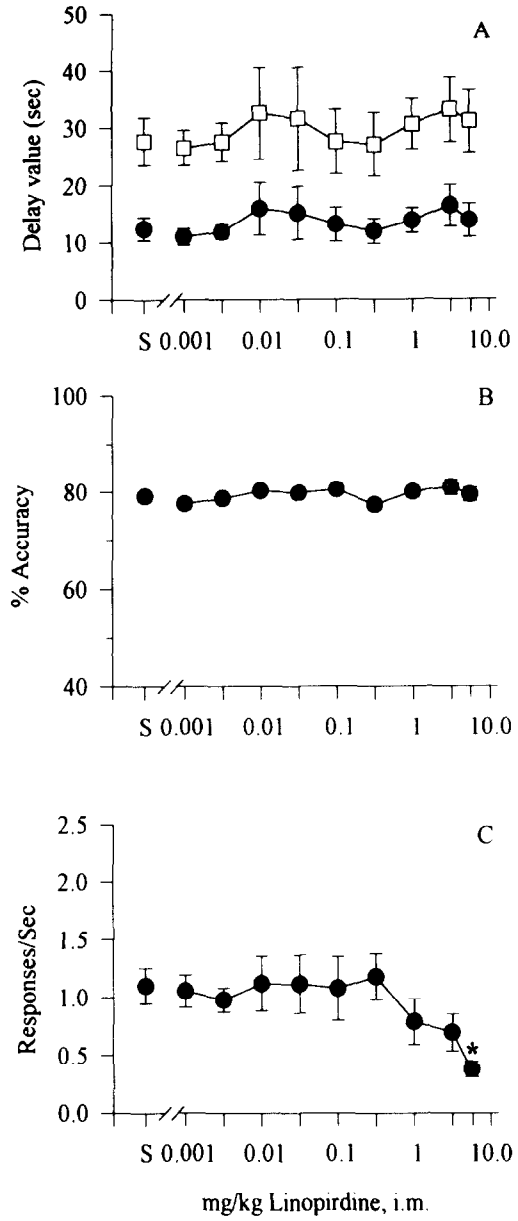

FIG. 1. Effects of linopirdine on (A) mean and maximum delay value, (B) percent accuracy, and (C) overall center key response rate in the pigeon. Abscissa: dose in milligrams per kilogram on a log scale; ordinate A: mean delay value achieved for the session in seconds (●) and maximum delay (□); ordinate B: percent accuracy for the entire session; ordinate C: rate of responding in responses/s. Points and brackets above S represent the saline injection control mean ± SE. Data points for the effects of linopiridine represent the mean ± SE of individual determinations in the six pigeons. *Statistically different from saline control.

barbital, phencyclidine, and scopolamine have all been shown to decrease matching accuracy in the pigeon (18–20), resulting in shorter mean and maximum delay values. In addition, cocaine, *d*-amphetamine, morphine, and physostigmine did not affect matching accuracy in the pigeon even at doses that reduced rates of responding. Similar results have been obtained in the squirrel monkey (9). Thus, the issue is not whether the baseline is sensitive to drug effects, but whether control performance under this baseline can be improved. This question is not limited to the titrating procedure, because drug-induced improvements in performance have not been consistently reported in animals responding under a matching-to-sample baseline using fixed delay values.

The present results confirm the lack of cognitive enhancement reported for linopirdine in animals responding under a repeated acquisition baseline (1). In contrast, linopirdine has been shown to improve place discrimination as measured by a water-maze task (1). This compound has also been shown to protect against hypoxia-induced retention deficits in a passive

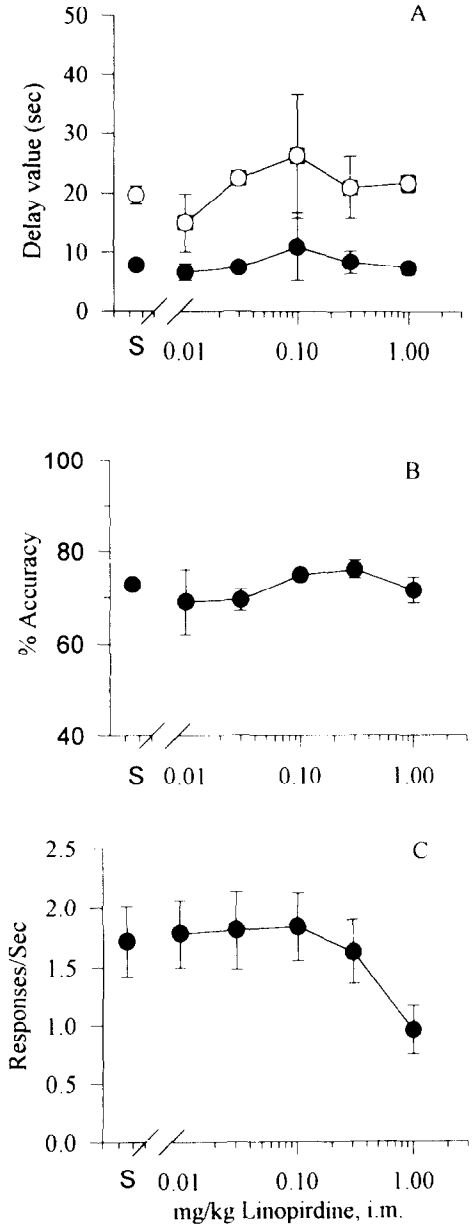

FIG. 2. Effects of linopirdine on (A) mean and maximum delay value, (B) percent accuracy, and (C) overall center key response rate in the squirrel monkey (n = 4). Data are presented as in Fig. 1.

avoidance task (6), and Brioni (3) demonstrated that linopirdine facilitated retention of avoidance training in mice and improved the performance of septal-lesioned rats in a water maze.

The reason for disagreement in results across test procedures is a cause for concern in the search for cognitive enhancers. Sarter (15,16) reviewed the current state of the field for cognitive enhancing agents. He correctly noted the lack of any agents with broad and consistent clinical efficacy despite the large number of agents that have been shown to improve memory function in laboratory animal tests. He suggested that the reasons for the rather high false-positive rate observed for many of the commonly used animal models of memory function (e.g., passive and active avoidance, water maze, radial arm maze, and T-maze) are the "fast and dirty" nature of many of these tests and a failure to show that the drug effect is mediated by a direct effect on memory rather than attention, discriminability, or motor function. In addition, many of these tests have not demonstrated either "face" or "construct" validity.

With respect to the TMTS procedure employed here, we believe that drug-induced decreases in the length of the mean and maximum delay do represent an effect on memory. Previous work showing drug-induced decrements (9,18–20) has demonstrated that at moderate dose levels the length of delay that the animal can continue to perform at approximately 80% accuracy changes. In addition, previous work has also shown that at high doses of drug, accuracy is decreased to the point at which the titration schedule can no longer maintain approximately 80% accuracy. Thus, at these high doses the specificity of the procedure for measuring memory deficits relative to attention and discriminability cannot be determined. We also believe that this procedure allows for a separation of motor function effects and cognitive effects, because our measure of motor function—response rate during presentation of the sample stimulus—is separated in time from the phase of the trial requiring the subject to choose between the stimuli, our measure of working memory. Furthermore, previous work has shown that the dose response curve for mean and maximum delay can be clearly different from the dose response curve for response rate (18–20).

With respect to "face" and "construct" validity, matching-to-sample performance has been studied in humans given triazolam and lorazepam (12). Both benzodiazepines produced decreases in accuracy and rate of responding; however, the effects of lorazepam were not as robust as those observed with triazolam. Interestingly, a TMTS procedure has also been used in humans (21). The focus of the study was to determine whether decreases in the chronic thioridazine dose would result in increased accuracy in mentally retarded adults responding under a delayed TMTS schedule. Withdrawal of chronically administered thioridazine resulted in increased accuracy and increases in delay length. Unfortunately, there are no reports in the literature correlating the matching-to-sample procedure with other procedures thought to measure working memory (e.g., digit span) in humans. However, these limited studies in humans are consistent with findings reported for laboratory animals responding under matching-to-sample schedules (9,18–20).

In conclusion, we have failed to demonstrate cognitive enhancement using the TMTS procedure in animals given linopirdine. Whether this represents a deficiency of the procedure or a lack of drug efficacy has yet to be determined.

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