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Studies on Desglycinamide Arginine Vasopressin and Scopolamine in a Modified/Lever-Touch Autoshaping Model of Learning/Memory in Rats

WILLIAM R. MUNDY*† AND EDGAR T. IWAMOTO*[1]

*Department of Pharmacology, *College of Medicine, the Graduate Center for Toxicology† and The Tobacco and Health Research Institute, University of Kentucky, Lexington, KY 40536*

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MUNDY, W. R. AND E. T. IWAMOTO. *Studies on desglycinamide arginine vasopressin and scopolamine in a modified/lever-touch autoshaping model of learning/memory in rats.* PHARMACOL BIOCHEM BEHAV **27**(2) 307–315, 1987.—Vasopressin administration has been reported to improve acquisition and retard extinction of both conditioned avoidance and food-reinforced behavioral tasks. In the present experiment the effects of a vasopressin analog (DGAVP) and scopolamine (SCOP) were tested in an autoshaped lever-touch model of learning and memory. Rats were food-deprived to 80% of original body weights and tested in modular cages which contained a retractable lever that was presented on a random interval 48 sec schedule. The lever retracted after 15 sec or when it was touched, at which time one 45 mg food pellet was delivered. Subcutaneous injection of 10 μg/kg DGAVP 1 hr prior to acquisition and extinction sessions did not alter responding compared to saline controls. DGAVP at doses of 10, 20, and 30 μg/kg also failed to affect responding in a more difficult task which included an 8 sec delay between lever retraction and reinforcement. Homozygous Brattleboro rats, which are deficient in vasopressin, did not differ from normal heterozygous littermates in the acquisition of the lever-touch response. Intraperitoneal injection of SCOP (0.1–0.8 mg/kg) 30 min prior to testing caused a dose-related impairment of acquisition compared to saline controls, but did not alter responding in animals which had previously acquired the lever-touch response. These data suggest that manipulations of vasopressin do not affect, while SCOP impairs, the acquisition of a positively reinforced lever-touch response in rats.

Desglycinamide arginine vasopressin Scopolamine Autoshaped behavior Lever-touch response

PAST literature suggests that vasopressin and its analogs can affect learning and memory processes of the mammalian central nervous system. The majority of this evidence has been obtained from studies using conditioned avoidance paradigms. Early work by DeWied and his associates showed that hypophysectomized rats were deficient in the acquisition of a shuttlebox avoidance task and less resistant to extinction [13]. Subcutaneous administration of microgram amounts of vasopressin reversed the acquisition deficit and restored resistance to extinction [7]. Similar effects were found using desglycinamide lysine vasopressin (DGLVP) [23], an analog which lacks vasopressor and antidiuretic effects [16].

The Brattleboro strain of rat, which exhibits congenital hypothalamic diabetes insipidus, provided another important model for testing the hypothesis that vasopressin is involved in learning and memory processes. Homozygous (HO) animals lack the ability to synthesize vasopressin, while their heterozygous (HE) littermates have a relatively normal vasopressin complement [36]. Using both passive and active avoidance paradigms, DeWied and coworkers reported that HO rats show memory deficits when compared with HE or normal animals [9,15] which can be ameliorated by the administration of vasopressin or vasopressin analogs. Other investigators, however, have not been able to demonstrate memory deficits in HO rats as compared to HE rats in conditioned avoidance paradigms [10,12], or have found performance of HO rats to be better than that of normal rats [3]. Experiments using positively reinforced operant procedures have also failed to detect learning and memory deficits in HO rats [24, 25, 31]. Thus, the use of the Brattleboro rat as a model in learning and memory studies is questionable.

In normal rats a single subcutaneous injection of vasopressin after an initial extinction session delayed the extinction of active avoidance tasks [14,22], and facilitated retention of passive avoidance tasks when administered immediately after training [1,8]. In other studies however, subcutaneous injection of vasopressin failed to improve passive avoidance behavior [20,33]. In a recent study, Sahgal *et al.* [34] did not find a reliable or consistent improvement in pas-

[1]Requests for reprints should be addressed to E. T. Iwamoto.

sive avoidance behavior after vasopressin administration, but rather a bimodal distribution of latency scores. The authors proposed that vasopressin acted to increase arousal, and assuming an inverted U-shaped relationship between arousal and performance, improved performance in under-aroused animals and impaired performance in over-aroused animals. Further studies using desglycinamide arginine vasopressin (DGAVP), a vasopressin analog with weak pressor activity, found no effect on latency scores in the passive avoidance paradigm [32].

Relatively few studies have used positively motivated paradigms to assess the effects of vasopressin on learning and memory. If the behavioral effects of vasopressin seen in aversely motivated tasks are due to an effect on learning and memory processes, then this property should be demonstrable using positively motivated tasks. Two studies have indicated that vasopressin may prolong the extinction of non-aversive, positively motivated tasks. Rats which received subcutaneous administration of vasopressin during the acquisition of a food-rewarded T-maze task performed the same as controls, while rats receiving vasopressin during extinction trials showed prolonged extinction [20]. In a similar study which utilized a sexually rewarded T-maze task [6], male rats which received desglycinamide lysine vasopressin (DGLVP) during acquisition showed an increased retention compared to controls. Sara *et al.* [35] trained rats in a semi-automated Y-maze to find food at the end of the lighted arm. Animals treated with 10 μg of vasopressin prior to acquisition trials learned the correct response significantly faster than saline-treated controls. Ettenberg *et al.* [18] reported that post-training vasopressin administration improved performance in a one-trial water-finding task. However, in the same study vasopressin was shown to disrupt locomotor activity and produce both taste and place aversions, while the analog DGAVP produced no aversive effects and did not improve performance in the water-finding task. The authors suggested that the memory-enhancing properties of vasopressin depend on its aversive properties since DGAVP had no effect. Additional work by Ettenberg *et al.* [17] demonstrated that the behavioral effects of vasopressin were prevented by peripheral administration of a pressor antagonist analog of vasopressin, supporting the authors hypothesis. Other studies have shown no effect [4,19] or an impairing effect [2] of vasopressin administration on the acquisition of positively motivated behavior.

Recently, Messing and Sparber [28,29] have studied the effect of DGAVP in a positively reinforced autoshaped lever-touch response. Unlike vasopressin, DGAVP has little antidiuretic or vasopressor activity [27] and does not affect locomotor activity or produce taste or place aversions [17]. Messing and Sparber reported that rats receiving subcutaneous injections of 5 μg/kg DGAVP prior to training slightly but significantly facilitated acquisition of the lever-touch response, while 5 and 10 μg/kg DGAVP slowed extinction, when compared to saline-treated controls [28]. Using a modified procedure in which food reinforcement was delayed, they showed a more robust improvement in acqusition after 15 μg/kg DGAVP [29].

Taking into account the paucity and conflicting nature of the data regarding the effects of vasopressin and vasopressin analogs in positively motivated behavioral tasks, the purpose of the present experiment was to confirm the facilitating effects of DGAVP observed by Messing and Sparber in the positively reinforced autoshaped lever-touch model of learning. The effects of DGAVP were assessed in both their original procedure [28] and in a modified procedure designed to make the task more difficult. In addition, the acquisition of the autoshaped lever-touch response was studied in vasopressin-deficient Brattleboro rats. Scopolamine, which has been shown to cause learning and memory deficits in a variety of behavioral tests [5, 23, 30], was used as a positive control in the modified autoshaping procedure.

METHOD

Animals

Adult, male Sprague-Dawley rats, weighing 265 to 345 g at the time of the experiments, were obtained from Harlan Industries (Indianapolis, IN) and held in a quarantine room for 10 days before experiments began. The animals were food-deprived and maintained at 80% of their initial body weight with free access to water. Rats were housed under automatically controlled conditions with consistent temperature (21°C) and humidity (35–55%) on a 12 hr/12 hr light cyle with lights on at 0700 hr. Experiments were performed between 0730 and 1530 hr. During the course of an experiment, body weights were monitored daily and maintained with the appropriate amount of Purina rat chow.

Apparatus

Experiments were carried out in modular operant test cages (E10-10, Coulbourn Instruments, Lehigh Valley, PA) housed in sound-attenuating enclosures. The test cages were equipped with one pellet delivery trough located in the center and 2 cm above the grid floor, one retractable lever 5 cm to the right and 3 cm above the floor, and triple cue lamps 12 cm above the lever. A centrally located house light 2 cm from the ceiling remained on during the session. Animal contact with the lever in the extended ("lever-touch") or in the retracted position ("nose-poke") was monitored via a high resistance contact input circuit. The reflected light intensity within the test cages was approximately 10 lux.

Autoshaping Procedure I: Acquisition and Extinction

This method is modeled after that of Messing and Sparber [28]. On Day 1, rats were magazine-trained by the delivery of one 45 mg food pellet (Bioserv, Frenchtown, NJ) every min for 30 min. (All animals ate the thirty pellets.) The animals were then randomly assigned to 8 groups of 6 rats each. On Day 2, rats were weighed and injected subcutaneously (SC) with either saline or 10 μg/kg DGAVP (Peninsula Laboratories, Inc.) 1 hr before autoshaping. After placement in the test cage, animals were given 12 lever presentations (trials). The retractable lever was presented on a random interval (RI) schedule where the average interval between presentations was 48 sec with a minimum interval of 24 sec. One yellow cue light was illuminated during each lever extension. The lever retracted, the cue light extinguished, and one food pellet was delivered after 15 sec had elapsed or after contact was made in the extended position. On Day 4, animals were readministered saline or DGAVP 1 hr before the sessions and given 36 trials. On Day 7, the rats did not receive any injections but were given 36 trials.

On Days 9 and 11, the extinction sessions, rats were weighed and injected with saline or 10 μg/kg DGAVP. One hr later, the rats were placed in the test cages and given 36 trials which were identical to acquisition trials except that the pellet-feeder was inactivated.

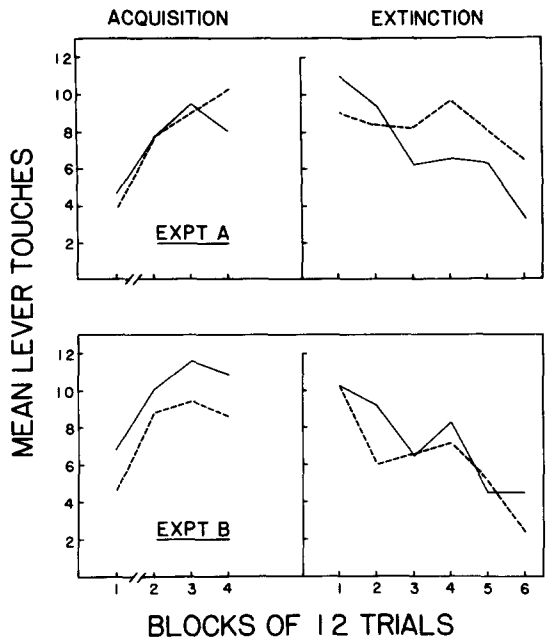

FIG. 1. Mean number of lever-touches during acquisition and extinction of the autoshaped lever-touch response using Procedure I. Groups of 6 animals received with saline (dashed line) or 10 μg/kg DGAVP (solid line) 1 hr before experimental sessions. During acquisition animals were exposed to one block of 12 trials in the first session, and three blocks 48 hr later. Three days after the last acquisition session, animals were reassigned (see text) and subjected to two extinction sessions of 36 trials, separated by 48 hr.

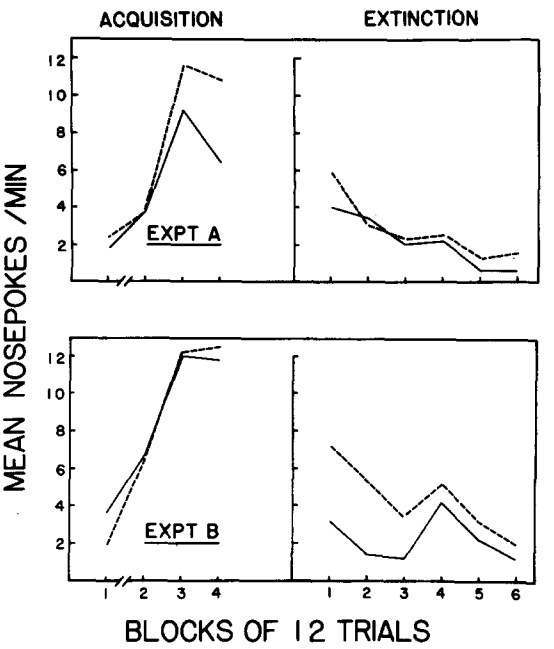

FIG. 2. Nose-poke behavior during acquisition and extinction of the lever-touch response. Animals received saline (dashed line) or 10 μg/kg DGAVP (solid line) 1 hr before experimental sessions. Data are presented as mean number of responses/min of duration for each block of 12 trials.

Experiments A and B of Procedure I were designed to reproduce the conditions used by Messing and Sparber [25]. Accordingly, before the extinction sessions of Experiments A and B (replicates) of Procedure I, the animals were reassigned to new saline and DGAVP groups each of which were comprised of 3 rats which had received DGAVP and 3 which had received saline during the acquisition sessions. The reassigned rats of the new saline and DGAVP groups were matched as evenly as possible with respect to the number of correct lever-touches. Because performance during extinction may be influenced by treatment during acquisition, an additional Experiment C using Procedure I was performed in which the animals were not reassigned before extinction.

In order to examine the usefulness of extinction as a measure of retention in the autoshaping paradigm, animals in Experiment D were subjected to Procedure I twice. All animals received saline 1 hr prior to acqusition and extinction sessions, and beginning on Day 13, were subjected to the entire acquisition/extinction paradigm for a second cycle. In all experiments, the number of correct lever-touches (touching the extended lever resulting in pellet delivery) were recorded for every RI and the data presented and analyzed in blocks of 12 trials. Nose-pokes (which had no behavioral consequence) were recorded and expressed as responses/min.

Autoshaping Procedure II: Testing A New Acquisition Paradigm

The same modular test cages, RI 48 sec and recording of lever-touches were used as in Procedure I, except that the rats were not magazine-trained. Instead, animals were weighed, placed in the test cages and exposed to 10 trials (lever presentations with pellet delivery at the end of 15 sec or after a successful lever-touch) per day. Autoshaping sessions continued until an animal attained the criterion of 10 out of 10 correct lever-touches in one day, or until 10 daily sessions had elapsed. After an animal attained the criterion of 10 lever-touches per day, it was removed from the experiment.

Experiment A of Procedure II examined the acquisition of the lever-touch response in heterozygous (HE) and homozygous (HO) Brattleboro rats (Blue Spruce, Albany, NY). Two experiments were conducted using 10 or 12 HO and HE rats per experiment. No injections were administered.

In Experiment B of Procedure II, the effect of 0.1, 0.25, and 0.8 mg/kg of scopolamine hydrobromide was examined in Sprague-Dawley rats. Drug was administered intraperitoneally 30 min before placement in the test cages. For each treatment group a saline-control group was run concurrently (N=10). The animals were given 10 trials per session for 10 sessions or until the criterion of 10 out of 10 correct lever-touches in one day was attained.

Experiment C of Procedure II tested for the effect of scopolamine in rats with an established lever-touch response. Twenty Sprague-Dawley rats with a previous history of autoshaped-behavior training with saline injections given 15 min before autoshaping were used. After all animals reached the 10/10 correct lever-touch criterion, training was discontinued and the animals were maintained at 80% of initial body weight for 3 weeks. The animals were then rein-

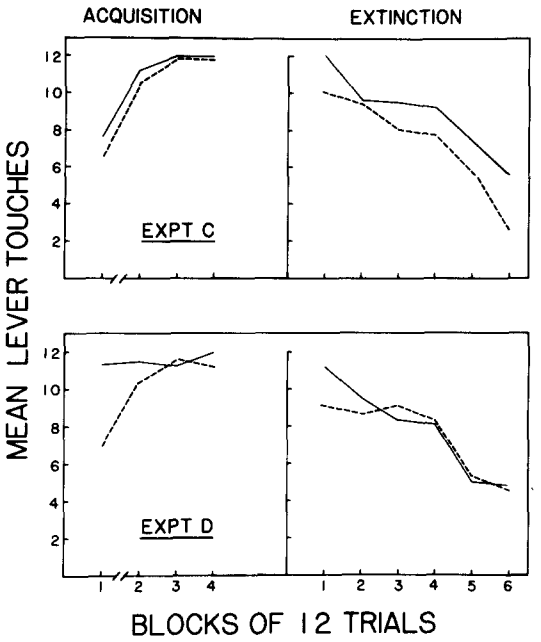

FIG. 3. Mean number of lever-touches during acquisition and extinction of the autoshaped lever-touch response. In experiment C, groups of 6 animals received saline (dashed line) or 10 μg/kg DGAVP (solid line) 1 hr before experimental sessions. During acquisition animals were exposed to one block of 12 trials followed by three blocks 48 hr later. Three days after the last acquisition session animals were subjected to two extinction sessions of 36 trials, separated by 48 hr. Animals were not reassigned prior to extinction sessions. In experiment D, one group of 12 animals received saline 1 hr before acquisition and extinction sessions (dashed line). Two days after the last extinction session, the same animals were resubjected to the acquisition and extinction sessions for a second time (solid line).

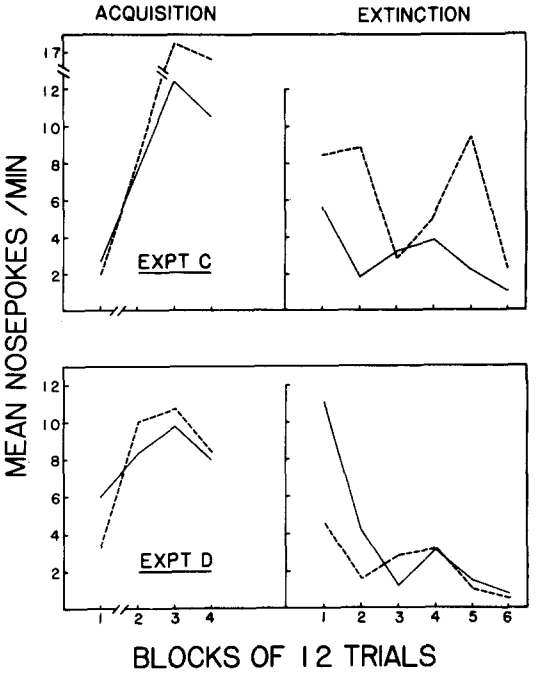

FIG. 4. Nose-poke behavior during acquisition and extinction of the lever-touch response. In experiment C animals received saline (dashed line) or 10 μg/kg DGAVP (solid line) 1 hr before experimental sessions. In experiment D all animals received saline 1 hr before the initial sessions (dashed line) and again 1 hr before being resubjected to acquisition and extinction sessions for a second time (solid line). Data are presented as mean number of responses/min of duration for each block of 12 trials.

troduced to daily sessions of 10 autoshaping trials; saline was administered 15 min prior to each session. After 2 days of retraining all animals reached the 10/10 criterion. The next day, the rats were randomly divided into 4 groups (N=5) and administered saline, 0.1, 0.25, or 0.8 mg/kg of scopolamine hydrobromide intraperitoneally 30 min before testing. Testing consisted of one session of 10 trials per animal.

Messing and Sparber [29] have shown that DGAVP-induced improvement in lever-touch performance is more robust when the task difficulty is increased by the addition of a delay between the lever-touch response and food reinforcement. Accordingly, Experiment D of Procedure II examined the effect of DGAVP (from Organon International) using an 8 sec delay between lever retraction and food reinforcement. On each of the two days prior to beginning autoshaping sessions forty Sprague-Dawley rats were allowed to eat ten 45 mg food pellets placed in their home cages so that the rats were familiar with the reinforcer when autoshaping began. Rats were randomly assigned to four groups (N=10) and received saline, 10.0, 20.0, or 30.0 μg/kg of DGAVP 1 hr before daily autoshaping sessions. Training continued for 10 days with all animals completing all 10 sessions.

Drugs

The DGAVP used in Procedure I was purchased from Peninsula Laboratories, Inc., Belmont, CA. DGAVP used in Procedure II was a gift from Organon International, OSS, the Netherlands. DGAVP solutions were dissolved in distilled H_2O on the day of use and kept on ice. Scopolamine hydrobromide was obtained from Sigma Chemical Co., St. Louis, MO, and dissolved in sterile normal saline. All drugs were administered in a volume of 1 mg/kg of body weight. Doses were expressed in terms of the salt.

Statistical Analysis

The proportion of extended lever-touches per block (Procedure I) or day (Procedure II) was calculated for each animal and transformed using the arcsine transformation. The resulting data was analyzed using a two-way (treatment × block or day) repeated measures analysis of variance. A significant treatment × day interaction, indicating that treatment groups were changing at a different rate over time, was used as a measure of learning rate. Individual comparison of means were made at a significance level of $p<0.05$.

RESULTS

Autoshaped Behavior, Procedure I

The effects of saline and 10 μg/kg of DGAVP on the acquisition and extinction of the lever-touch response in Experiments A and B are shown in Fig. 1. Analysis of variance

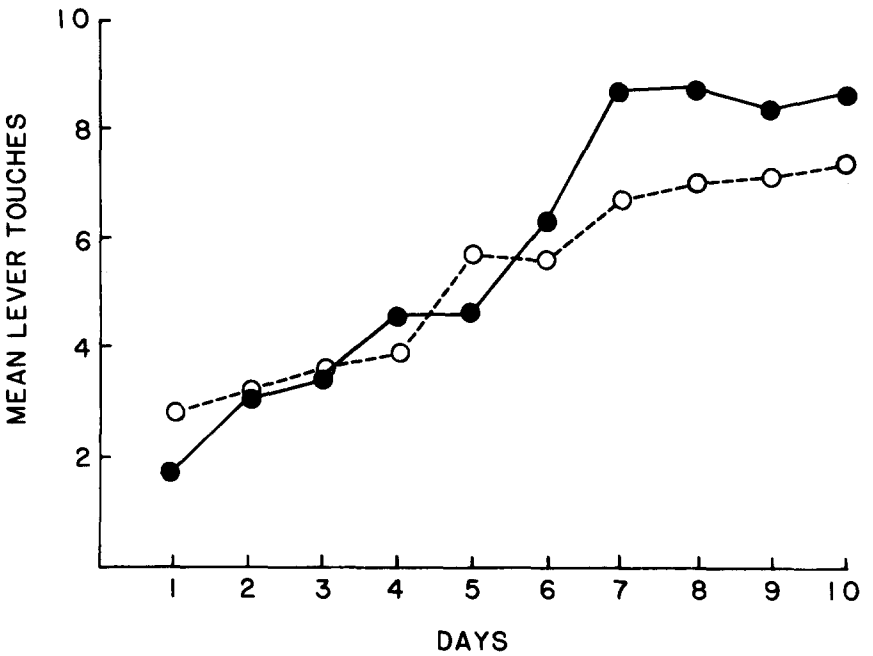

FIG. 5. Acquisition of the lever-touch response in Brattleboro rats using Procedure II. Heterozygous (○) and homozygous (●) animals (n=10) were subjected to daily sessions of 10 trials. Acquisition training continued until animals attained the criterion of 10/10 correct lever-touches in one session, or 10 days had elapsed.

revealed no significant effect of treatment or treatment × block interaction during the acquisition of the response in Experiments A and B. However, the overall increase in responding over blocks (effect of training) was significant in both Experiment A, $F(3,30)=8.1, p<0.0005$, and Experiment B, $F(3,24)=10.4, p<0.0001$. There were no significant differences in performance during the 36 trials given on day 7.

The decline of the mean number of lever-touches during extinction of the lever touch response in the two experiments is shown in the right-hand side of Fig. 1. Analysis of variance indicated that there was no significant effect of treatment or treatment × block interaction during extinction of the lever-touch response. There was a significant decrease in responding over blocks in both Experiment A, $F(5,50)=10.7$, $p<0.0001$, and Experiment B, $F(5,50)=10.5, p<0.0001$.

The frequency of nose-pokes, a reflection of activity engaged in near the retracted lever, during acquisition and extinction sessions for the same groups used in Fig. 1 is shown in Fig. 2. Analysis of variance did not show any significant effect of treatment or treatment × block interaction during the acquisition of the nose-poke response in Experiments A and B. The increase in mean number of nose-pokes over blocks was significant in both Experiment A, $F(3,30)=7.5$, $p<0.001$, and Experiment B, $F(3,24)=7.1$ $p<0.002$.

The decline in the mean number of nose-pokes during extinction sessions of Experiment A and B is shown in the right-hand side of Fig. 2. Analysis of variance indicated that there was no significant effect of treatment or treatment × block interaction during the extinction of the nose-poke response in either experiment. The decline in mean number of nosepokes over blocks was significant in both Experiment A, $F(5,50)=4.2$, $p<0.005$, and Experiment B, $F(5,50)=7.1$, $p<0.0001$.

The mean number of lever-touches during acquisition and extinction of the lever-touch response in Experiment C is shown in Fig. 3 (top graphs). In Experiment C, animals were not reassigned prior to extinction of the lever touch response. Analysis of variance revealed no significant difference between saline and DGAVP treated animals in the acquisition of the lever-touch response. The increase in responding over blocks was significant, $F(3,30)=20.8$, $p<0.0001$. Extinction of the lever-touch response during Experiment C is shown in the top right-hand graph of Fig. 3. Analysis of variance indicated no significant effect of treatment or treatment × block interaction during the extinction of the lever-touch response. The decrease in responding over blocks was significant, $p<0.0001$. DGAVP had no effect on nose-pokes during acquisition or extinction (Fig. 4, top).

In Experiment D, the only treatment rats received was a saline injection 1 hr before the acquisition and extinction trials (Figs. 3 and 4, bottom graphs). The group of 12 rats exhibited the typical increase in lever-touches and nose-pokes during acquisition, and the decline in the number of lever-touches and nose-pokes during extinction. Two days after the last extinction trial, the same group of animals were resubjected to the acquisition and extinction sessions of Procedure I (no group reassignments were made). Retention of the lever-touch response is evidenced by the high number of lever-touches in the first block of acquisition trials (Fig. 3, Experiment D, solid lines). Analysis of variance indicated a significant effect of treatment, $F(1,22)=6.9, p<0.025$, due to the greater number of lever-touches on the first block of acquisition during the second exposure to the procedures as compared to the first ($p<0.05$). The decrease in the number of lever-touches during extinction after the second exposure to the procedure was not significantly different from one exposure to the procedure. Analysis of variance indicated

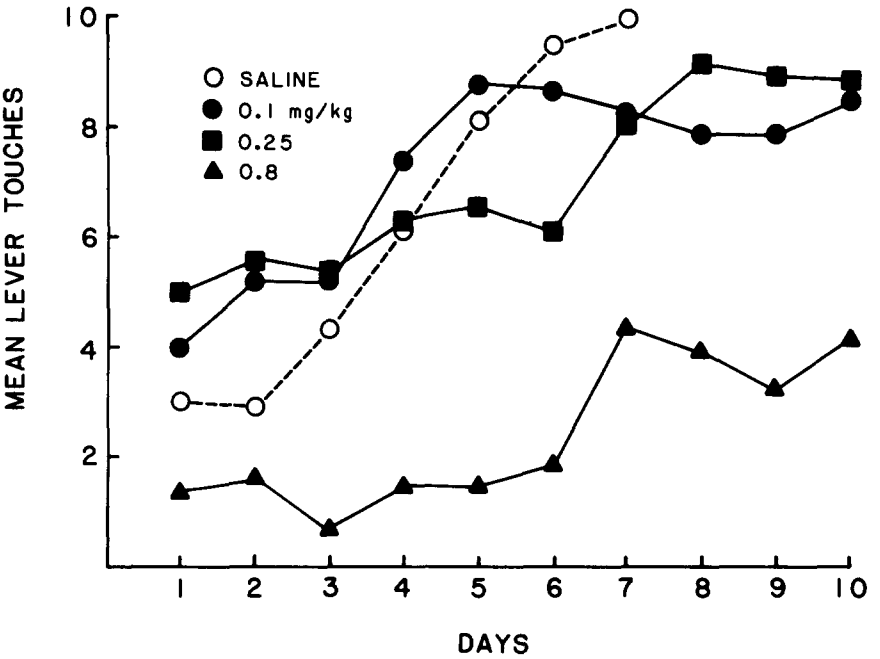

FIG. 6. Effect of scopolamine on the acquisition of the lever-touch response using Procedure II. Groups of 10 animals received saline or scopolamine (0.1, 0.25, and 0.8 mg/kg) 30 min before daily sessions of 10 trials. Acquistion training continued until animals attained the criterion of 10/10 correct lever/touches in one session, or 10 days had elapsed.

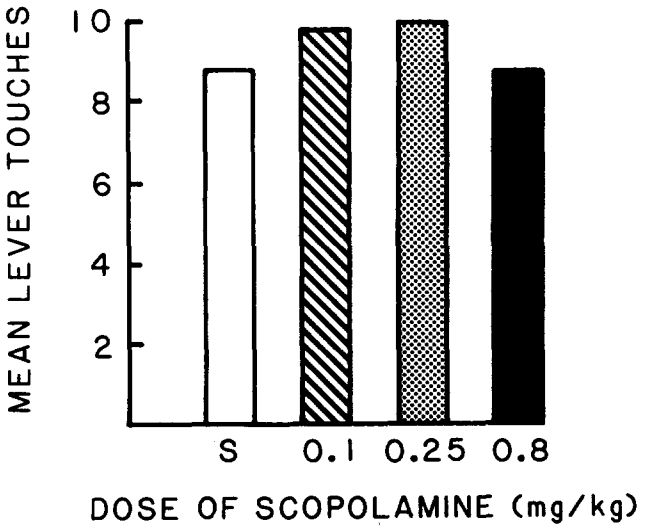

FIG. 7. Effect of scopolamine on an established lever-touch response. Animals had a previous history of autoshaped behavior training using Procedure II. After a period of 3 weeks during which animals received no training but remained food deprived, all animals were retrained to a criterion of 10/10 correct lever-touches in one session. The next day, the animals were randomly divided into 4 groups of 5 animals and received saline (S) or scopolamine (0.1, 0.25, and 0.8 mg/kg) 30 min before a single session of 10 trials. The standard errors of the means for S, and 0.1, 0.25, and 0.8 mg/kg of scopolamine were ±1.2, 0.2, 0, and 1.2, respectively.

that the decline in lever-touches over blocks was significant during both the first and second exposure to the procedure, $F(5,110)=14.6$, $p<0.0001$.

The frequency of nose-pokes for Experiment D is shown in Fig. 4 (bottom). There was no significant difference in the acquisition of the nose-poke response between the first and second exposure to the procedure. Analysis of variance showed a significant difference on the first block of extinction, $F(1,22)=8.4$, $p<0.01$, but not at any other time.

Autoshaped Behavior, Procedure II

Experiment A using procedure II examined the acquisition of the autoshaped lever-touch response by Brattleboro rats. Rats of this strain which are homozygous (HO) are vasopressin deficient and exhibit signs of diabetes insipidus (polydipsia and polyuria) while heterozygous (HE) littermates have near normal levels of vasopressin. Urine volumes over 24 hr were determined for both groups as an indirect measurement for the presence of vasopressin. The mean urine volume (ml/100 g body weight ± SE) over 24 hr for HO rats was 83.9±5.7, and for HE rats, 20.9±8.8.

The acquisition of the lever-touch response by Brattleboro rats is shown in Fig. 5. Analysis of variance indicated that there was no significant effect of treatment or treatment × day interaction. Lack of treatment × day interaction suggests that the learning rates of HE and HO rats were the same. This experiment was repeated using groups of 12 HE and HO rats, and the results were identical to those of the first experiment (data not shown).

Experiment B using the new Procedure II, tested the effects of scopolamine hydrobromide on acquisition of the lever-touch response (Fig. 6). Because a comparison of the saline control groups associated with each dose of

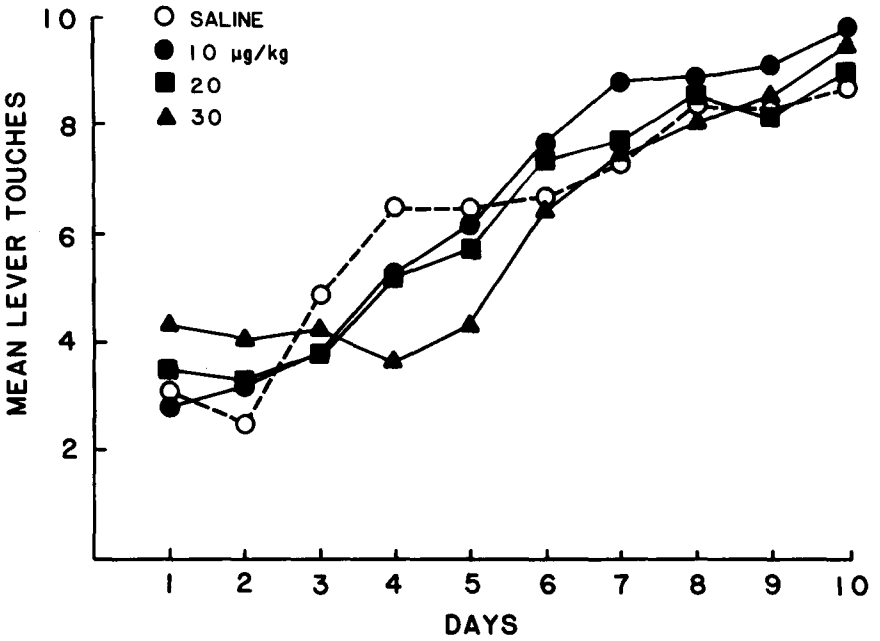

FIG. 8. Effect of DGAVP on acquisition of the lever-touch response using Procedure II with an 8 sec delay between lever retraction and food reinforcement. Groups of 10 animals received saline or DGAVP (10.0, 20.0, and 30.0 µg/kg) 1 hr before daily sessions of 10 trials. Acquisition training continued for 10 sessions for all animals.

scopolamine indicated that responding was not significantly different, the results were pooled and represented as a single control group (N=30). Using the treatment × day interaction as a measure of relative rates of learning, scopolamine caused a dose-related decrease in the rate of learning compared with saline-pretreated rats. After 0.1 mg/kg of scopolamine, repeated measures analysis of variance indicated that the difference from the concurrent saline-control rate of learning was marginally significant, $F(9,162)=3.6$, $p<0.10$. The main effect of treatment was not significant.

The 0.25 mg/kg dose of scopolamine caused a significant decrease in the rate of learning relative to concurrent saline controls, $F(9,162)=4.21$, $p<0.0001$. Individual comparison of means indicated a significantly greater number of lever-touches for saline control animals on day 6 only ($p<0.05$). The highest dose of scopolamine used, 0.8 mg/kg, resulted in a rate of learning that was significantly less than concurrent saline controls, $F(9,162)=4.7$, $p<0.0001$, repeated measures analysis of variance and about equal to that observed for a dose of 0.25 mg/kg. Individual comparison of means indicated that the 0.8 mg/kg scopolamine-treated rats had significantly less number of lever-touches on sessions 3 through 10 compared with saline controls ($p<0.025$).

Using Procedure II, Experiment C tested the effects of scopolamine in rats which had previously established the lever-touch operant. Scopolamine, at doses of 0.1, 0.25, and 0.8 mg/kg, had no significant effect on lever-touch performance compared with saline controls during a single session of 10 trials per animal (Fig. 7).

In Experiment D of Procedure II, the effects of DGAVP were examined in a more difficult procedure, in which an 8 sec delay was inserted between lever retraction and food pellet delivery. Figure 8 shows the mean number of lever-touches for rats treated with saline of DGAVP 1 hr prior to daily autoshaping sessions. A comparison of the saline-treated controls in Fig. 6 (no delay) and Fig. 8 (8 sec delay) shows the expected decrease in performance after inserting a delay between lever-retraction and reinforcement. However, analysis of the data depicted in Fig. 8 revealed no significant effect of treatment or treatment × day interaction. Thus, DGAVP failed to improve acquisition of the lever-touch response using the more difficult procedure.

DISCUSSION

In the present study we were unable to confirm the findings of Messing and Sparber [28,29] which indicated that DGAVP facilitates the acquisition and retards extinction of a positively reinforced lever-touch task. In Procedure I, a paradigm identical to that of Messing and Sparber [28], subcutaneous injections of 10 µg/kg DGAVP had no effect on the acquisition or extinction of the lever-touch response. Repeating the experiment gave similar results. In Procedure I, Experiment C, the procedure was altered in that animals were not reassigned to new treatment groups before extinction testing; it was reasoned that reassignment could introduce a complicating factor since DGAVP administered during acquisition may have effects which carry over to extinction testing. In fact, there are several studies which suggest that the effects of vasopressin can persist for several days [13,16]. However, even without reassignment of animals, there was no effect of DGAVP during acquisition or extinction.

The behavioral processes involved in the extinction of an operant response are not entirely clear. During the early stages of extinction, the level of responding may reflect the strength of retention of the conditioned response, so extinction could be considered as a measure of memory for the original task. Clearly, the studies which present delayed extinction of conditioned avoidance paradigms as evidence for

a retention-enhancing effect of vasopressin rely on such an interpretation. However, as extinction continues, the decline in responding over time can itself be considered as a form of learning, so extinction is likely a measure of both retention of the original response and the acquisition of new responses [11,30]. In order to examine the usefulness of extinction as a measure of retention in the autoshaping paradigm, animals in Experiment D were subjected to Procedure I twice. The performance of the animals on the first cycle was compared to their performance on the second cycle. Retention of the lever-touch response is evidenced by the high number of lever-touches during the acquisition phase of the second cycle. If extinction is a good measure of retention then it would be expected that the rate of extinction should be prolonged on the second cycle. However, our data shows that responding during extinction on the first and second cycle was nearly identical (Fig. 3). These results suggest the rate of extinction is probably not a good measure of retention in the autoshaping paradigm.

In a second study, Messing and Sparber [29] reported that the facilitating effects of DGAVP were more robust when the task difficulty was increased. Accordingly, in Procedure II animals were exposed to only 10 trials a day over a 10 day period. In addition, a delay of 8 sec was inserted between lever retraction and food reinforcement. This procedure was similar to that of Messing and Sparber, and the increase in difficulty resulted in a slower learning rate, with saline-treated animals attaining a mean of 8.7 lever-touches per day by day 10 (Fig. 8). However, using DGAVP obtained from the same supplier and in a dose range similar to that of Messing and Sparber, we were unable to show a significant drug effect. This discrepancy is difficult to explain, and suggests that the memory enhancing properties of DGAVP may not be as robust as has been reported. It is possible that the negative findings of the present study are due to differences in the experimental subjects. In both Procedure I and II we used rats of the Sprague-Dawley strain with a mean weight of 305 g, while Messing and Sparber used both Holtzman and Long-Evans rats weighing up to 625 g. Thus, strain and age differences in learning ability or vasopressin sensitivity may have rendered our animals less susceptible to the behavioral effects of the drug. Another factor that may explain the discrepancy between the present study and those of Messing and Sparber is the commercial brand of experimental equipment used in the operant chamber. The present study used retractable levers obtained from Coulbourn Instruments (Lehigh Valley, PA) while the levers used by Messing and Sparber were obtained from BRS/LVE (Laurel, MD). Differences in the stimuli which accompany the extension of the two brands of levers may alter the acquisition of the operant response. The differences outlined above may be reflected in the performance of control animals in the delayed reinforcement procedure. Using a 6 sec delay between lever retraction and reinforcement, Messing and Sparber found no increase in performance in control animals over 8 training sessions. In contrast, control animals in the present study clearly learned the lever-touch response in the presence of an 8 sec delay. While it is possible that the increased learning rate of the control animals obscured the observation of a drug effect, this is considered unlikely since Messing and Sparber also showed DGAVP-induced facilitation of learning using a no-delay procedure which produced substantial learning in control animals.

To further investigate the role of vasopressin in learning and memory processes, we examined the acquisition of the lever-touch response in Brattleboro rats which lack the ability to synthesize vasopressin. DeWied and coworkers have reported that rats of this strain show acquisition and retention deficits when compared to normal rats using conditioned avoidance procedures [9,15]. However, we and other investigators have had difficulty in replicating these results [3, 10, 12]. In two separate but identical experiments we found no significant differences in learning between vasopressin-deficient (HO) and normal (HE) Brattleboro rats. Urine volumes were determined over a 24 hr period as an indirect measure of the level of vasopressin. Urine volume was four times greater in HO rats than in the normal HE rats, suggesting that the HO rats used in our experiments were deficient in vasopressin compared to the HE controls. Our results showing no difference between normal and vasopressin-deficient rats in the acquisition of a positively reinforced lever-touch task are consistent with those of Laycock *et al.* [24,25] and Sahgal [31] who found no learning impairment in Brattleboro rats using positively reinforced operant procedures. Thus, studies using both active and passive avoidance procedures as well as positively reinforced tasks have failed to consistently detect learning and memory impairments in vasopressin-deficient rats, and suggest that vasopressin is not necessary for all types of learning behavior.

That our procedure is sensitive to drug-induced deficits in acquisition is evidenced by the effect of the cholinergic antagonist scopolamine. Scopolamine has been shown to disrupt memory and learning in a variety of tasks [5, 26, 37]. We found that 0.1, 0.25, and 0.8 mg/kg of scopolamine administered 30 min prior to daily autoshaping sessions caused a dose-related decrease in learning rate compared to saline-treated controls. When animals that had previously acquired the lever-touch response (Fig. 7) were treated with the same doses of scopolamine there was no effect on lever-touch performance. These results suggest that the impairment of acquisition after scopolamine administration was not the result of drug-induced alterations in motor performance, motivation, or sensory processes, but rather a drug effect on learning and/or memory.

To conclude, the present findings indicate that the autoshaped lever-touch procedure is sensitive to increasing task difficulty. Decreasing the number of trials per day and inserting a delay between lever-retraction and food reinforcement resulted in slower learning rates. The procedure is also sensitive to the disruptive effects of scopolamine, which caused a dose-related decrease in learning rate compared to saline-treated controls. The autoshaping procedure is quite reproducible as evidenced by the two experiments using Brattleboro rats which gave identical results, and in the results of the scopolamine experiments which showed no significant difference between three separate saline control groups. Finally, we could not confirm the facilitating effects of DGAVP using the autoshaped lever-touch task.

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