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An Evaluation of the Mechanism of Scopolamine-Induced Impairment in Two Passive Avoidance Protocols

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ELROD, K. AND J. J. BUCCAFUSCO. *An evaluation of the mechanism of scopolamine-induced impairment in two passive avoidance protocols.* PHARMACOL BIOCHEM BEHAV **29**(1) 15–21, 1988.—The effects of several doses of the centrally-acting muscarinic antagonist, scopolamine, on subsequent learning and memory were examined using two versions of a standard light versus dark passive avoidance paradigm. The first protocol was employed primarily to examine the effects of scopolamine on the acquisition component of learning and memory as subject performance was measured during five successive (repeated) training trials. The second protocol employed a one-trial twenty-four hour retention task in which subjects were given one training trial followed by one testing trial twenty-four hours later. This latter test emcompasses acquisition, retention, and recall components of learning and memory. Dose response studies indicated an effective dose range of 0.4–1.2 mg/kg with 0.8 mg/kg producing maximal performance decrement. Differential scopolamine treatment demonstrated that the drug's primary effect was on the acquisition component only under the present experimental protocols. Furthermore, scopolamine was not found to produce state-dependent learning. Animals administered scopolamine before training and testing failed to perform better than animals receiving pre-training administration only.

Scopolamine Learning Memory acquisition Passive avoidance State dependency Rat

ACETYLCHOLINE has been implicated for many years as a primary neurotransmitter involved in the processes of learning and memory. Pharmacological manipulation of central cholinergic systems can produce significant changes in performance of several types of tasks. For example, administration of centrally-active cholinolytics produces profound effects on performance of a wide battery of behavioral tasks, depending upon the species of the animal, behavioral paradigm, drug, dose, and time of drug administration (relative to performance).

Heise [27] has reviewed the results of several studies on the effects of the cholinolytic, scopolamine, and other cognitive debilitating drugs in various species and in various behavioral paradigms. In most cases, a decrement in learning and/or memory of the study-specific task was afforded by scopolamine. However, the effect of scopolamine was not always directed at the same stage of the learning and memory processes. Such discrepancies with regard to the mechanism of anticholinergic action were noted in a recent review by Warburton and Wesnes [45] in which the authors, citing their own studies, observed a decrement by atropine on the input of information only (and not the process of memory storage). Yet in another study, these authors demonstrated an effect of scopolamine on information storage.

Other more recent studies employing atropine and/or scopolamine have shown disruption in eyelid conditioning in rabbits [25], acquisition and retention of object discrimination in marmosets [37], acquisition of spatial memory in the radial maze in rats [23, 28, 43, 46], acquisition of the Morris water maze in rats [48], acquisition of spatial working memory in rats [3, 15, 33], discrimination performance in a go-no go task in rats [31], and conditioned suppression in rats [5]. Pretreatment with anticholinergics has also yielded impairment in delayed matching-to-sample and delayed nonmatching-to-sample performance in primates [1, 36, 40]. In man, scopolamine administration has been shown to interfere with both verbal and nonverbal learning and memory [4, 10, 12, 17–19, 30, 34, 35, 38].

Passive avoidance is a behavioral task widely employed to assess learning and memory. Variations of this task afford a focus on one or more of the components of the learning and memory processes. The ability of scopolamine to impair passive avoidance learning and memory has been demonstrated often, although the precise nature of its action is still unknown [7, 9, 11, 13, 14, 21, 22, 24, 29, 31, 44, 47]. Evidence is conflicting, for example, concerning which components of passive avoidance learning and memory are affected by scopolamine. Results from several studies have indicated

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primary interference by scopolamine with either the acquisition component of learning [7, 9, 14], or with the acquisition and consolidation (or retention) components of memory [22]. At least one study has indicated impairment of the acquisition, retention and retrieval components of memory [21]. Debate also exists concerning the possibility that state-dependent learning [32] occurs in the presence of scopolamine [9, 21, 29, 42, 49].

The present study is the first, to our knowledge, in which two protocols have been employed to elucidate which components of passive avoidance learning and memory are influenced by scopolamine. The first protocol was designed to measure primarily acquisition, or training ability, of the passive avoidance response. The second protocol was the standard one-trial twenty-four hour task which encompasses all components of learning and memory. As test-specific responses are known to occur in behavioral testing, both the repeated acquisition and one-trial 24 hour retention tasks were employed to co-substantiate the results obtained with scopolamine administration. Multiple doses of scopolamine were employed for each protocol. Also, we examined the possibility that learning under the influence of scopolamine was state-dependent.

ANIMALS

Subjects were male 225–275 g outbred Wistar rats (Harlan-Sprague Dawley, Indianapolis, IN) housed in standard fashion on a twelve hour light-dark cycle (lights on at 7 a.m.) in a temperature constant (22°C) room. Animals had free access to food and water except during testing and were handled by the experimenter the day before training began. Animals were randomly assigned to treatment groups and were trained and tested in the same order.

DRUG ADMINISTRATION

Scopolamine hydrobromide (Sigma Chemical Co.) was dissolved in sterile normal saline. Drug solutions were prepared daily, kept light-protected, and administered in a volume of 0.1 ml/100 g. Numerous previous investigations have failed to find any effect of peripherally-acting anticholinergic agents such as methylscopolamine on performance of several behavioral tasks, including the passive avoidance task [2, 4, 6, 7, 11, 22, 29]. Thus, rats were injected subcutaneously with drug solution or saline vehicle in a room separate from where behavioral testing occurred. Thirty minutes was allowed after injection before training or testing began since the maximal response to scopolamine occurred at this time and, in fact, lasted for approximately two hours.

BEHAVIORAL APPARATUS

Rats were allowed at least fifteen minutes to adapt to the dark testing room each day before behavioral testing began. A standard shuttle cage (Coulbourn Instruments, Lehigh Valley, PA), 6 1/2″×14″×8″, was employed with a guillotine door dividing the cage into equal compartments, one of which was made darker and designated the unsafe, shocking side. A three minute acclimation period in the safe compartment was allowed for each rat to become accustomed to the cage before beginning the first training trial and the testing (retention) trial. A trial was initiated by automatically raising the guillotine door and turning on a light in the safe side. When the rat crossed over to the dark, unsafe side, the guillotine door was lowered and an inescapable 0.8 mA, 5 sec scrambled footshock was delivered through the grid floor.

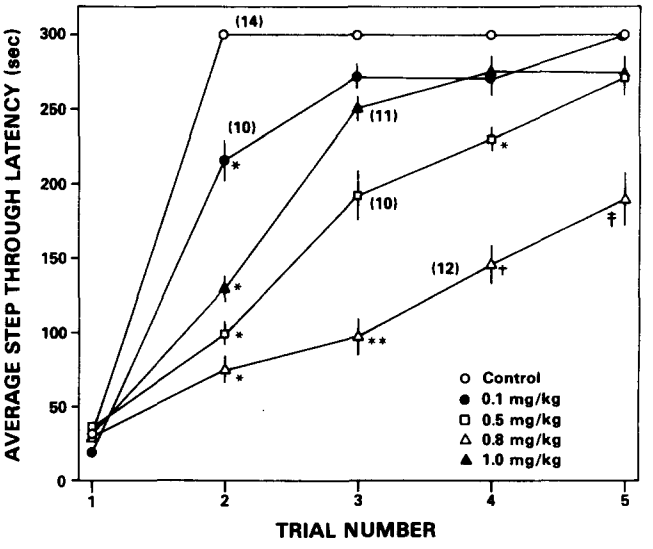

FIG. 1. Effect of scopolamine on test step-through latency during a five-trial repeated passive avoidance acquisition task. Results are shown as mean±S.E. Values in parentheses indicate the number of rats per group. *$p<0.05$ level of significance vs. control group. **$p<0.05$ level of significance versus control, 0.1 and 1.0 mg/kg groups. †$p<0.05$ level of significance vs. control and 1.0 mg/kg groups. ‡$p<0.05$ level of significance vs. control and 0.1 mg/kg groups.

The amount of time required for the rat to cross through to the dark side (step-through latency) was recorded automatically by an electronic timer. Any rat which did not cross over within two minutes during the first (or training) trial was eliminated from the study. Animals were considered to have learned the task if they remained in the safe, lit side for a minimum of 300 sec (cut-off latency).

STATISTICS

Parametric data are expressed as the mean±S.E. and means for several groups or treatments were compared using an analysis of variance (ANOVA), with Tukey's HSD test employed for *post hoc* analysis and the Mann Whitney U-test. Frequency data were compared using a Chi Square analysis. In both cases, data between groups were considered significantly different at the $p<0.05$ level.

EXPERIMENT 1

Method

Five groups of rats (10–15 per group) received either saline, 0.1, 0.5, 0.8 or 1.0 mg/kg scopolamine, respectively. Thirty minutes later, each animal was given five repeated trials for acquisition of the passive avoidance task. A five minute intertrial interval in a resting cage was allowed between each trial. A thirty second acclimation period was allowed prior to the beginning of each subsequent trial. Step-through latencies (sec) and the percentage of animals reaching the 300 sec cutoff (learning frequency) at each dose were recorded.

Results

Figure 1 and Table 1 illustrate the effect of pre-trial injec-

TABLE 1

EFFECT OF SCOPOLAMINE ON FIVE-TRIAL REPEATED ACQUISITION PASSIVE AVOIDANCE LEARNING FREQUENCY

	Percent Rats Learning Task				
		Dose (mg/kg)			
Trial No.	Control (saline)	0.1	0.5	0.8	1.0
1	—	—	—	—	—
2	100	70†	20†	17*	36†
3	100	90	60	25‡	82
4	100	90	70	42‡	91
5	100	100	90	58‡	91
N	14	10	10	12	11

All drugs administered 30 minutes prior to the first trial.
*=$p<0.05$ level of significance vs. control and 0.1 mg/kg groups.
†=$p<0.05$ level of significance vs. control group.
‡=$p<0.05$ level of significance vs. control, 0.1 and 1.0 mg/kg groups.

tion of scopolamine on test latencies and learning frequencies, respectively, for each dose across the five trials. Treatment with 0.5 mg/kg scopolamine resulted in a slightly higher, though significant, increase in first trial latency ($U=21, p<0.05$). Scopolamine was effective in disrupting the acquisition of the passive avoidance response measured either as a decrease in step-through latency, or as an increase in the frequency of avoidance failure. All doses were significantly different from control by the second trial ($U=49$, $p<0.0001$ for 0.1 mg/kg; $U=14$, $p<0.001$ for 0.5 mg/kg; $U=14$, $p<0.0001$ for 0.8 mg/kg; $U=28$, $p<0.001$ for 1.0 mg/kg and $\chi^2=25.34$, $p<0.0001$). The impairment was most marked at the second trial when a dose of 0.8 mg/kg was used ($U=14$, $p<0.001$ and $\chi^2=21.41$, $p<0.001$). During the third trial, a dose of 0.8 mg/kg produced latencies significantly lower than control ($U=17$, $p<0.001$), 0.1 mg/kg ($U=22.5$, $p<0.01$) and 1.0 mg/kg ($U=28.5$, $p<0.05$). In fact, only the dose of 0.8 mg/kg was significantly effective in maintaining disruption through the fifth trial ($U=37$, $p<0.01$ versus control; $U=40$, $p<0.05$ versus 0.1 mg/kg scopolamine; and $\chi^2=13.13$, $p<0.02$). A further increase in dosage (1.0 mg/kg) did not induce a greater performance decrement.

EXPERIMENT 2

Method

The purpose of this experiment was to rule out test-specific effects of scopolamine for the two versions of the passive avoidance paradigm which we employed, particularly with respect to the dose-response relationship. Four groups of rats (12–18 per group) received either saline, 0.4, 0.8 or 1.2 mg/kg scopolamine, respectively, thirty minutes before training. Following the training trial, animals were returned to a holding cage for at least ten minutes before being returned to their standard housing cages. Twenty-four hours later, rats were given one retention (testing) trial conducted in the same manner as before, except that a saline injection was administered as placebo to each rat. Step-through latencies and cumulative learning frequencies at each dose were recorded.

Results

Treatment with 0.4 mg/kg and 1.2 mg/kg scopolamine resulted in slight, though significant, increases in training step-through latencies ($U=50$, $p<0.05$ and $U=59.5$, $p<0.01$, respectively). The test latencies and learning frequencies are plotted in Fig. 2. All doses of scopolamine impaired performance in the twenty-four hour retention task ($U=54$, $p<0.001$ for 0.4 mg/kg; $U=36$, $p<0.0001$ for 0.8 mg/kg; $U=45$, $p<0.001$ for 1.2 mg/kg scopolamine; and $\chi^2=21.36$, $p<0.0001$). No dose response effect for scopolamine was observed; however, the lowest mean testing step-through latencies and learning frequency were observed in the group that received 0.8 mg/kg. As in Experiment 1, a further increase in dosage (1.2 mg/kg) was not more effective in disrupting performance in terms of step-through latency and learning frequency.

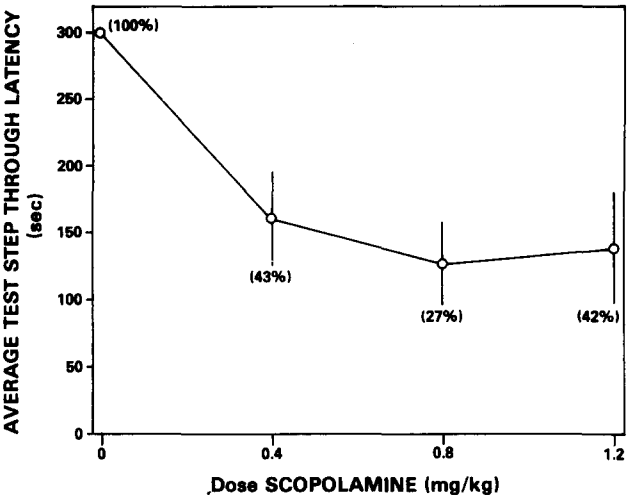

FIG. 2. Effect of scopolamine on test step-through latency in a one-trial twenty-four passive avoidance task. Results are shown as mean±S.E., n=12–18 per group. Values in parentheses indicate cumulative learning frequencies. Test latencies and learning frequencies for each dose of scopolamine were significantly lower than control values, $p<0.05$ level of significance. There were no significant differences between doses.

EXPERIMENT 3

Method

Three groups of rats (9–14 per group) received scopolamine treatment at different times relative to training and testing in a one-trial twenty-four hour retention task. All groups received a dose of 0.8 mg/kg. This dose was chosen based on the results of the first two experiments, which indicated that 0.8 mg/kg was a maximal dose producing a performance decrement. The one-trial twenty-four retention task was employed (as in Experiment 2) to assess the effects of differential scopolamine treatment on the acquisition, retention and recall components of learning and memory, respectively. The first group received drug treatment thirty minutes prior to training and saline thirty minutes prior to testing. The second group received drug immediately (within thirty seconds) after training. The third group received saline thirty minutes prior to training and drug thirty minutes prior to testing. Step-through latencies were recorded for each group. The control groups (n=10 per group) for the three experimental groups were pooled into one large control group since their performances (step-through latencies and learning frequencies) were not significantly different.

TABLE 2

EFFECT OF DIFFERENTIAL SCOPOLAMINE (0.8 mg/kg) TREATMENT ON ONE-TRIAL 24 HOUR PASSIVE AVOIDANCE LEARNING AND MEMORY

Scopolamine Treatment	Step-Through Latency (sec) Training	Testing	N
Control (saline)	16.55 ± 1.99	300.00 ± 00.00	30
30 min prior to training (acquisition)	21.69 ± 4.18	72.49 ± 15.69*	12
Immediately after training (retention)	23.64 ± 6.17	300.00 ± 00.00	9
30 min prior to testing (recall)	15.34 ± 3.44	300.00 ± 00.00	14

Values are expressed as mean ± S.E.
*=$p<0.001$ level of significance vs. other groups.

Results

There were no statistically significant differences in training step-through latencies between groups, $F(3,59)=8.33$, $p<0.4$. Table 2 summarizes the results obtained from Experiment 3. Of the three groups, only animals of the group that received scopolamine before training showed a performance deficit during testing ($U=28, p<0.001$ for control versus pretraining administration; $U=9, p<0.001$ for pretraining versus posttraining administration; and $U=14, p<0.001$ for pretraining versus pretesting administration). The groups receiving scopolamine after training or before testing performed as well as controls with regard to step-through latency, i.e., they all reached the cut-off latency and were considered to have learned the task.

EXPERIMENT 4

Method

Four groups of rats (15–17 per group) were used in a one-trial twenty-four retention task to examine the possibility that state-dependent learning occurred in the presence of 0.8 mg/kg scopolamine. A factorial 2×2 design was employed as follows: the first group (SALINE/SALINE) received saline before both training and testing; the second group (SCOPOLAMINE/SALINE) received scopolamine before training and saline before testing; the third group (SALINE/SCOPOLAMINE) received saline before training and scopolamine before testing; and, the fourth group (SCOPOLAMINE/SCOPOLAMINE) received scopolamine before both training and testing. All drugs or vehicles were administered thirty minutes before training or testing. Step-through latencies and cumulative learning frequencies were recorded.

Results

Training latencies for the SALINE/SALINE and SALINE/SCOPOLAMINE groups were slightly lower than training latencies for the SCOPOLAMINE/SCOPOLAMINE group ($U=45$ and $U=42$, $p<0.01$, respectively). Table 3 summarizes the results of Experiment 4. All animals trained in the absence of drug (SALINE) reached the cut-off latency during testing, regardless of whether testing occurred in the absence or presence of drug (SCOPOLAMINE). Animals receiving scopolamine before training exhibited a significant deficiency in performance during testing, which was reflected by (1) much lower testing latencies ($U=22.5$ and $U=45, p<0.001$ for SCOPOLAMINE/SALINE and SCOPOLAMINE/SCOPOLAMINE versus SALINE/SALINE, respectively; $U=21$ and $U=42, p<0.001$ for SCOPOLAMINE/SALINE AND SCOPOLAMINE/SCOPOLAMINE versus SALINE/SCOPOLAMINE, respectively); and (2) a lower frequency of rats learning the task $\chi^2=32.91$, $p<0.001$). The two groups trained in the presence of drug (SCOPOLAMINE/SALINE and SCOPOLAMINE/SCOPOLAMINE) did not differ, however, in their performances.

DISCUSSION

The possible selectivity of muscarinic antagonists on all components of learning has not often been addressed in a single study. The apparent discrepancies reported for state dependency and the involvement of various components of learning and memory in the action of scopolamine on passive avoidance behavior (see Introduction) may be related to slightly different training and testing paradigms, different doses of scopolamine, and the strain and age of animals employed. The present study is a first step towards eliminating many of these variables at least with respect to one classical paradigm of learning and memory.

Results from three studies in which single components of passive avoidance learning and memory have been examined have revealed that pre-training (acquisition) administration of scopolamine disrupted performance twenty-four hours later [11,24] and that post-training administration of scopolamine (retention) did not alter subject performance [47]. The results of our first three experiments clearly support these findings in that the effect of scopolamine occurred only when the drug was administered prior to training, thus suggesting an action directed primarily at the acquisition component, or the ability of the animal to learn the task. The results of the five trial repeated task (Experiment 1) indicate that the effect of scopolamine on passive avoidance performance does occur within five minutes of the first training trial. Processing of a stable memory trace has been reported to occur within this time period [20, 26, 41]. Therefore, this paradigm may not allow us to distinguish between an effect of scopolamine on acquisition and retention components. The results of the one-trial twenty-four hour task (Experiment 3), however, are not consistent with an action of the muscarinic antagonist on retention. In Experiment 3, scopolamine was able to disrupt performance only when

TABLE 3

LACK OF PRODUCTION OF STATE DEPENDENCY BY SCOPOLAMINE (0.8 mg/kg) IN ONE-TRIAL 24 HOUR PASSIVE AVOIDANCE LEARNING

Pretreatment (train/test)	Step-Through Latency (sec) Training	Testing	Percent Rats Learning Task	N
SALINE/SALINE	17.77 ± 1.95†	300.00 ± 00.00	100	15
SCOPOLAMINE/SALINE	38.29 ± 9.53	105.68 ± 29.33*	24‡	17
SALINE/SCOPOLAMINE	23.41 ± 8.69†	300.00 ± 00.00	100	15
SCOPOLAMINE/ SCOPOLAMINE	43.27 ± 6.99	164.00 ± 31.78*	40‡	15

Values are expressed as mean ± S.E. All drugs administered SC 30 minutes prior to training and testing.

*=$p<0.001$ level of significance vs. SALINE/SALINE and SALINE/SCOPOLAMINE groups.

†=$p<0.05$ level of significance vs. SCOPOLAMINE/SCOPOLAMINE group.

‡=$p<0.05$ level of significance vs. SALINE/SALINE and SALINE/SCOPOLAMINE groups.

administered prior to training. We observed no interference with performance when scopolamine was administered immediately (within 30 seconds) after training. The fact that significant blockade of brain muscarinic receptors would occur at this early time after subcutaneous scopolamine administration was demonstrated in parallel cardiovascular studies in our laboratory. Physostigmine is a centrally-active inhibitor of acetylcholinesterase, the catabolic enzyme of acetylcholine. Intravenous administration of physostigmine induces a centrally-mediated increase in blood pressure in the conscious rat, with an onset of approximately ten seconds [8]. Rats pretreated with 0.8 mg/kg scopolamine failed to respond to intravenous administration of physostigmine (in doses up to 0.3 mg/kg), injected two minutes later. Also, in saline-pretreated rats, we observed a near immediate (within one minute) reversal of the physostigmine-induced pressor response upon subcutaneous administration of 0.8 mg/kg scopolamine. To our knowledge, the exact temporal course for acquisition or consolidation of the passive avoidance response in the rat is not known at this time; however, the physostigmine experiments indicate that significant central muscarinic blockade occurs within a few minutes after scopolamine injection. Thus, we deduced from both experiments that acquisition was being influenced by scopolamine and that there seems to be no prominent effect on the information storage process. Lastly, we observed no effect of scopolamine on the recall component of memory, as pre-testing administration of the drug failed to induce a performance decrement.

The inability of doses of scopolamine higher than 0.8 mg/kg to produce further performance decrement in either protocol is not clear at this time. One possible explanation is that higher doses of scopolamine may block presynaptic muscarinic autoregulatory receptors to cause an increase in acetylcholine release. Scopolamine-induced release of acetycholine has been observed in the hippocampus and brainstem of cats [16]. In our studies, an increase in acetylcholine release induced by the higher doses of scopolamine could potentially counter the pharmacological blockade, resulting in less impairment than seen with the lower doses of scopolamine. An alternative possibility is that higher doses of scopolamine could impair motor activity of the animals, yielding longer latencies that would falsely indicate less severe disruption. The short training trial latencies argue against this possibility, however.

The results of the state dependency experiment (Experiment 4) clearly illustrate the lack of dissociative learning in the presence of scopolamine in the passive avoidance model. If symmetrical state-dependent learning were present, then the administration of scopolamine to rats before training and testing should have yielded a performance better than that observed in rats receiving scopolamine before training alone. One would expect that the performances of the SCOPOLAMINE/SCOPOLAMINE and SALINE/SALINE groups would be very similar. Likewise, if state-dependent learning was present, the SCOPOLAMINE/SALINE and SALINE/SCOPOLAMINE groups' performances should be similar. However, in neither case was this observed. Rats trained and tested in the presence of scopolamine did not perform better than rats in which drug was administered before training alone. Rats trained in the absence of drug performed equally well, whether or not drug was present during testing. Our results support those of previous reports which have indicated that scopolamine does not produce symmetrical dissociative learning [9,42].

Scopolamine-induced asymmetrical dependency has been reported [6]. In this case, retention was reported to occur in the conditions of NO DRUG/DRUG and DRUG/DRUG but not in the DRUG/NO DRUG condition. This type of dissociation was not observed in our experiments, as performance of DRUG/DRUG animals was not significantly different from that of DRUG/NO DRUG animals. Clearly, there is a lack of dissociation of learning by scopolamine in our studies.

Lastly, previous work has shown that administration of scopolamine up to doses of 2 mg/kg does not alter footshock sensitivity [39]. Also, we have observed no differences between saline- and scopolamine-injected rats' responses to tail flick testing of analgesia. Thus, the observed effects of scopolamine are not thought to be attributable to an alteration in sensory threshold.

In conclusion, our studies support the widely-growing body of evidence that a pharmacologically intact central cholinergic system is critical for passive avoidance learning to occur. Specifically, our results further substantiate the findings by other investigators that the muscarinic antagonist scopolamine influences primarily the acquisition component of the employed model of learning and memory. This effect

of scopolamine was evident in the performances of animals subjected to either protocol of inhibitory avoidance. The mechanism(s) of action by which scopolamine seems to preferentially influence acquisition is (are) not known. Lastly, our studies strengthen previous reports that scopolamine impairs acquisition of passive avoidance in a non-state-dependent manner.

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