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Different Temporal Effects of Serotonergic Antagonists on Passive Avoidance Retention

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ALTMAN, H J AND H J NORMILE *Different temporal effects of serotonergic antagonists on passive avoidance retention* PHARMACOL BIOCHEM BEHAV **28**(3) 353–359, 1987 —The experiments examined the effects of acute administration of three different serotonergic receptor antagonists (ketanserin, pirenperone and mianserin) on one-trial passive avoidance retention in mice Administration of each antagonist 30 min before training produced a dose-dependent impairment in retention In contrast, administration of each of the antagonists immediately after training produced a dose-dependent improvement in retention The time-dependent effects of pre- and post-train antagonist administration were assessed using pirenperone In both cases, the effects on test performance were determined to be time-dependent The results provide additional evidence suggestive of a differential role of the serotonergic nervous system in the processes underlying learning and memory

Memory Learning Serotonin Ketanserin Mianersin Pirenperone Receptor antagonist Inhibitory avoidance Mice

ONLY a few studies have examined the effects of acute serotonergic antagonist administration on learning and memory Interestingly, the combined data suggest that the serotonergic nervous system may play a differential role in the various phases thought to underlie the processing of information For example, cyproheptadine (but not methysergide) has been reported to impair retention of a one-trial inhibitory avoidance response in mice when administered 1 hr prior to training [3] In contrast, intrahippocampal infusions of mianserin immediately following training improved the performance of rats in a shock-motivated, brightness discrimination task [12] In previous reports from this laboratory, it has been suggested that the interference with serotonergic neurotransmission prior to retention testing facilitates retrieval of a previously learned aversive habit in mice This suggestion is based on the observation that administration of any one of a number of serotonergic receptor antagonists prior to testing of a previously learned inhibitory avoidance response (lick suppression task) elevated test latencies [1, 2, 8]. The antagonist-induced response appeared to be due to a direct effect on mnemonic processes since non-contingently shocked animals (administered shock outside the training apparatus and later tested under the influence of the highest dose of each antagonist) failed to exhibit a similar elevation in test latency scores

Interpretation of these results, however, is difficult due to differences in the types of tasks used as well as differences in the species employed The purpose of the present series of experiments was to compare and contrast the effects of pre- and post-train administration of serotonergic receptor antagonists in one species (mice) using the same behavioral task (lick suppression task). The lick suppression task was selected because this task has previously been used by this laboratory to assess the effects of pre-test administration of serotonergic receptor antagonists on memory [1, 2, 8].

METHOD

Animals

A total of 676 male Swiss Webster mice (28–35 g) were used The animals were obtained from West Jersey Biological Supply Farm (Wenonah, NJ) and arrived at 10 weeks of age The animals were not used in any experiment for at least 2 weeks following arrival. The mice were housed 4 per cage, maintained on a 12/12 hr light/dark cycle (lights on at 0700 hr) and allowed free access to food and water until the onset of the experiment, after which time the animals were placed on a limited drinking schedule Food was freely available throughout the experiments.

Behavioral Task

The behavioral task used was the lick suppression task [11] in which thirsty mice were trained to avoid drinking from a tube located in a dimly lit chamber. Retention of the original avoidance response was assessed 48 hr later under extinction (no shock) conditions.

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Apparatus

Animals were trained in 4 identical chambers (10 cm square, 6 cm high) constructed of black Plexiglas walls, a clear Plexiglas top and a stainless steel floor. A drinking tube was positioned 2 0 cm above the floor through one wall of the chamber Detection of drinking, recording of latencies, and administration of shock were accomplished using solid state programming and recording equipment

General Procedure

There were three phases to the behavioral procedure adaptation, training and testing Following 24 hr of water deprivation, mice were given an adaptation session in the lick suppression chamber during which time they were allowed to freely explore the apparatus and learn the location of the water spout The session was terminated when the animals completed a total of 5 sec of drinking (in all cases less than 50 sec). Following completion of the adaptation session, the animals were returned to their home cages and allowed free access to water for 2 hr During the training session 24 hr later, the mice were again permitted 5 sec access to the drinking tube, after which time a shock circuit was automatically activated and all subsequent contacts with the tube were punished In all of the experiments, training was terminated when the mice either failed to touch the tube for at least 60 sec or when the animals received the maximum number of shocks (see below for details) Any animal failing to complete 5 sec of drinking within 300 sec or that only received 1 shock during the training session (less than 1 0%) was discarded from the experiment In Experiment 1 the mice were trained using a relatively high shock level (2 0 mA, 7 shock maximum) These shock parameters were selected based on the extant literature and the experience of this laboratory. For example, pre-train administration of cyproheptadine has been reported to produce short test latencies when compared to control animals [3] This laboratory has found that the 2 0 mA shock level results in long test latencies (indicative of good memory) and thus allows the assessment of drug-induced retention impairments In Experiment 2 the mice were trained using a lower shock level (0.75 mA, 3 shock maximum) The 0 75 mA shock level results in short latencies (indicative of a weak memory) and allows the assessment of a drug-induced facilitation of memory Therefore, this shock level was used based on the extant literature [12] and preliminary data from this laboratory indicating that post-train antagonist administration facilitated passive avoidance performance.

Following training, all animals were returned to their home cages and given free access to water for 24 hr, followed by 24 hr of water deprivation. All animals were tested for retention of the original avoidance habit 48 hr later under extinction conditions. Retention was measured as the latency (sec) to complete 5 sec of drinking. Any animal failing to complete 5 sec of drinking within 2000 sec (ceiling) was removed from the apparatus and assigned a maximum test latency score of 2000

The only other procedural deviation from the above was the addition of specific groups of non-shocked (NS) control animals to Experiment 2. Animals in the NS control groups were placed into the lick suppression chamber on the training day and allowed to drink freely from the tube located in the chamber However, all subsequent contacts with the drinking tube beyond the initial 5 sec were not punished (i e , shocked). Instead, the animals were immediately removed from the apparatus, injected with one of the antagonists and returned to their home cages to await testing As with all other animals, animals in the NS groups were tested 48 hr following the training session

Drugs

All drugs were dissolved in 0 9% physiological saline (SAL) The drugs used for these experiments were. pirenperone dihydrochloride (PIREN), ketanserin tartrate (KETAN) and mianserin hydrochloride (MIAN) PIREN and KETAN were a gift from Janssen Pharmaceuticals, New Jersey MIAN was a gift from Organon International, Holland. Control animals received 10 ml/kg of 0 9% SAL All drugs were made fresh daily and administered intraperitoneally

Statistics

The behavioral results were expressed and analyzed as ordinal data The overall significance of the difference was calculated using the Kruskal-Wallis one-way analysis of variance (ANOVA) Post-hoc, individual pairwise comparisons were performed using the Mann-Whitney U-test (two-tailed) with the minimum acceptable level of significance set at $p<0$ 05

Experiment 1 Pre-Train Antagonist Administration

The purpose of Experiment 1 was to determine the dose- and time-dependent effects of *pre-train* serotonergic receptor antagonist administration on retention of an inhibitory avoidance habit

All mice were adapted, trained (2 0 mA, 7 shock maximum), and tested as described in the General Procedure section The dose-dependent effects of the antagonists were determined by injecting either SAL or one of several doses of KETAN (0 42, 0 56, 1 0, 4 2, 7 5 mg/kg), PIREN (0 1, 1 0, 1 3, 1 8 mg/kg) or MIAN (0 24, 1 0, 2 4, 5 6, 10 0 mg/kg) 30 min prior to training In addition, the training data were analyzed for potential drug-induced differences in the latency to drink and the number of shocks received during the training session. This analysis was conducted in order to determine whether changes in test performance could be directly attributed to drug-induced effects occurring during training (e g , altered shock sensitivity, illness)

The time-dependent effects of *pre-train* antagonist administration on retention were determined by injecting PIREN (1.8 mg/kg) at various times (15, 30, 45, 60, 90 min) before training and then assessing the performance of the animals (i e , latency to complete 5 sec of drinking) during the retention test 48 hr later

Experiment 2 Post-Train Antagonist Administration

The purpose of Experiment 2 was to determine the dose- and time-dependent effects of *post-train* serotonergic receptor antagonist administration on retention of the inhibitory avoidance habit

With the exception of the procedure for training the NS-trained mice described below, the procedures for adapting, training (0 75 mA, 3 shock maximum), and testing of the mice is as described in the General Procedure section. The dose-dependent effects of the various antagonists were assessed by injecting either SAL or one of several doses of KETAN (1 0, 5 6, 10 0 mg/kg), PIREN (0.56, 1.0, 3 2 mg/kg) or MIAN (1 0, 10 0, 13 0 mg/kg) immediately following train-

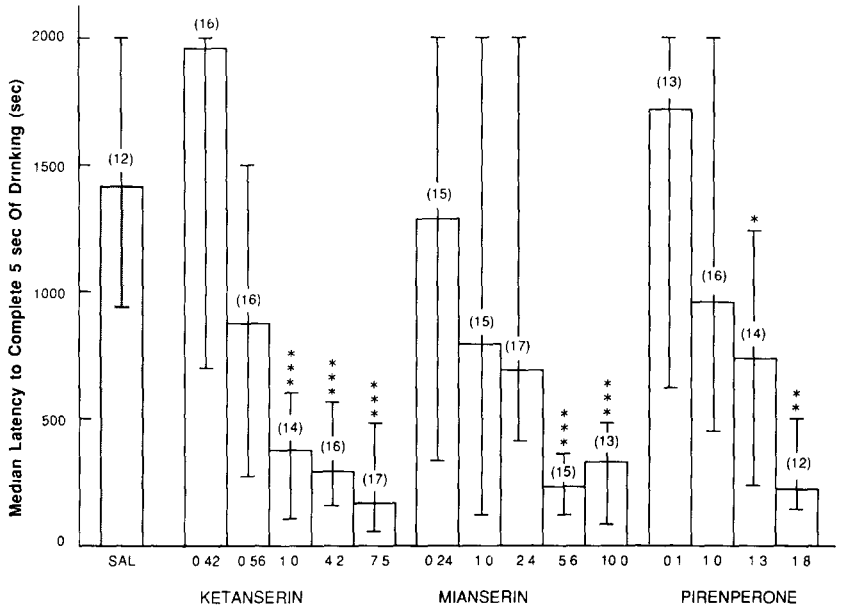

FIG 1 Median test latency (and interquartile ranges) to complete 5 sec of drinking for individual groups of mice injected with saline (SAL) or one of several doses (in mg/kg) of either ketanserin, mianserin or pirenperone 30 min before training Number of mice in each group indicated in parentheses $*p<0\ 05$, $**p<0\ 02$, $***p<0\ 002$ vs SAL

TABLE 1

EFFECTS OF PRE-TRAIN (30 MIN) SEROTONERGIC RECEPTOR ANTAGONIST ADMINISTRATION ON THE LATENCY TO COMPLETE 5 SEC OF DRINKING AND THE NUMBER OF SHOCKS RECEIVED DURING TRAINING

Drug (N)	Dose (mg/kg)	Median Latency (sec)	Range Q_1–Q_3	P vs SAL	Median No of Shocks	Range Q_1–Q_3	P vs SAL
SAL (12)	—	19 7	8 9–28 9	—	2	2 0–3 0	—
KETAN (17)	7 5	22 1	20 1–34 4	NS	3	2 0–3 7	NS
MIAN (13)	10 0	36 5	15 4–67 8	NS	2	2 0–2 7	NS
PIREN (12)	1 8	23 6	16 6–53 3	NS	2	2 0–3 0	NS

ing. In addition, three separate groups of NS-trained mice (one group for each drug studied) were included in order to determine whether the antagonist-induced response could be attributed to non-specific effects of the drugs on behavior in general (e.g., learned aversion) which could eventually confound the final interpretation of the data As with all other animals, mice in the NS-trained groups were injected immediately after the training session. However, unlike the rest of the mice in this experiment, the mice in the NS-trained group were only injected with the highest dose of the antagonists under investigation.

The time-dependent effects of *post-train* antagonist administration on retention were determined by injecting PIREN (3.2 mg/kg) at various times (0, 5, 10, 15 or 30 min) following training and then assessing the performance of the animals (i e., latency to complete 5 sec of drinking) during the retention test 48 hr later

RESULTS

Experiment 1 Pre-Train Antagonist Administration

Dose-response As can be seen from an examination of Fig. 1, administration of each of the antagonists 30 min prior to training resulted in a significant dose-dependent decrease in the latency to complete 5 sec of drinking in the lick suppression chamber during the retention test, KETAN $H(5)=35.95$, $p<0\ 001$; MIAN $H(5)=25.27$, $p<0.001$; PIREN $H(4)=14.30$, $p<0\ 01$ Post-hoc, pairwise comparisons revealed that the test latencies of the mice injected with the highest two doses of MIAN and PIREN and the highest three doses of KETAN were all significantly shorter than that of comparably treated SAL-injected controls

Analysis of the performance of the mice at training (i.e , latency to complete 5 sec drinking and the total number of shocks received) indicated that neither the latency to com-

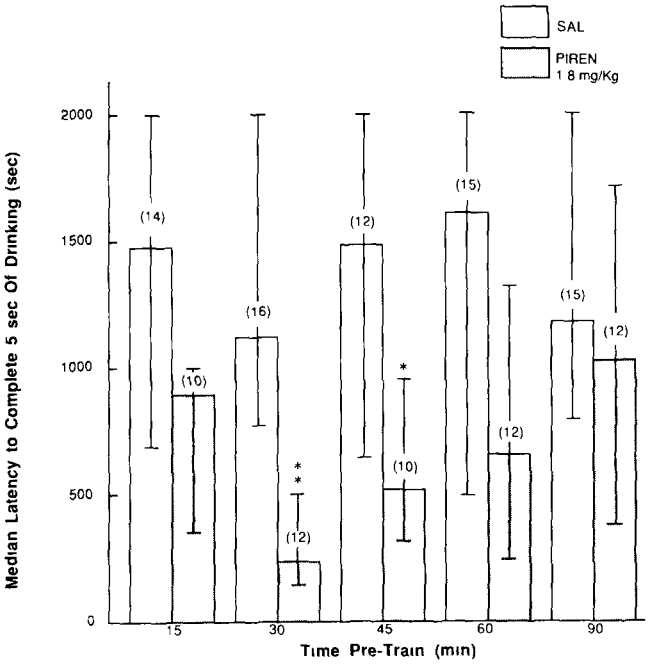

FIG 2 Median test latency (and interquartile ranges) to complete 5 sec of drinking for individual groups of mice injected with saline (SAL) or 1 8 mg/kg pirenperone (PIREN) at various times before training Number of mice in each group indicated in parentheses *$p<0$ 05, **$p<0$ 02 vs SAL

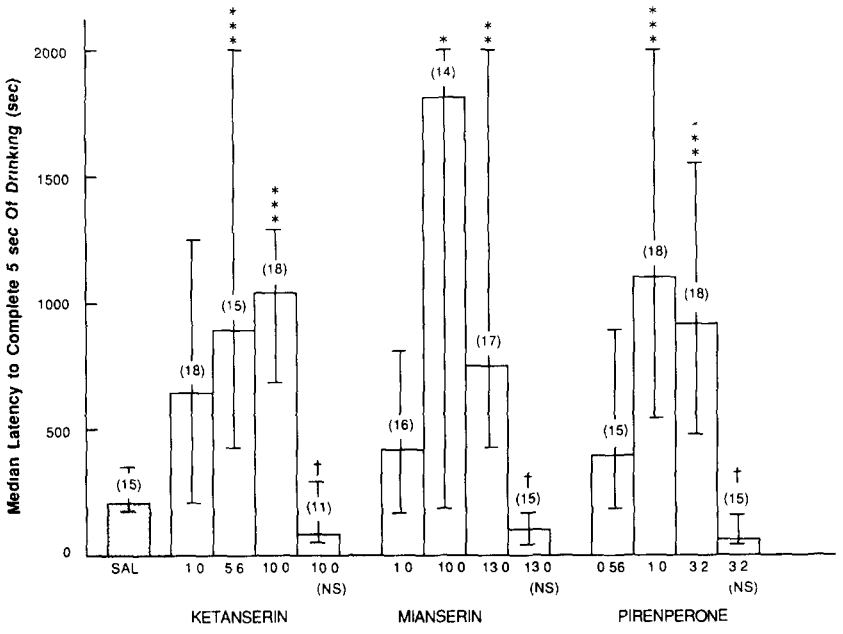

FIG 3 Median test latency (and interquartile ranges) to complete 5 sec of drinking for individual groups of mice injected with saline (SAL) or one of several doses (in mg/kg) of either ketanserin, mianserin or pirenperone immediately following training Number of mice in each group indicated in parentheses NS=non-shocked *$p<0$ 05, **$p<0$ 02, ***$p<0$ 002 vs SAL †$p<0$ 002 vs same dose administered to shocked mice

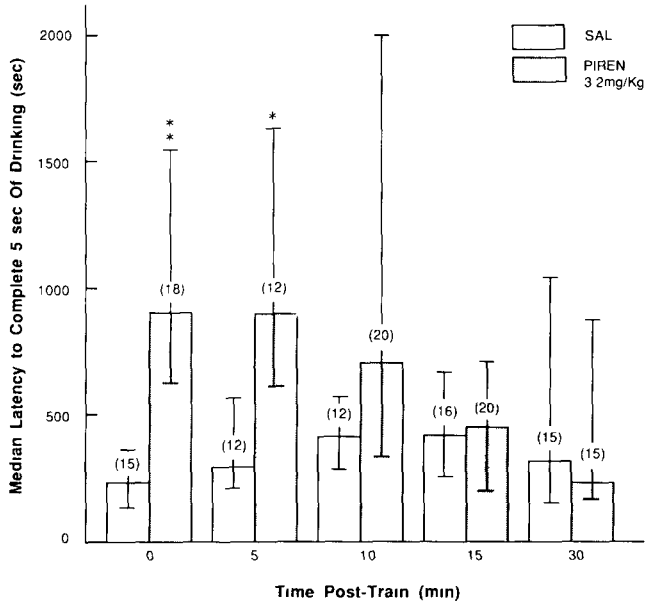

FIG 4 Median test latency (and interquartile ranges) to complete 5 sec of drinking for individual groups of mice injected with saline (SAL) or 3 2 mg/kg pirenperone (PIREN) at various times following training Number of mice in each group indicated in parentheses *$p<0$ 05, **$p<0$ 02 vs SAL

plete 5 sec of drinking nor the number of shocks received by the mice differed significantly between drug and SAL-injected animals (Table 1)

Time course The results of the time course study are depicted in Fig. 2 While an analysis of variance failed to support the conclusion that time of injection prior to training was a significant factor in the effectiveness of the drugs to affect subsequent test performance, $H(4)=5.97$, $p>0.05$, independent, post-hoc, pairwise comparisons did reveal that the performance of the mice was significantly different from comparably treated SAL-injected controls at both the 30 and 45 min time points. A likely explanation as to why the ANOVA failed to reach statistical significance probably has to do with the U-shaped nature of the curve That is, early and late effects probably cancelled out intermediate ones The subsidiary calculations were conducted in spite of the failure to demonstrate a significant main effect because it would be improper to disregard the performance of the animals at certain time points

Experiment 2 Post-Train Antagonist Administration

Dose-response As can be seen from an examination of Fig. 3, administration of each of the antagonists immediately after training resulted in a significant dose-dependent *increase* in the latency of the mice to complete 5 sec of drinking during the retention test· KETAN $H(3)=14.78$, $p<0.01$, MIAN $H(3)=10$ 10, $p<0.02$; PIREN $H(3)=13.81$, $p<0.01$ Independent, post-hoc, pairwise comparisons revealed that the highest two doses of each of the antagonists resulted in a significant elevation in the latencies of the mice to complete 5 sec of drinking compared to SAL-injected controls. The increased suppression of responding was not attributed to non-specific effects of the drugs on behavior in general, as the latencies of the NS-trained animals, injected with the highest dose of each drug studied, were significantly less than that of comparably treated shocked animals injected with the same dose of the drug

Time course The results of the time-dependent effects of post-train PIREN administration on subsequent test performance are depicted in Fig 4 Unlike the results of Experiment 1, a clearly significant difference in test performance was established as a function of the time between training and administration of the antagonist after training, $H(4)=10$ 54, $p<0$ 05 Independent, post-hoc, pairwise comparisons revealed, however, that only the latencies of the mice injected with PIREN either immediately after or 5 min after training significantly affected the performance of the mice 48 hr later during the retention test

DISCUSSION

The present study was conducted to further compare and contrast the effects of serotonergic receptor antagonists on retention of a one-trial inhibitory avoidance habit in mice The results suggest a differential effect on retention, depending on the times the drugs were administered with respect to training Administration of KETAN, PIREN or MIAN 30 min prior to training resulted in a dose-dependent *decrease* in the latency to complete 5 sec of drinking (amnesia) during the subsequent retention test In contrast, immediate post-train administration of these same drugs produced a dose-dependent *increase* in the latency to complete 5 sec of drinking (memory enhancement) The effects of pre-train drug administration on test performance could not be attributed to differences in response to the training parameters as the latencies to complete 5 sec of drinking and the total number of shocks received by the mice during training were not significantly different from comparably treated SAL-injected control animals Similarly, the effects of post-train drug administration could not be attributed to drug-induced learned aversion as the latencies of NS-trained animals were significantly different from shocked animals injected with the same dose of the drug The time-dependent nature of the effects of serotonergic receptor blockade on memory were assessed with PIREN The results of these studies indicated that the effects of both pre- or post-train receptor antagonists administration were time-dependent

Only a few studies have examined the effects of serotonergic receptor blockade on memory in animals The results are not, however, consistent For example, pre-train administration of cyproheptadine, but not methysergide, has been reported to impair retention of a one-trial inhibitory avoidance habit in mice [3] It is curious that both receptor antagonists did not have the same effect on memory for this task. However, other factors (e g., receptor affinity profiles, ability to cross the blood brain barrier) could have been responsible for the apparent differences In the present study, PIREN was only effective if it was administered within 45 min of training In the previous study both methysergide and cyproheptadine were administered 60 min prior to training It is possible, therefore, that the negative results with methysergide could have been due, in part, to differences in temporal factors

Other studies have found that pre-train administration of serotonergic receptor antagonists fail to affect avoidance retention [7,9]. However, in these studies the drugs were essentially used as tools to attenuate the behavioral effects

produced by procedures that augmented serotonergic activity Consequently, neither the dose- or time-dependent effects of the antagonists on their own were examined For example, metergoline (1.0 mg/kg) has been reported to block the effect of p-chloroamphetamine-induced serotonin release on one-way active avoidance learning in the rat [9] Metergoline had no effect on its own. However, only one dose of the drug at only one time point was examined Similarly, it has been reported that while methysergide (2 0 mg/kg) blocked the ECS-induced amnesia for a one-trial passive avoidance habit, the drug did not effect the performance of sham-ECS controls [7]. Again, only one dose of methysergide was examined and only at one time point (45 min prior to training)

Interpretation of the effects produced by pre-train administration of serotonergic receptor antagonists is further complicated by certain methodological problems inherent in studies that train animals under the influence of a drug [5,6] That is, the drug may effect performance by its action on processes other than those directly involved in learning and memory (e.g , nonspecific or non-associative effects). This problem was addressed, in part, within the present series of studies by examining the training latencies and the number of shocks received by drugged and non-drugged animals While there were no statistically significant differences in either parameter, it is virtually impossible to completely rule out all of the drug-induced non-specific factors (e g., motivational or perceptual) that may have contributed to the observed differences in the performance of the animals during the retention test. Another problem associated with attempting to evaluate the effects of the antagonists on mnemonic processes is presented by a possible establishment of "state dependency" [10] That is, the mice were under the influence of the drugs during training, but not during retention testing Therefore, the performance deficits observed when the animals are trained under the influence of the drug could be related to a difference in state between training and testing The approach commonly used to address this problem is to train and test the animals while under the influence of the drug [10]. However, as indicated previously, this laboratory has already reported that the administration of serotonergic receptor antagonists prior to retention testing appears to facilitate memory retrieval Therefore, the effects of pre-train vs. pre-test antagonist administration appear to be in opposite directions Consequently, combined pre-train and pre-test administration would appear to offer little in the way of additional clarity regarding the resolution of this issue

On the other hand, the present study demonstrates that post-train administration of serotonergic receptor antagonists significantly facilitates memory. Similar results have been reported in the past using aversively motivated tasks [1,12]. However, it should be noted that negative results have been reported for a positively reinforced task. For example, methysergide (5–10 mg/kg) injected either immediately or 2 hr after rats completed the first 4 choices in an 8-arm radial arm maze failed to affect performance [4] Taken together, these results suggest that the antagonist-induced response may be dependent on differences in the nature of the task (e g., reward contingencies, levels of processing, etc)

Post-train drug administration avoids many of the problems associated with evaluating the effects of drugs on performance as the animals are trained and tested under the same non-drugged state However, it is still possible that other factors such as the injection procedure or certain discriminable qualities of the drugs may have been responsible for the observed effects This would appear to be unlikely as NS-trained mice, injected with the highest dose of each of the antagonists studied, failed to significantly affect the latencies of the mice at testing.

Taken together, the results of the present study, combined with those of previous studies by this laboratory, suggest that serotonergic receptor blockade may differentially effect avoidance retention as a function of when the drugs are administered with respect to training and/or testing The retention deficit observed following pre-train administration of the serotonergic antagonists was likely not due to impaired learning since responding was suppressed during the training session in a fashion similar to SAL-injected controls Accordingly, pre-train antagonist administration may interfere with some post-learning memory process only if the drugs are present during or immediately after acquisition The effects of post-train antagonist administration would appear to argue against this interpretation as immediate post-train administration of the antagonists has the opposite effect However, it should be noted that the central effects of peripheral drug administration will take some time to develop As a result, post-train drug administration may fail to affect critical processes occurring immediately after training

An important issue to be resolved, therefore, would be to determine whether pre-train, post-train and pre-test administration of serotonergic antagonists differentially effect performance in other types of learning and memory tasks A systematic examination of the temporal effects of these drugs using a variety of behavioral situations should provide a more clear understanding of the role serotonin plays in learning and memory

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