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Effects of DDAVP, a Vasopressin Analog, on Delayed Matching Behavior in the Pigeon[1]

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TEAL, J. J. AND H. L. EVANS. *Effects of DDAVP, a vasopressin analog, on delayed matching behavior in the pigeon.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1123–1127, 1982.—Five pigeons were tested in a delayed matching-to-sample task after receiving an acute injection of DDAVP (1-desamino-8-D-arginine), scopolamine or d-amphetamine. A feeding test also was used to document non-specific drug effects. Scopolamine produced a marked dose-related decrement in accuracy of matching, regardless of delay, indicating that scopolamine impairs both discrimination and short-term memory. Neither DDAVP nor d-amphetamine produced consistent changes in delayed matching. Thus, an experimental model of short-term memory with pigeons did not confirm the findings of others of a positive effect of DDAVP upon cognitive performance in humans.

Matching-to-sample Short-term memory Stimulus control d-Amphetamine, memory
Scopolamine, memory DDAVP Vasopressin

CLINICAL reports indicate that analogs of vasopressin may improve memory [18, 19, 21]. Initial reports were criticized on methodological grounds [22] and were not always replicated [15]. However, a recent, carefully-planned study of both cognitively impaired and unimpaired adults demonstrated a significant augmentation of learning and memory with 1-desamino-8-D-arginine vasopressin (DDAVP) [29]. These positive clinical reports plus one report with aged monkeys [2] indicate the need for experiments using a standard animal model of short-term memory, such as delayed matching-to-sample in the pigeon. The experiments reported here compared DDAVP with scopolamine and amphetamine, two reference drugs known to affect stimulus control and occasionally reported to affect memory [11, 13, 16, 26, 27].

In the delayed matching procedure, a sample stimulus is briefly presented, then extinguished, and after a variable time delay, the subject must identify the sample in a group of stimuli. Evidence of short-term memory is found in the subject's selection of the sample stimulus more frequently than explicable by chance, with the accuracy of choice declining as a function of the delay between sample and choice stimuli [12]. This procedure provides good definition and control of the stimulus and minimizes non-specific variables such as motor impairment, which can confuse the interpretation of memory tests such as the step-through passive avoidance [11,12].

METHOD

Animals

The subjects were 5 adult male White Carneaux pigeons (Palmetto Pigeon Plant, Sumter, SC), housed individually in a room illuminated between 6 a.m. and 6 p.m. and dark at other times. Birds had water available ad lib but were maintained at 80% of their free-feeding body weight (450 to 550 g) by measured feeding of Purina Pigeon Checkers given after the daily behavioral tests. All birds were previously trained in an operant temporal discrimination and had been injected with water, amphetamine or pentobarbital (Daniel and Evans, in preparation) more than 6 months prior to the start of this experiment.

Apparatus

Four identical operant conditioning chambers (No. E10-10, Coulbourn, Inc., Lehigh Valley, PA), were enclosed in ventilated isolation boxes. Each chamber had an automatic grain feeder, a house-light and 3 transparent disk-shaped response transducers with corresponding display units (I.E.E., No. E21-18) capable of uniformly illuminating each disk with either white, red or green light. The three disks were mounted in a row on one wall 25.2 cm above the floor; the grain hopper was positioned below the center disk. The "observing" and "sample" stimuli were illuminated upon the center disk and the "choice" stimuli were illuminated upon

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the two side disks. Programming of events, the collection and computations of data were controlled by a PDP-8A computer and SKED interface.

Matching-to-Sample Procedure

A variable delay, matching-to-sample procedure was used as a measure of short-term memory. The procedure can be described as a discrete-trial, 2-choice procedure. Trials were initiated by a flashing white light on the center disk. A peck on the disk (i.e., observing response) or expiration of a 10-sec limited hold caused the sample stimulus to be illuminated upon the center disk simultaneously with a 0.2 sec tone (10,000 Hz Sonalert). The sample, either the red or green light (randomly determined), remained illuminated for 3.0 sec. Pecks during the sample presentation had no consequences. A variable delay of 0.01 (hereafter referred to as zero), 4.0 or 8.0 sec (randomly determined) separated the termination of the sample and the illumination of the red and green choice stimuli. The positions of the 2 choice stimuli were randomized. The brief tone described above also signalled the illumination of the choice stimuli. At this point the bird had 3.0 sec (limited hold) to peck either the left or right disk; a response on the center disk had no programmed consequences. A peck on the choice disk illuminated by the color that matched the sample stimulus was reinforced with 2.5 sec access to grain. A peck on the disk illuminated by the color that did not match the sample extinguished the lights for a 5.0 sec timeout without reinforcement. Failure to peck either choice stimulus within the 3-sec limited hold also caused a 5-sec timeout. The errors of omission were recorded separately from errors of commission. An incorrect response was followed by a "correction trial" in which the sample and choice stimuli were repeated so as to minimize position biases. A maximum of 5 consecutive correction trials was permitted in training sessions and one in drug sessions *(vide infra)*.

For each session the following data were recorded: % correct and response probability for 0, 4 and 8-sec delays; the probability of an observing response; the reaction time (i.e., the time between the illumination of the choice stimuli and the choice response) and the number of responses (with no programmed consequences) made during the delay interval. Data from correction trials were excluded from these calculations.

During training, the delays were initially set at zero and then gradually increased when the bird's accuracy of choice consistently exceeded 50%. Drug tests were scheduled after accuracy of choice exceeded 90% for the 0-sec delay, 80% for the 4-sec delay and 70% for the 8-sec delay. This level of stimulus control was maintained throughout the study by interdispersing training sessions (at least 3 per week) with the drug testing sessions.

Daily training sessions and scopolamine sessions concluded after either 85 min or 305 trials, whichever came first. Test sessions with d-amphetamine and with DDAVP lasted 35 min or 200 trials, whichever came first.

Feeding Test

This test is sensitive to changes in visual, motor and motivational functions, and thus provides evidence of drug effects not specifically involving memory [10]. A glass dish (10.6 cm) containing 20 grains of unpopped popcorn was attached to the pigeon's homecage. The time from the pigeon's first peck until all of the grains of corn were eaten was recorded along with the total number of pecks required to consume all 20 grains. Two observers independently recorded these data with the mean being used as the score. Pigeons received a few practice sessions before testing started. Only two tests were given per week to ensure no accumulation of the drugs. Pigeons were tested following injections of saline and drugs. Since the time course for DDAVP was unknown, pigeons (n=3) were tested 3 times within a 30 min period after the injection and once 24 hours later. Since the time course of scopolamine was known, and the feeding test is completed within 1 minute, birds (n=5) were all given the feeding test 20 min after scopolamine injection.

Drugs

Injections were given IM in the pectoral muscle in a volume of 1.0 ml/kg of body weight except where indicated otherwise. Scopolamine hydrobromide (Sigma Chemical Co., St. Louis, MO), d-amphetamine sulfate (Penwalt Corp., Rochester, NY) and 1-desamino-8-D-arginine vasopressin acetate (DDAVP, Desmopressin, Ferring Pharmaceuticals, Inc., NY) were dissolved in 0.9% saline and stored in the dark at 5°C. Scopolamine, d-amphetamine and saline were injected 10 min before the delayed-matching test session. The sequence of dosing was randomly determined for each of 5 birds, with each bird receiving all doses. Due to our limited supply of DDAVP, fewer subjects were used and not every bird received every dose. We used a stock solution of 0.3 mg/ml so the volume injected varied depending on the desired dose (small volumes were given with a microsyringe and large volumes given in multiple sites); a 5-min pretreatment period was used for DDAVP in the matching test.

Statistical Analyses

Data from scopolamine and d-amphetamine sessions were analyzed using an analysis of variance with two trial factors (i.e., dose and delay interval) [3]. Since all birds did not receive all doses of DDAVP, these data were evaluated by *t*-tests. The criterion for statistical significance was $p<0.05$.

RESULTS

Scopolamine produced a dose-related decrease in accuracy of delayed matching (Fig. 1, left panel). Accuracy was significantly related to delay under both control and drug conditions, but there was no significant interaction between scopolamine and delay. This lack of a selective decrease in accuracy at the longer delays can be observed graphically in the right panel of Fig. 1. All of the curves are relatively parallel with a slight exception of the highest dose of scopolamine (0.1 mg/kg) at which the accuracy was approaching the floor of 50% correct (random guessing).

Scopolamine was tested in doses (0.1 mg/kg) sufficient to completely inhibit responding in 2 of 5 birds and to prolong the response time in the other 3 birds. Scopolamine produced a significant dose-related decrease in the probability that the bird would initiate a trial with an observing response and the probability of making a choice response (Fig. 2). The magnitude of this decrease was independent of the delay interval so data were combined for the 3 delays in Fig. 2.

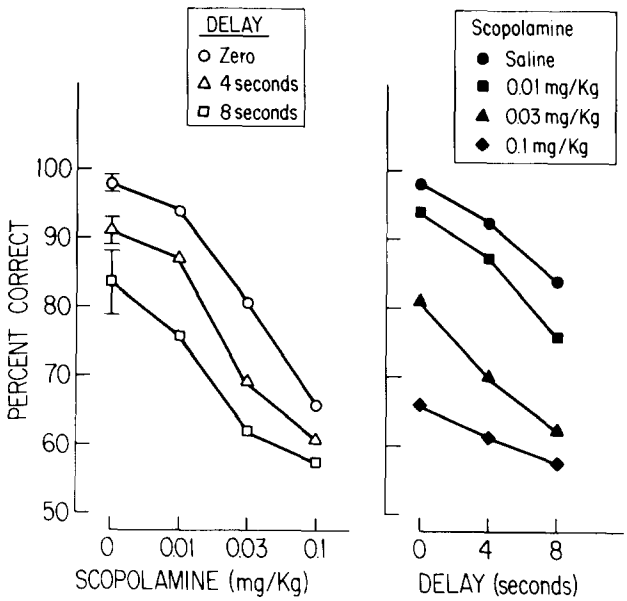

FIG. 1. Effect of scopolamine upon the percentage of correct responses made in the delayed matching-to-sample test. Data are expressed both as a function of dose (left panel) and as a function of delay interval between the presentation of the sample and the choice stimuli (right panel). Points represent a mean of 5 birds except at the 0.03 and 0.1 mg/kg doses in which only 4 out of 5 and 3 out of 5 birds, respectively, were able to respond. Scopolamine or saline was injected IM, 10 min before the start of the session. Data for saline (0 mg/kg) are shown with SEM bars. Percentage of correct responses was based on approximately 87, 62, 36 and 31 trials completed for each delay with the 0, 0.01, 0.03 and 0.1 mg/kg doses of scopolamine, respectively.

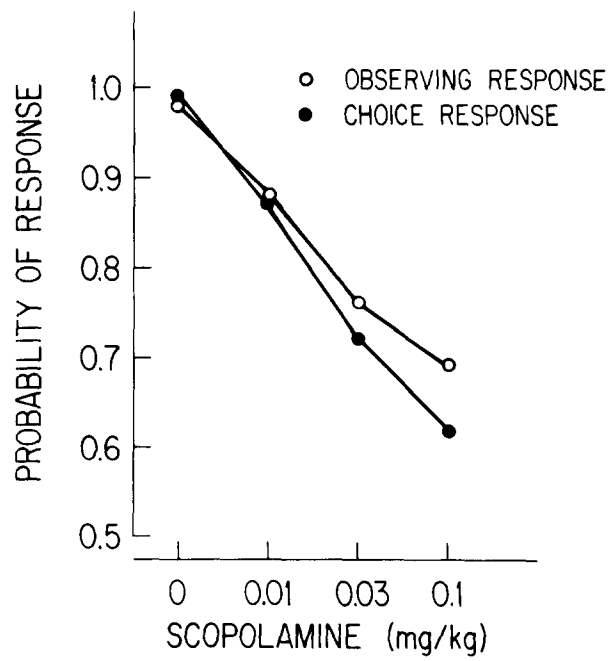

FIG. 2. Effect of scopolamine on the probability of making an observing response or a choice response in the delayed matching-to-sample task. Data for all 3 delays were combined as there was no significant difference between them. All other details are the same as in Fig. 1.

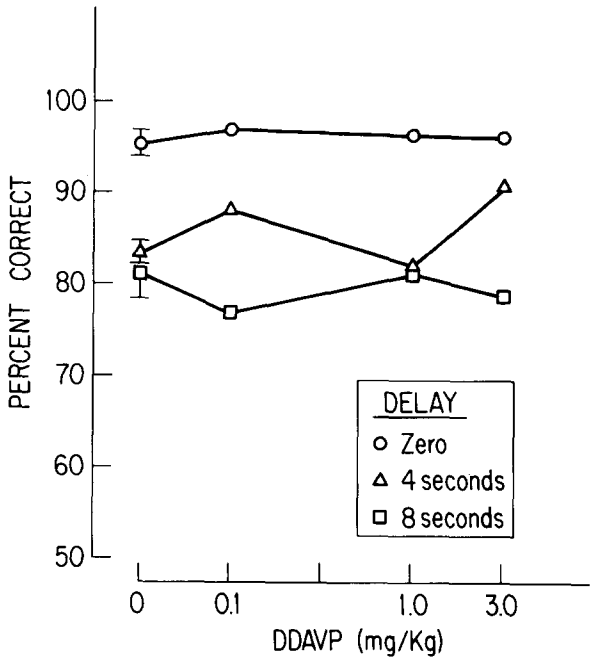

FIG. 3. Effect of DDAVP upon the percentage of correct responses made in the delayed matching-to-sample test with the 0, 4 and 8-sec delay intervals. Two birds were tested with saline (5 replications; ±1 SEM), 0.1 (replicated in 1 bird) and 1.0 mg/kg; one bird was tested with 3.0 mg/kg of DDAVP. Drugs were injected IM, 5 min before the start of the session. Percentage of correct responses was based on approximately 45 trials completed for each delay per session.

DDAVP had no significant effect on the accuracy of delayed matching (Fig. 3). There was, however, a trend toward a slight improvement at the 4-sec delay with 0.1 and 3.0 mg/kg of DDAVP but these same doses produced a slight decrement in accuracy at the 8-sec delay. Response time and the probability of both observing and choice responses were unaffected by DDAVP (data not shown).

DDAVP was also relatively inactive in the feeding test (Fig. 4) indicating that the doses tested do not cause nonspecific behavioral changes in the pigeon. Pecking rate was slightly increased with the lowest dose (0.01 mg/kg) which might explain the drop in accuracy at this dose. In general, DDAVP slightly decreased the accuracy, but this effect was inversely related to the dose of DDAVP.

In contrast, scopolamine produced a dose-related decrease both in accuracy and in pecking rate in the feeding test (Fig. 4). Unlike the memory test, all birds were able to complete the feeding test after doses as high as 0.3 mg/kg of scopolamine.

Dextro-amphetamine also produced relatively flat dose-response curves in the delayed matching procedure (Fig. 5). No significant changes in accuracy were observed. Since the highest dose of d-amphetamine (1.0 mg/kg) increased reaction time, higher doses were not tested as we were not interested in non-specific effects.

DISCUSSION

These results indicate that DDAVP has relatively little behavioral activity when administered intramuscularly to the

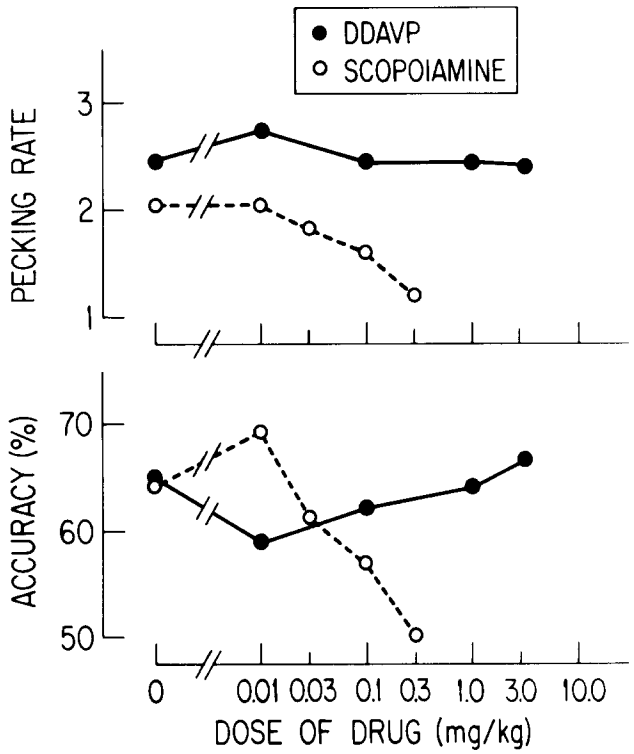

FIG. 4. Effects of scopolamine and DDAVP on the feeding test. Percent accuracy is based on 20 grains of popcorn. Pecking rate was determined by dividing the number of pecks by the time to consume all 20 grains of popcorn. Points represent a mean of 5 birds for scopolamine and one for DDAVP (data for the 3 trials within 30 min were averaged). Data are not shown for the 24 hr test after DDAVP as they did not differ from control.

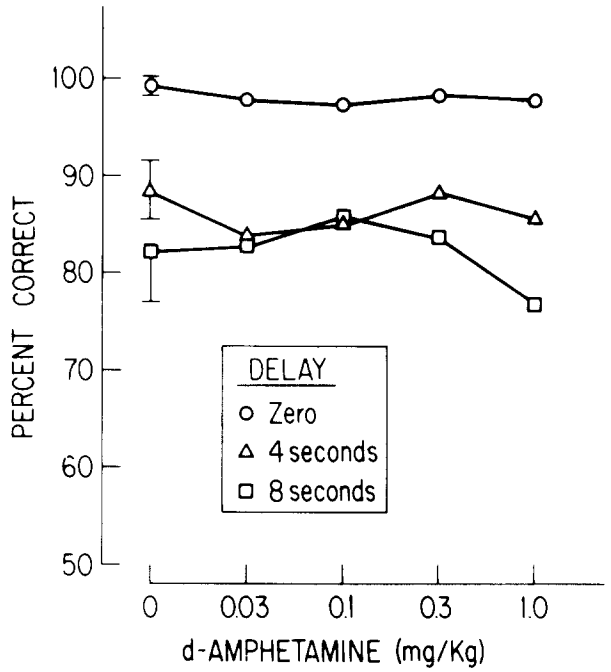

FIG. 5. Effect of d-amphetamine upon the percentage of correct responses in the delayed matching to sample test with the 0, 4 and 8-sec delay intervals. Each point is a mean of 5 birds. The SEM bars are shown for saline. Drugs were injected IM, 10 min before the start of the session. The percentage of correct responses was based on approximately 45 trials completed for each delay per session.

pigeon. Neither short-term memory (delayed matching, Fig. 3), appetite nor motor coordination (Fig. 4) were affected. DDAVP also is free of non-specific behavioral effects in humans at doses used both therapeutically for diabetes insipidus and in memory studies [7, 24, 29]. The ineffectiveness of DDAVP in a standard animal model of short-term memory is not surprising in light of the inconsistent effects of lysine- and arginine-vasopressin [2] and suggests limitations to the claim that DDAVP improves several aspects of human cognition [29]. Several factors, discussed below, deserve additional investigation before concluding that DDAVP is inactive in experimental tests of cognitive behavior.

First, the magnitude of the doses of DDAVP must be considered. The wide range of doses (0.1–3.0 mg/kg, IM) producing negative results with our pigeons are comparable to those reported to improve memory when given SC either to mice [28] or to monkeys [2]. Moreover, our doses extended well above those found to affect human memory (60 μg/day intranasally; approximately 0.86 μg/kg) [14]. Doses above 60 μg are impractical because they induce significant antidiuretic effects [23,24]. We cannot, however, rule out the possibility that the pigeon is less sensitive to DDAVP than are human or non-human primates or mice.

Second, although delayed matching may be considered the best test of short-term memory in animals, the type of cognitive improvement that DDAVP induces in humans probably involves factors other than memory. The matching procedure was designed to be specifically sensitive to changes in short-term memory. Effects due to changes in learning, vision, motor activity, position biases and attention were minimized by the use of stringent training criteria, highly discriminable stimuli, a two-choice task, the random positioning of stimuli, an observing response and by auditory cues. In contrast, the types of human memory tests in which DDAVP has been active, serial learning and recall, prompted free recall [29] and the digit symbol test of the Wechsler Adult Intelligence Scale [19] are sensitive to changes in learning, attention [20], speed [27] and mnemonic association in long-term memory [8] in addition to any changes in short-term memory. The problem of interpretation is magnified in patients who are cognitively-impaired because of disease or injury and who also have deficits in attention as well as in motivation. Depressed patients whose condition may be improved by DDAVP [14] further cloud the actual cause of improvement in the memory tests. Our results suggest that DDAVP may affect some aspect of cognition other than short-term memory.

The effects of DDAVP upon short-term memory have not been studied previously with animal models. However, the positive findings that in mice DDAVP reverses puromycin-induced amnesia in a 1–2 week memory retention test [28] and that other vasopressin analogues increase consolidation [4, 5, 6] suggest that DDAVP can affect long-term memory. Other aspects of cognition that DDAVP might affect are yet to be determined.

Memory is a complex function which cannot be defined adequately by a single test. The delayed matching-to-sample

procedure has, on occasion, been reported to detect drug-impaired memory in animals [1] as well as drug-enhanced memory [25]. Our finding with scopolamine showed a dose-related decrement of accuracy of matching which occurred uniformly, regardless of the delay, confirming a general impairment of discriminative behavior [9,10] that also affected our test of short-term memory. These same doses of scopolamine altered performance on the feeding test, further demonstrating the lack of specificity of scopolamine for short-term memory. Dextro-amphetamine produced a flat dose-response curve in our memory test similar to that observed in other behavioral tests with pigeons [17,30]. Since our animals performed at 80% correct under control conditions at the longest retention interval, this baseline was adequate to document either a drug-induced improvement as was observed with other drugs in a similar procedure [25] or a drug-induced impairment, e.g., Fig. 1. In research of this type, it is important to provide a baseline level of performance capable of registering either improvement (80 to 100%) or impairment (80 to 50%, i.e., the chance level of accuracy) [9, 11, 16]. Thus, these results and those reviewed above provide little evidence of pharmacological enhancement of short-term memory in animals.

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