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Atropine, Scopolamine and Hippocampal Lesion Effects on Alternation Performance of Rats[1]

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WHITE, S. R. *Atropine, scopolamine and hippocampal lesion effects on alternation performance of rats.* PHARMAC. BIOCHEM. BEHAV. 2(3) 297–307, 1974. – Interactions between effects of hippocampal lesions and cholinergic blocking agents were examined using two behavioral tasks, delayed spatial alternation and go-no go temporal alternation. Atropine and scopolamine were administered to control rats and to rats with lesions in three areas of the hippocampal formation. Large hippocampal lesions disrupted performance on both tasks as did administration of atropine or scopolamine to control rats and to rats with lesions in restricted areas of the hippocampus. However, the effects of atropine and scopolamine on performance of rats with large lesions involving all regions of the hippocampal formation depended upon the alternation task used. Atropine and scopolamine disrupted delayed spatial alternation performance of all groups tested, but atropine failed to significantly disrupt go-no go alternation performance of the group with large unrestricted hippocampal lesions. Both the size of the lesion and the behavioral task were found to be important determinants of the effects of cholinergic blockers on the performance of rats with hippocampal lesions.

Hippocampus Lesion Atropine Scopolamine Spatial alternation Go-no go alternation

A VARIETY of evidence suggests that the hippocampus may be an important site of action of cholinergic blocking drugs. Hippocampal lesions have effects on many behavioral tasks which are very similar to those produced by injections of cholinergic blockers (see Douglas [3] and Carlton [1] for reviews of hippocampal lesion effects and cholinergic blocking drug effects). In addition, applications of cholinergic blocking drugs directly to the hippocampus via cannulae have been found to cause the same performance changes on behavioral tasks as did peripheral injections of the drugs [9,17].

If cholinergic blocking agents disrupt performance on behavioral tasks by acting on the hippocampus, one would expect the drugs to be ineffective on the performance of animals from which most of the hippocampus has been removed. Warburton [15,16] found this to be the case. Atropine, which disrupted asymptotic single alternation performance and repeated extinction performance of unoperated rats, did not disrupt performance of rats with large hippocampal lesions. However, in contradiction to the hypothesis that performance effects of cholinergic blocking drugs are caused by actions of the drugs on the hippocampus, Suits and Isaacson [13] found that scopolamine continued to modify avoidance learning in rats with hippocampal lesions. Scopolamine has also been found to produce activity increases in rats with large hippocampal lesions [2]. This conflicting evidence about the effects of cholinergic blocking drugs on the performance of animals with hippocampal lesions may result from differences in the location of lesions within the hippocampus, in the particular cholinergic blocking agent used, or in the requirements of the behavioral tasks. The purpose of the present set of experiments was to determine how these three variables affect cholinergic blocking drug – hippocampal lesion interactions.

EXPERIMENT 1

Large lesions in the hippocampal formation severely impair performance on delayed spatial alternation tasks [11,12]. Delayed spatial alternation is also disrupted by administration of the cholinergic blocking agent, scopolamine hydrobromide [5]. Both deficits may result from impaired cholinergic neurotransmission in the hippocampus. If this hypothesis is correct, administration of the cholinergic blockers, atropine sulfate and scopolamine hydrobromide, should cause little or no deficit in the asymptotic performance of animals with extensive hippocampal lesions.

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In the following experiment the effects of scopolamine and atropine on the delayed spatial alternation performances of unoperated rats, rats with neocortical lesions and rats with extensive hippocampal lesions were compared. In addition, performances of animals with lesions restricted to the anterior–dorsal or posterior–ventral regions of the hippocampus were also examined. Jackson and Strong [7] found that rats with lesions involving primarily the anterior–dorsal hippocampus were facilitated rather than impaired in the acquisition of a spatial alternation task. Therefore, the spatial alternation deficits found in rats with extensive hippocampal lesions may result from damage to the posterior–ventral portions of the hippocampus.

Method

Animals. Male Sprague-Dawley rats, supplied by Hormone Assay Laboratories (Chicago), were used. The rats were approximately 120 days old at the onset of training and weighed between 300 and 350 g. Six were unoperated (Group NORM), 7 had neocortical lesions in the area overlying the anterior–dorsal hippocampus (Group NEO), 6 had anterior hippocampal lesions (Group A), 7 had posterior hippocampal lesions (Group P) and 6 had lesions in both regions of the hippocampus (Group A+P). The animals were maintained on a 23 hr water deprivation schedule following a 2 week postoperative recovery period.

Surgery. During surgery the rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Lesions were made by radiofrequency current oscillating at approximately 5 megacycles per sec. The current was delivered through stereotaxically placed electrodes connected to a Wappler cold cautery scalpel (American Cytoscope Makers, Inc.). The power selector was set at 4. The rat's head was held firmly in a horizontal position by a mouth bar and nose clamp. A stainless steel plate electrode placed under the body of the animal served as the indifferent electrode. Group A lesions were made by applying current at 4 sites: 2.5 mm posterior to bregma, 2 mm lateral to the midline on each side, and 3.5 mm ventral to the skull surface for one set of 2 sites; and 4.0 mm posterior, 2.0 mm lateral on each side, and 3.8 mm ventral for the other set. Current was applied for 30 sec at each site. Group P lesions were also made by applying current at 4 sites: 5.5 mm posterior to bregma, 5.0 mm lateral to the midline on each side, and 6.5–3.5 mm ventral to the skull surface for one set; and 7.0 mm posterior, 5.0 mm lateral on each side, and 6.5–3.5 mm ventral for the other set. Current was applied for 15 sec at the 6.5 mm depth. Then, applying current constantly, the electrode was slowly moved up to 3.5 mm below the skull surface where current was applied for an additional 10 sec. Group A+P lesions were made by combining the procedure for Group A and Group P. The neocortical control group (Group NEO) had current applied for 30 sec at the same 8 placements as Group A+P except that the ventral setting was only 1.5 mm from the skull surface for each placement. When surgery was completed in an animal, the scalp was closed with wound clips, sulfathiazole was applied, and the animal was injected i.m. with 100,000 units of Bicillin.

Histology. All animals with hippocampal or neocortical lesions were perfused intracardially first with physiological saline, then with a 10% Formalin solution. The brains were frozed on dry ice and sliced into 40 μ sections. Every fifth section through the lesion was mounted and stained with cresyl violet.

Apparatus. Bar press training took place in chambers containing a panel mounted with a single Gerbrands lever, a light, and a cup for delivering reinforcement. Reinforcements throughout the experiment were 0.1 cc drops of 9% sucrose in water solution. Discrete trial training and delayed spatial alternation training occurred in chambers containing 2 Gerbrands levers mounted on an end panel, one on the left and one on the right. A cup which delivered the reinforcement was mounted between the levers. A 6 W white light was mounted above the cup; and a loudspeaker which delivered a 1000 cps, 80 dB tone was mounted beside the chamber.

Procedure. A compound stimulus of light and tone was constantly present during shaping of the bar-press response. When a stable response was established, discrete trial training began. Discrete trial procedure consisted of alternating 5 sec light-tone periods (trials) with 10 sec dark-silent periods (inter-trial intervals). A single lever press in the presence of the trial stimulus (light and tone) delivered a reinforcement, terminated the trial stimulus and initiated the inter-trial interval (ITI). A lever press during the last 5 sec of the ITI delayed the onset of the next trial by 5 sec. No reinforcement was available during the ITI. To equalize experience on each lever, a partition confined the animal to the left lever until it obtained 100 reinforcements. Then the partition was shifted and the animal was confined to the right lever for 100 reinforcements. Daily sessions of 200 reinforcements continued in this manner until the animals responded on at least 90% of the trials during 2 consecutive sessions. This phase of training took 4 sessions for most animals.

On the subsequent day delayed spatial alternation training began. Trial and ITI cues and durations were the same as during discrete trial training, but the partition was removed to allow access to both levers. Optimum performance consisted of alternating levers on successive trials. An error response occurred when the animals pressed the same lever during a trial which it had pressed during the previous trial. Error responses terminated the trial but did not deliver reinforcement. Trials on which error responses occurred were repeated; and, failing to respond on a trial also caused the trial to be repeated. Consequently, animals were always reinforced for switching the response to the lever opposite the one on which the most recent trial response was made. Bar presses occurring during the intertrial intervals (ITI responses) were not reinforced. Two hundred reinforcements terminated daily sessions until the animals reached asymptote. The sessions were then shortened to 100 reinforcements for the drug portion of the experiment.

At least 2 days always separated drug sessions. Each animal received i.p. injections of atropine sulfate (2.0, 6.0 and 12.0 mg/kg), scopolamine hydrobromide (0.25, 0.5 and 1.0 mg/kg), physiological saline and methylatropine bromide (12 mg/kg). Drugs were dissolved in distilled water and given in an injection volume of 1 cc/kg 15 min prior to beginning the test session. Half the animals received scopolamine before atropine and this order was reversed for the other half. The order of doses was randomly assigned except that all animals received the saline treatment first. The number of error responses was divided by the number of trial responses for each session. Since an error could occur on every trial, this measure gave the conditional probability of error responses.

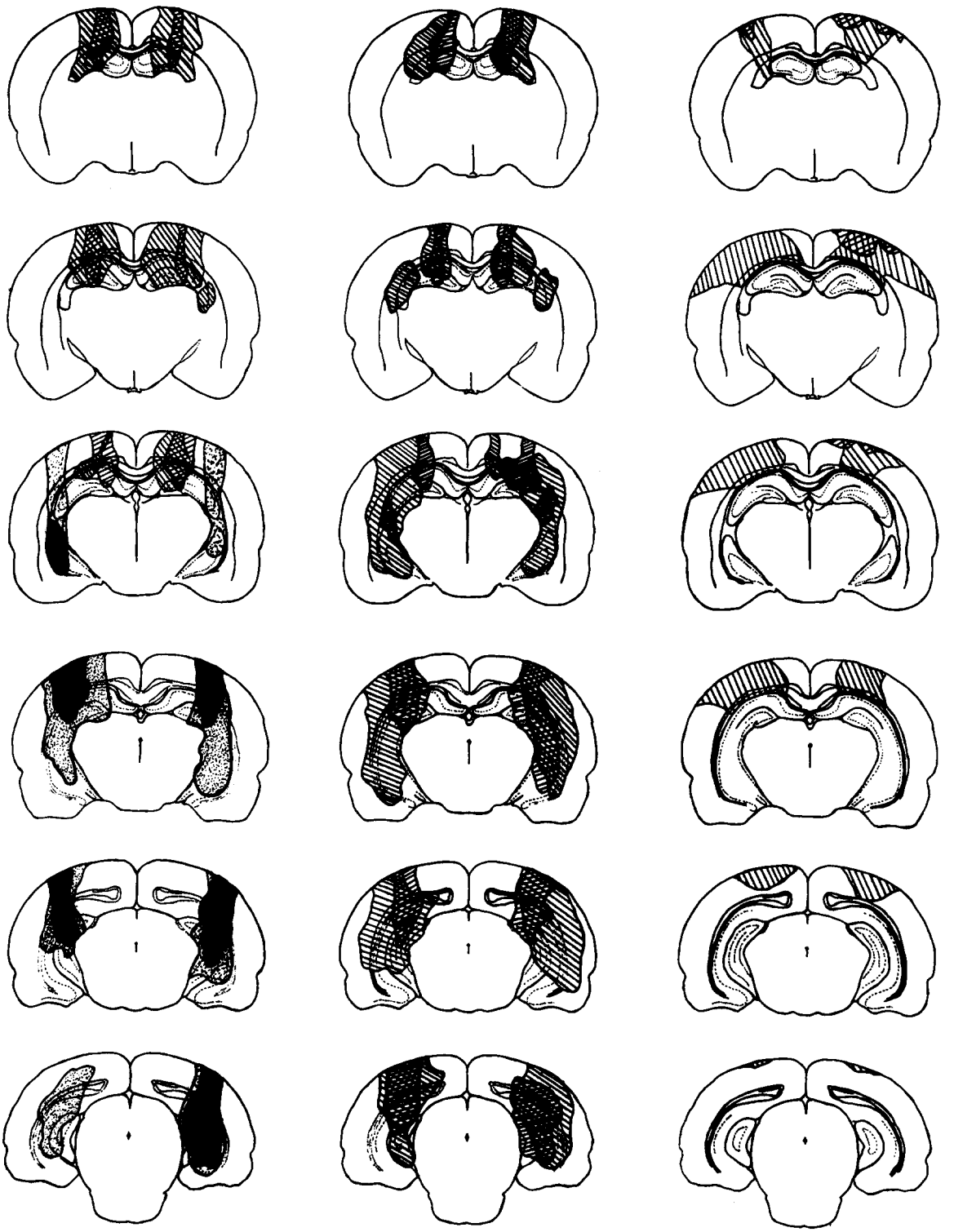

FIG. 1. Lesion extent for Group A (upper 3 diagrams, left column), Group P (lower 4 diagrams, left column), Group A+P (middle column) and Group NEO (right column). ▩: largest lesions for Groups A, A+P, and NEO. ▩ : smallest lesions for Groups A, A+P, and NEO. ▨: largest lesions for Group P. ▬ smallest lesion for Group P. Diagrams taken from atlas of König and Klippel [8].

Results

Histology. The brain damage sustained by the animals with the largest and the smallest lesion in each group is depicted in Fig. 1. The mean amount of hippocampal formation damage was 58% for Group A+P, 40% for Group P and 11% for Group A. Hippocampal damage in all Group A animals was confined to the anterior–dorsal region of the hippocampus, while damage in Group P animals was restricted to the posterior–lateral and ventral regions.

In addition to hippocampal damage, 2 Group A+P rats and 1 Group P rat sustained slight unilateral damage to the dorsal thalamus. Three of the neocortical animals had slight unilateral hippocampal damage.

Lesion effects on acquisition. During the initial acquisition session the performance of the 5 groups did not differ, but, by Session 4, Groups P and A+P showed a marked deficit (Fig. 2). Group A and the control groups (NORM and NEO) reached an asymptotic performance level of 10 to 15% errors by the end of Session 12. Group P was delayed in acquisition but reached the same asymptotic level of 10 to 15% errors by the end of Session 15. Group A+P never performed better than 25 to 30% errors throughout the experiment. A lesion group × session analysis of variance was performed on the number of errors made by each group on each of the first 10 acquisition sessions. The group effect, the session effect and the group × session interaction were all significant; $F(4,270) = 56.62$, $p<0.001$, $F(9,270) = 82.09$, $p<0.001$ and $F(36,270) = 1.70$, $p<0.05$ respectively. Post hoc (Scheffé) comparisons revealed that while the groups did not differ significantly on the first 3 sessions, Group P made significantly more errors at the $p<0.05$ level than controls and Group A on Sessions 4, 5, 6 and 9; and Group A+P made significantly more errors than controls and Group A on Sessions 4, 6, 7, 8, 9 and 10 ($p<0.05$). Group NORM, Group NEO and Group A did not differ significantly on any session. Although Groups P and A+P made more error responses than the other groups during acquisition, the differences in the number of ITI responses made by the 5 groups (Table 1) were not statistically significant, $F(4,27) = 0.44$.

Drug effects on performance. One animal each from Groups NORM, NEO and A+P did not complete the drug

TABLE 1

ITI RESPONSES DURING FIRST 10 SPATIAL ALTERNATION ACQUISITION SESSIONS

Group		NORM	NEO	A	P	A + P
N		6	7	6	7	6
ITI	mean	1236	949	1205	1292	1691
Responses	σ	261	513	813	1502	1013

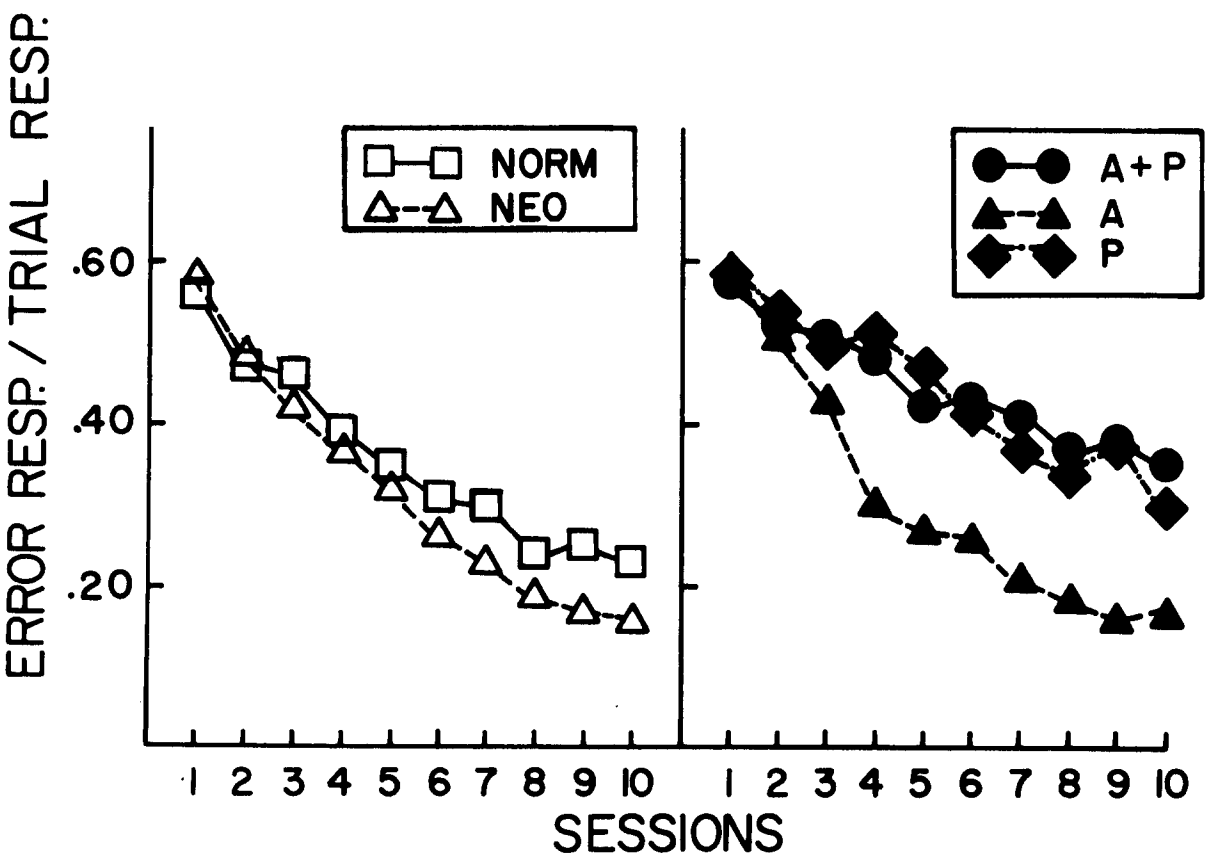

FIG. 2. Delayed spatial alternation acquisition. Mean performance for each group during first 10 sessions.

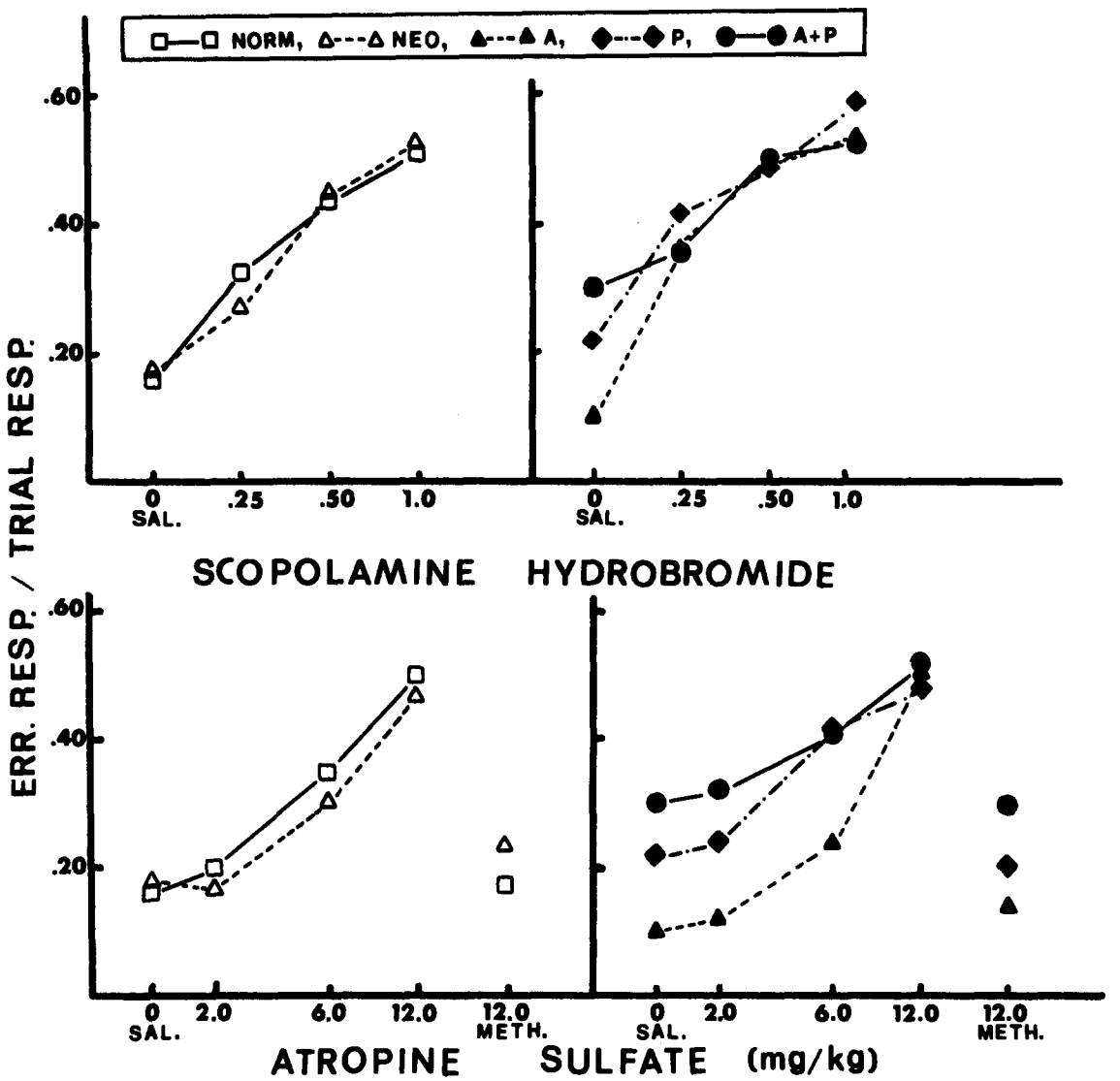

FIG. 3. Effects of atropine and scopolamine on delayed spatial alternation performance. (SAL. = physiological saline, METH. = methylatropine bromide).

series and was not included in the drug results. Figure 3 shows that both atropine sulfate and scopolamine hydrobromide increased error responses for all three hippocampal lesion groups as well as for the two control groups. The fact that Group A+P did not reach the same baseline level of performance as the other groups is apparent in Fig. 3. The higher proportion of errors made by Group A+P at the 0 dose of the drugs reflects this impaired baseline performance. Physiological saline alone did not change performance from the preceeding no injection day. Methylatropine bromide, a cholinergic blocker that does not readily enter the central nervous system when injected i.p., also had little affect on performance when compared with no injection performance. A lesion group × drug dose analysis on the number of errors made by the groups while under the influence of saline and the doses of scopolamine indicated that the group effect, $F(3,92) = 9.02$, $p<0.001$, the dose effect, $F(3,92) = 53.86$, $p<0.001$, and the group × dose interaction, $F(9,92) = 2.01$, $p<0.05$, were all significant. The data from the control groups (Groups NORM and NEO) were pooled for the drug analyses. Post hoc (Scheffé) comparisons revealed that the numbers of errors made under the influence of the 0.50 and 1.0 mg/kg doses of scopolamine were significantly greater than the numbers of errors made under the influence of saline for all groups ($p<0.01$ in each case). The 0.25 mg/kg dose increased errors compared to saline for Group A ($p<0.05$) but not for any other group. None of the hippocampal lesion groups were significantly different from controls in the number of errors made at any dose given.

A lesion group × drug dose analysis of variance on the number of errors made while under the influence of atro-

pine sulfate or saline revealed that, as with scopolamine, the group effect $F(3,96) = 53.30$, $p<0.001$, the dose effect, $F(3,96) = 157.11$, $p<0.001$, and the interaction, $F(9,96) = 10.18$, $p<0.001$, were all significant. The 12.0 mg/kg dose of atropine sulfate increased errors compared to saline for all groups ($p<0.01$). The 6.0 mg/kg dose increased errors for controls and Group P ($p<0.01$). Group A+P made significantly more errors than Group A and the controls at the saline dose and at the 2.0 mg/kg atropine sulfate dose ($p<0.01$). However, this difference is a result of the impaired nondrug asymptotic performance level of Group A+P. The hippocampal lesion groups and the controls did not differ significantly in the number of errors made under the influence of the two higher doses of atropine.

High doses of both atropine and scopolamine brought all groups to nearly random performance on this task. On each trial there were two possible responses, a correct response or an error response. Under the influence of high doses of the drugs the probability of an error response was approximately 0.50 for all groups. Although scopolamine and atropine increased errors for all groups, they failed to increase ITI responses for any group.

Discussion

The deficit in delayed spatial alternation acquisition found with Group A+P and Group P rats corresponds with the findings of other studies investigating large hippocampal lesions [11,12]. However, the fact that Group A was not significantly different from controls during acquisition conflicts with Jackson and Strong's [7] report that hippocampal lesions (mainly in anterior–dorsal regions) facilitated spatial alternation acquisition. The final asymptotic performance level of Group A animals was slightly better than controls in the present experiment, but the difference was not statistically significant. Differences in the spatial alternation tasks may account for the discrepancy. Jackson and Strong reinforced animals for a sequence of two responses rather than reinforcing each switch from one lever to the other, and no delays were programmed between alternations.

The hypothesis that cholinergic blockers would have little effect on the performance of animals with large hippocampal lesions was not supported. Both atropine and scopolamine increased errors for all groups tested. This could indicate that the drug effects were caused by actions on a central structure other than the hippocampus or that the drugs were acting on the small amount of hippocampal tissue left intact even in the group with the largest lesions. The interpretation of the effects of the drugs on Group A+P is clouded by the fact that Group A+P never reached the same asymptotic performance level as the other groups. However, although Group A+P had a higher baseline level of errors than controls, the number of errors made was significantly increased by the higher doses of both scopolamine and atropine.

Contrary to the results of this experiment, Warburton [15,16] found that go-no go alternation performance and repeated extinction performance of rats with large hippocampal lesions were not disrupted by atropine while control animals were severely disrupted. The discrepancy in the results of the studies could arise from differences in the hippocampal lesions or from differences in the requirements of the behavioral task. Experiment 2 was conducted to differentiate between these possibilities.

EXPERIMENT 2

The procedures of Experiment 1 were repeated using a go-no go alternation task very similar to that used by Warburton [15]. Since the hippocampal lesions were made in a manner indentical to Experiment 1, any difference in drug effects could be attributed to differences in the requirements of the delayed spatial alternation and go-no go alternation tasks.

Method

Animals. Thirty male Sprague-Dawley rats, supplied by Hormone Assay Laboratories, were used. They were approximately 120 days old and weighed between 300 and 350 g at the onset of training. Six were unoperated (Group NORM), 8 had anterior hippocampal lesions (Group A), 7 had posterior hippocampal lesions (Group P), 5 had lesions in both the anterior and posterior hippocampus (Group A+P), and 4 had lesions in the neocortex overlying the hippocampus (Group NEO). A 2 week postoperative recovery period preceded the first training session. Surgical and histological procedures were the same as in Experiment 1.

Apparatus. Bar press training took place in the training chambers described in Experiment 1. All other training took place in chambers containing a single lever, a light and a cup for delivering reinforcement. The cup that dispensed 9% sucrose in water solution in 0.1 cc drops was in the center of the chamber wall with the 6 W white light mounted above it. A 1000 cps, 80 dB tone was delivered through a loudspeaker mounted near the ceiling of the chamber.

Procedure. The bar-press response was shaped in the presence of continuous light and tone. When the response was stable, 10 sec periods of light and tone (trials) were alternated with 10 sec periods of darkness and silence (inter-trial intervals). A single lever press during a trial terminated the light-tone stimulus and delivered a reinforcement. Lever presses during the inter-trial interval (ITI) were not reinforced and postponed the onset of the next trial by 10 sec. Daily sessions continued until the animal collected 150 reinforcements. When the animal responded on 90% of the trials during 2 sessions, this phase of training ended. Most animals met this criterion in 5 sessions.

During go-no go alternation training, trials were also signalled by the light-tone compound stimulus and were separated by ITI of 10 sec duration. Trials on which reinforcement could be obtained (go trials) alternated throughout the session with trials on which no reinforcement was available (no go trials). A no go trial always lasted 10 sec. A go trail was terminated by a bar press or terminated automatically after 10 sec. Lever pressing during a go trial was counted as a correct response; while lever pressing during a no go trial counted as an error. Only the first bar press in a trial was recorded. An error occurring during the trial immediately following a correct response was counted as an alternation error. The ratio, alternation errors/correct responses, was calculated for each rat on every session. Acquisition training continued until this ratio varied by no more than 0.05 during 3 successive sessions. Daily sessions terminated after 150 reinforcements.

When the animals reached acquisition criterion, sessions were shortened to 80 reinforcements and the drug regimen was begun. Drugs and dosages used were the same as in Experiment 1. High doses of atropine and scopolamine sometimes caused animals to quit responding for long

periods of time on this task. Since the effects of drugs wear off with time, data from sessions in which animals collected less than 70 reinforcements during 400 trials (approximately 2 hr) were not included in the results. The drug dose given in these sessions was repeated. This occurred for the 12.0 mg/kg dose of atropine sulfate, the 12.0 mg/kg dose of methylatropine bromide and the 1.0 mg/kg dose of scopolamine hydrobromide. It was never necessary to repeat a dose level more than once in any animal. The conditional probability of alternation errors was calculated for each session. Since in this task an alternation error could occur only after a correct response, the conditional probabilities were found by dividing the number of alternation errors by the number of correct responses for each session.

Results

Histology. The hippocampal lesions in this experiment were very like those in Experiment 1. The amount of damage for all groups fell within the ranges illustrated in Fig. 1. Mean hippocampal damage sustained was 12% for Group A, 40% for Group P and 55% for Group A+P. All 4 animals in the neocortical control group had slight unilateral damage to the dorsal surface of the hippocampus.

Lesion effects on acquisition. All groups with lesions in the hippocampus were impaired in acquiring the go-no go alternation task (Fig. 4). A lesion group × session analysis of variance on the alternation errors made during the first 5 sessions showed that the lesion effect, $F(4,125) = 78.60$, $p<0.001$, the session effect, $F(4,125) = 231.52$, $p<0.01$, and the lesion × session interaction, $F(16,125) = 9.46$, $p<0.001$, were all significant. Post hoc (Scheffé) comparisons indicated that all 3 hippocampal lesion groups made significantly more alternation errors than controls on Sessions 2 and 3 ($p<0.05$). Group A+P made more alternation errors than controls on sessions 4 and 5 as well ($p<0.05$). Although the groups with hippocampal lesions were deficient in acquiring this task, all 5 groups tested reached the same asymptotic performance level of alternation errors/correct responses = 0.05 to 0.10 within 9 sessions.

The ITI responses made during acquisition are shown in Table 2. An analysis of variance indicated that only Group A+P made significantly more ITI responses than controls ($p<0.05$). Most ITI responses occurred in the intervals before correct responses for all animals. The groups did not differ significantly in the number of ITI responses occurring during intervals preceding error responses.

Drug effects on performance. One animal each from Groups NORM, NEO and A did not complete the drug series and was not included in the results. Group A+P was markedly less affected by atropine sulfate than the other groups (Fig. 5). While increasing doses of atropine resulted in increased errors for the other groups, the dose-response curve for Group A+P remained essentially flat. Drug data for the 2 control groups were combined and compared to the 3 hippocampal lesion groups. A lesion group × drug

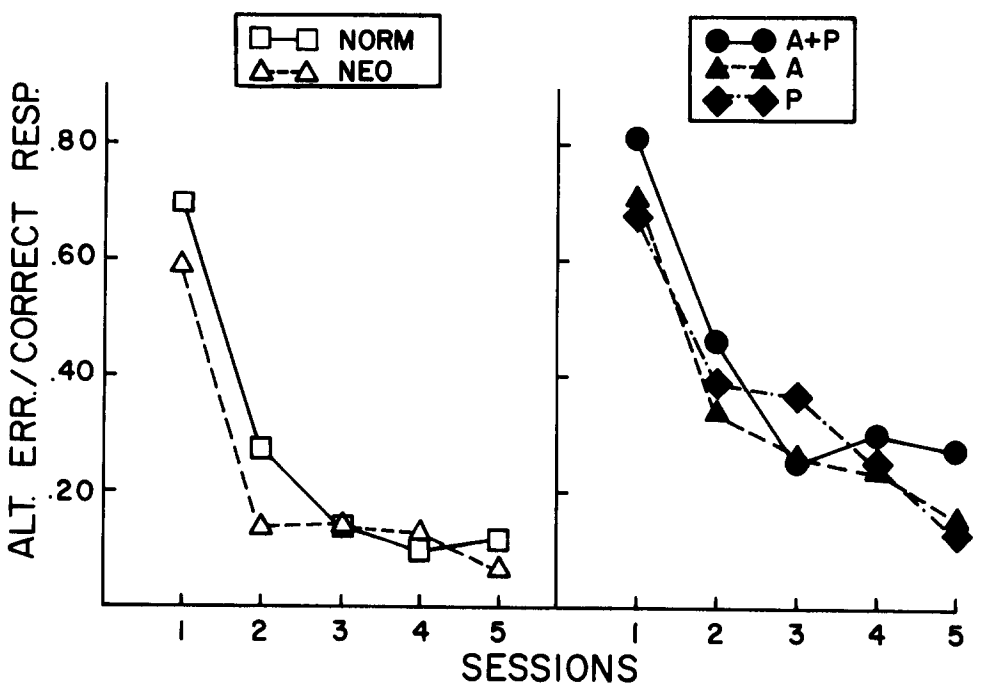

FIG. 4. Go-no go alternation acquisition. Mean performance for each group during first 5 sessions.

TABLE 2

ITI RESPONSES DURING FIRST FIVE GO–NO GO ALTERNATION ACQUISITION SESSIONS

Group		NORM	NEO	A	P	A + P
N		6	4	8	7	5
Total ITI	mean	289	155	289	380	491
Responses	σ	83	107	121	148	139
Pre-reinforcement	mean	237	123	201	246	325
ITI Responses	σ	77	91	88	69	112
Pre-error	mean	19	10	36	59	60
ITI Responses	σ	14	4	32	55	52

dose analysis of variance on the alternation errors made while under the influence of atropine or saline revealed that the group effect $F(3,16) = 61.61$, $p<0.001$, the dose effect, $F(3,16) = 104.21$, $p<0.001$, and the group x dose interaction, $F(9,16) = 15.15$, $p<0.001$, were all significant. Post hoc (Scheffé) comparisons showed that while the 6.0 and 12.0 mg/kg doses of atropine sulfate increased alternation errors above saline levels for controls and Groups A and P ($p<0.001$ in each case), no dose of atropine tested significantly increased alternation errors for Group A+P ($p>0.05$ for each dose). Group A+P made significantly fewer errors than Groups A, P and controls at the 12.0 mg/kg dose ($p<0.01$). Neither Group A nor Group P were significantly different from controls at any dose. Methylatropine bromide had little effect on performance.

In contrast to the atropine results, scopolamine increased alternation errors for all groups (Fig. 5). A lesion group x drug dose analysis of variance on the scopolamine data showed that the group effect, $F(3,92) = 17.53$, $p<0.001$, the dose effect, $F(3,92) = 57.36$, $p<0.001$, and the interaction, $F(9,92) = 3.34$, $p<0.01$, were all significant. The 0.50 and 1.0 mg/kg doses of scopolamine increased alternation errors over saline levels for controls, Group A and Group P ($p<0.01$). Only the 1.0 mg/kg dose significantly increased errors for Group A+P ($p<0.01$). The 0.50 mg/kg dose was not different from saline ($p>0.05$). Group A+P was significantly less affected by the 0.50 mg/kg dose than controls ($p<0.01$) but was not different from controls in the number of alternation errors made with the 1.0 mg/kg dose of scopolamine ($p>0.05$).

Atropine and scopolamine, like large hippocampal lesions, increased ITI responses occurring prior to correct responses ($p<0.001$ and $p<0.1$ respectively), but did not significantly increase ITI responses occurring before errors.

Discussion

The acquisition deficit found in animals with hippocampal lesions agrees with the deficit found by Warburton [15], but is in contradiction to the facilitated go-no go alternation acquisition found by Means, Walker and Isaacson [10]. This discrepancy may arise from differences in ITI activity in the studies. Means, *et al.* [10] used a retractable lever to separate trials so there was no opportunity for inter-trial interval responding. Walker, Messer, Freund, and Means [14] observed that alternation performance of rats with hippocampal lesions was much more disrupted than control animal performance by bar pressing occurring between trials.

Although all 3 hippocampal lesion groups were impaired in acquiring this task, only the group with the largest lesions was refractory to the effects of a cholinergic blocker. Atropine, in accord with Warburton's [15] finding, failed to disrupt performance of Group A+P animals. However, the largest dose of scopolamine did impair performance of Group A+P animals, suggesting that a cholinergic system was still important for mediating performance in these animals.

The cholinergic blocking drugs and the largest hippocampal lesions increased ITI responses as well as alternation errors. However, the ITI response increase occurred only in the intervals preceding correct responses. Very few responses occurred in the ITI preceding error responses, and virtually none occurred in the ITI preceding trials on which no response was made. Consequently, the increased ITI responses seem to reflect an increased anticipation of reward rather than a general inability to inhibit responding.

GENERAL DISCUSSION

Large hippocampal lesions and drugs which block cholinergic muscarinic neurotransmission were found to have similar effects on alternation performance. Both go-no go alternation errors and delayed spatial alternation errors were increased by the lesions and the drugs. This similarity of effect on performance between animals with large hippocampal lesions and animals given cholinergic blocking drugs has been found with several behavioral tasks [2,13]. Consequently, it is not surprising that similar functions have been attributed to the hippocampal formation and to central cholinergic neurotransmission. Carlton [1] has proposed that a central cholinergic system is important in mediating

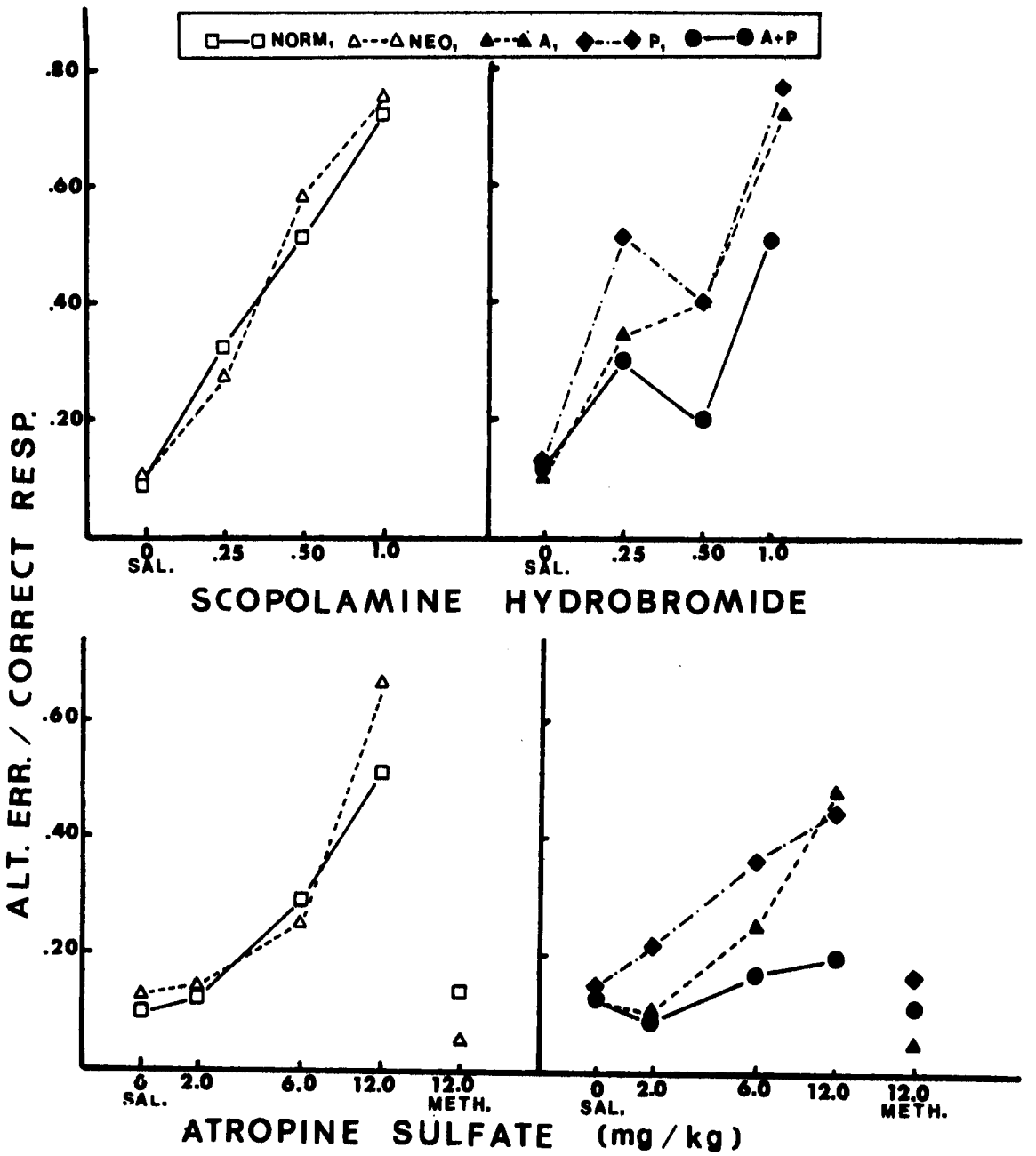

FIG. 5. Effects of atropine and scopolamine on go-no go alternation performance. (SAL. = physiological saline, METH. = methylatropine bromide).

inhibitory processes engendered by nonreward, and Douglas [3] has suggested that the hippocampus is important for response inhibition. In the present study, a deficit in ability to inhibit nonreinforced responses could account for the increased go-no go and spatial alternation errors accompanying hippocampal lesions and cholinergic blocking drugs. The animals were required to refrain from bar pressing entirely during alternate trials on the go-no go task, and to refrain from pressing the lever which had delivered reward on the previous trial in the spatial alternation task. However, impaired short term memory could also explain the increased errors. No external cue was available at the time of the trial to signal the correct response in either alternation task. Appropriate responding required using reinforcement cues from the previous trial.

Although both memory deficits and response inhibition deficits could account for the increased alternation errors, neither seems to adequately explain the ITI responding. Large hippocampal lesions and cholinergic blocking drugs significantly increased ITI responding only on the go-no go

task and only during the intervals preceding trials on which correct responses occurred. This patterning of the ITI responses in relationship to reinforcement on the go-no go task resembles the response patterns of rats with large hippocampal lesions on fixed interval reinforcement schedules [4]. The bursts of responding occurring immediately prior to reinforcement seem to be more an expression of increased arousal than a general inability to inhibit responding or an impaired short term memory. No one single function attributed to the hippocampus or to cholinergic neurotransmission seems to adequately account for all the effects on behavior of damage to the systems.

A variety of evidence indicates that the hippocampus is not a homogeneous structure [6]. Nevertheless, most hippocampal lesions involve only the anterior–dorsal regions in studies using rats as subjects. In the present experiment, effects of lesions restricted to the posterior–ventral hippocampus and lesions restricted to the anterior–dorsal hippocampus were compared to the effects of lesions involving both regions. The importance of the various regions of the hippocampus in mediating performance was found to depend upon the task. Lesions restricted to the posterior–ventral area increased errors on both alternation tasks, but lesions in the anterior–dorsal region increased errors only on the go-no go task. While go-no go alternation acquisition was disrupted by all 3 hippocampal lesions, the hippocampus was not indispensable for adequate performance on the task. Even the animals with the largest lesions eventually reached the same baseline performance level as controls. This suggests that some other structure is capable of taking over mediation of the task when the hippocampus is damaged. The central nervous system seems to be less plastic in respect to delayed spatial alternation performance. During many weeks of testing, rats with large hippocampal lesions (Group A+P) were never able to reduce errors on this task to the level of controls. Thus, the hippocampus seems to be necessary for shifting responses from one lever to another, but not for completely refraining from responding on alternate trials.

The similarity between the behavioral effects of hippocampal lesions and cholinergic blocking drugs suggests that the hippocampus may be the site of action of the drugs. Evidence on this point is contradictory. Studies have shown that scopolamine continues to affect behavior in rats with hippocampal lesions [2,13] and that atropine has no effect on the performance of rats with hippocampal lesions [15,16]. The latter results would be expected if, indeed, the drugs do act upon the hippocampus to produce their behavioral effects and the system mediating performance when the hippocampus is damaged is not cholinergic. In the present study, interactions between the effects of hippocampal lesions and cholinergic blocking drugs were found to depend upon the amount of hippocampal damage, the particular drug used, and the behavioral task. Performance of rats with lesions restricted to the anterior or the posterior regions of the hippocampus was just as disrupted by atropine and scopolamine as performance of control rats. The drugs may have been affecting the undamaged areas of the hippocampus or they may have been acting on some other structure. In any case, neither region of the hippocampus, by itself, was indispensable for the drug effect.

Results for rats with large lesions involving both the anterior and the posterior hippocampus depended upon the drug and the behavioral task. Both scopolamine and atropine disrupted performance on delayed spatial alternation, but only scopolamine increased the go-no go alternation errors of Group A+P rats. Although Group A+P was not disrupted by the 0.50 mg/kg dose of scopolamine while the other groups were, it was disrupted by the 1.0 mg/kg dose. Atropine, in accord with Warburton's finding [15], failed to increase go-no go alternation errors in the group with large hippocampal lesions. It was not possible to examine the effects of higher doses of atropine sulfate because the rats ceased responding entirely when given doses larger than 12 mg/kg. However, the same dose of atropine that increased Group A+P spatial alternation errors had no effect on go-no go performance.

Although both scopolamine and atropine are competitive inhibitors of acetylcholine at muscarinic receptor sites, scopolamine is a more potent centrally active drug than atropine. It may be that cholinergic blocking drugs do disrupt go-no go alternation performance by acting on the hippocampus, as the atropine results suggest, but that scopolamine is more capable of affecting the small amount of hippocampal tissue left intact even in the animals with the largest lesions. It is also possible that in the intact animal the cholinergic blockers affect performance on this task by acting on the hippocampus, but that in animals with hippocampal lesions, another cholinergic brain region takes over mediation of the task.

The fact that both atropine and scopolamine disrupted spatial alternation performance of Group A+P rats suggests that the hippocampus is not the only mediator of the drug effect on this task. Direct injection of cholinergic blocking agents into other brain areas, the septal area for example [9], changes performance on behavioral tasks. It is likely that cholinergic blocking agents have their effects on most behaviors by acting on several brain areas, of which the hippocampus is only one.

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