Chinese Journal of Physiology 41(1): 33-44, 1998

Buspirone Impaired Acquisition and Retention in Avoidance Tasks: Involvement of the Hippocampus

Keng-Chen Liang, Kuo-Yu Tsui, Yih-Min Tyan and Tzu-Ching Chiang

Department of Psychology
National Taiwan University
Taipei 107, Taiwan, ROC

Abstract

This study investigated the effects of buspirone on acquisition as well as formation and expression of memory in three different types of avoidance tasks. Rats were trained and tested on a one-trial inhibitory avoidance task, an 8-trial active avoidance task or the Morris water maze. Buspirone (5.0 mg/kg) was administered subcutaneously 30 min before training, immediately after training or 30 min before testing. Retention was tested at various times after training. In the inhibitory avoidance task, pretraining injections of buspirone produced a marked impairing effect on retention, posttraining injections of buspirone produced a moderate but time-dependent memory deficit. Pretest injections of buspirone suppressed retention performance. Such an effect was more pronounced in the 1-day test than in the 21-day test. Intra-hippocampal infusion of buspirone (5.0 μg) before testing suppressed expression of the 1-day, but not the 21-day, memory. In the active avoidance task and the Morris water maze, an injection of buspirone before training or testing also impaired acquisition or suppressed retention performance. These findings suggest that buspirone given at various times could compromise acquisition, consolidation and retrieval of affective memory and the hippocampus was involved in the retrieval effect.

Key Words: anxiolytics, 5-HT_{1A} receptor, inhibitory avoidance, active avoidance, Morris water maze, memory, rats

Introduction

Cumulative evidence implicates the central serotonergic system in cognitive functions, particularly that of learning and memory (38). Manipulation of the serotonergic function alters performance in various aversive learning tasks (42, 43): Administration of p-chloro-amphetamine (PCA) significantly impaired learning in the active avoidance task (3), which may be due to the excessive release of serotonin caused by an early action of this drug (36). In the inhibitory avoidance task, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a selective 5-HT_{1A} agonist, impaired retention when given subcutaneously before training, 5 min after training or before testing (8). In the same task, posttraining infusion of 8-OH-DPAT into the lateral septum also caused a retention deficit (22). These findings suggest that the 5-HT_{1A} receptors are critical for memory processing of affective events, although the roles of other types of 5-HT receptors can not be excluded (4).

Buspirone, a partial agonist for the presynaptic or postsynaptic 5-HT_{1A} receptor (10), belongs to a new generation of anti-anxiety drugs and is less sedative and more resistant to tolerance and dependence than benzodiazepines (41). Animal studies have yielded evidence that buspirone possesses a strong anxiolytic effect in various anxiety-induced models such as the conflict test in pigeons (1), shock probe burying and elevated plus-maze tests in rats (40). Additionally, buspirone is able to suppress the action of various anxiogenic agents including the corticotropin releasing factor (Liang, unpublished data) or a conditioned stimulus previously paired with electric shocks (19).

Corresponding author: Dr. Keng Chen Liang, Department of Psychology, National Taiwan University, 1 Roosevelt Rd. Sec. 4, Taipei 107, Taiwan, ROC.

In view of its action on 5-HT_{1A} receptors which are implicated in affective learning and memory, buspirone should have a marked effect on acquisition or retention in aversive learning tasks. However, in contrast to the abundant results showing that benzodiazepines impaired learning and memory (11), evidence on such effects of buspirone is less conclusive. Early studies have reported that pretest systemic injections of buspirone blocked expression of conditioned fear in a potentiated acoustic startle task (19) and suppressed retention performance in an inhibitory avoidance task (35). Pretest injections of buspirone had no effect on the Morris water maze if the rat had mastered the task (35) but impaired working memory in a novel swimming task (2). Rats receiving pretraining injections of buspirone acquired the Morris water maze more slowly than the controls (29), but their retention of the inhibitory avoidance response appeared to be normal (35).

In all these studies, buspirone was administered before the memory task, which renders it very difficult, if not impossible, to rule out the influence of buspirone on sensory, motor or motivational functions as well as to dissociate the effects on acquisition and memory consolidation. A retention deficit caused by posttraining buspirone injections, which could strongly argue for an influence on memory processes per se, is yet to be shown. In view of the evidence that posttraining systemic injections or intra-septal infusion of 8-OH-DPAT impaired retention (8, 23), it would be of interests to investigate whether posttraining injections of buspirone likewise induced an impairing effect.

It is obscured where in the brain buspirone acts to affect conditioned fear or anxiety in various learning tasks. A theory has proposed that the septal-hippocampal system subserves, among others, anxiogenic functions on the basis of that many anxiolytics alter its neural activities (15). Perturbing hippocampal functions shortly after training affected retention in an inhibitory avoidance task (23). The hippocampus has a high density of 5-HT_{1A} receptors (20, 34), which could mediate the inhibitory effect of buspirone or other anxiolytics on innate or learned fear responses. Intra-hippocampal microinfusion of WAY 100635--a 5-HT_{1A} antagonist--attenuated a deficit induced by scopolamine in performing the Morris water maze (7) or that caused by transecting the fornix to retrieve a visuospatial memory (17). In view of these data, it would be important to examine whether intra-hippocampal infusion of buspirone affected memory in aversive learning tasks. The present study was designed to address all the issues raised above.

Materials and Methods

Subjects

Male Wistar rats weighing 300 to 350 grams were used in this study. After arriving from the National Breeding and Research Center for Experimental Animals, rats were housed individually and maintained at 21° to 25 °C with 50 % relative humidity. Food and water were available all the time. A 12:12 light:dark cycle was adopted with lights on at 7:00 a.m. throughout the study.

Surgery

Approximately one month after arrival, some rats were subjected to the stereotaxic brain surgery. Under sodium pentobarbital (50 mg/kg) anesthesia, 23 gauge stainless steel thin-wall cannulae (12 mm long) were implanted bilaterally into the dorsal hippocampus based on the coordinates: AP -3.0 mm from the bregma, ML ±3.0 mm from the midline, DV -3.0 mm below the skull surface. Two cortical screws serving as anchors were implanted over the right frontal and left posterior cortices. The cannulae were affixed on the skull with dental cement. A stylet was inserted into each cannula to maintain patency.

Behavioral Tasks

Inhibitory Avoidance

The apparatus was a trough-shape alley divided by a sliding door into a safe compartment lit by a 20 W light bulb and a shock compartment which was dark. The procedure, unless otherwise specified, followed that described elsewhere (27). The rat was placed into the lit side facing against the door. As the rat turned around, the door was opened. After the rat stepped into the dark compartment, the door was closed and an inescapable footshock (1.0 mA/1.0 s) was administered to the floor by a constant current shocker connected to a timer (Lafayette Instruments, Model 80240 and Model 58010, Lafayette, IN). The shock intensity was calculated as the root mean square of the sinusoidal alternating current.

After shock administration, the rat was removed from the alley and returned to his home cage. In the retention test given 1 or 21 days later, the rat was reintroduced into the alley and its latency to step into the dark compartment was taken as a retention score. If a rat did not step through in 10 min, the test was ended and a ceiling score of 600 was assigned.

Active Avoidance

The one-way active avoidance training followed the procedure of a previous study (25). The apparatus

was the same alley described above. The rat was placed in the dark compartment, facing toward the door. The door was then opened and 10 seconds later, a 0.6 mA footshock was delivered onto the floor in the dark side. To escape from the shock, the rat had to run into the lit safe compartment. An avoidance response was scored if the rat ran into the lit side before the shock onset. The rat stayed there for the 30 s inter-trial intervals. If the rat failed to escape within 30 s after the shock onset, it was placed into the lit side by the experimenter. Rats received 8 such trials on training and also on testing given at various times later. Performance was indicated by the number of avoidance responses made in the 8-trial training or testing session.

Morris Water Maze

The task, as described in a former study (24), was performed in a circular plastic pool (224 cm diameter, 46 cm height) located in a room with distinctive visual cues. Water was filled to a depth of 36 cm and a transparent plastic platform (25×25 cm^2, 32 cm height) was located at the center of a fixed quadrant beneath the water level. Training started by acclimating the rat to the task environment with 2-min free swimming in the pool for two days. Rats then received 4 daily training trials consecutively for 6 days. On each trial, the rat entered the water randomly from one of the four quadrants. The rat had to swim until it climbed onto the platform submerged in the water. The escape latency, which equals to the duration from entering water to reaching the platform, was taken as a measure of acquisition/retention performance. If the rat failed to reach the platform by 120 s, it was picked up and placed onto the platform by the experimenter. The rat stayed on the platform during the 60 s inter-trial interval. On the test session, four similar trials were given and followed by a probe trial, in which the hidden platform was withdrawn from the pool and the rat was given 2 min of free swimming in the pool. The amount of time spent in searching of the quadrant which previously contained the target platform and those which did not was recorded.

Drugs and Drug Administration

Buspirone hydrochloride was obtained from Sigma (St. Louis, MO) and dissolved into a vehicle to the appropriate concentrations. The vehicle for systemic injections was distilled water and that for the central infusion was a specific brain buffer which in 100 ml contained 0.9 g of NaCl, 4.5 ml of 0.2 M Na_2HPO_4, and 0.95 ml of 0.2 M $NaH_2PO_4 \cdot 2H_2O$. Concentration was calculated as the salt weight. Buspirone was injected either subcutaneously at a dose of 5.0 mg/kg or infused into the dorsal hippocampus in 5.0 μg.

The intra-hippocampal infusion device was constructed as follows: A piece of 0.5 m polyethylene tubing (PE-20, Clay Adams) connected to a 10 μl Hamilton microsyringe on one end and cemented to a 30 G dental needle on the other. The syringe and the tubing were first filled with distilled water. The drug solution was then introduced into the injection needle and separated by a tiny air bubble from the distilled water. Drug infusion was administered to a conscious rat with care not to stress the animal. To facilitate diffusion of drugs, the infusion needle protruded 1.5 mm beyond the tip of the cannulae. Bilateral infusion was administered through a microinfusion pump (CMA/100, Carnegie Medicin, Stockholm) at a rate of 0.5 μl per minute. The infusion volume in each side was 1.0 μl. After infusion, the needle stayed in the cannula for an additional minute before withdrawn and the stylet was immediately replaced to prevent back flow.

Histological Verification

At the conclusion of the last experiment, rats were sacrificed with an overdose of sodium pentobarbital (50 mg per rat, i.p.) and perfused through the heart with saline and then 10 % formalin. The brain was removed, stored in formalin for at least 48 hrs and was later sectioned into 40 mm slices and stained with cresyl violet. Placements of cannulae were examined and the location of cannula tips was recorded on series of coronal plains according to the brain atlas by Paxinos and Watson (33).

Results

Effects of Pretraining, Posttraining and Pretest Systemic Injections of Buspirone on Acquisition, Retention and Memory Expression in the Inhibitory Avoidance Task

Six groups of rats were trained on the task and four of them were tested on both 1 and 21 days after training. They received vehicle or buspirone at one of the following times: 30 min before training, immediately or 4 hrs after training. Rats receiving vehicle at various times showed comparable retention, they were thus pooled into a single control group. The remaining two groups received injections of buspirone 30 min before testing and were tested only on 1 or 21 days (but not both) after training. Retention scores for the five groups tested 1 day after training are shown in Figure 1 (the dotted pattern bars). Rats given buspirone before training or testing had poor retention. Immediate posttraining buspirone injections induced a moderate deficit, but the delayed

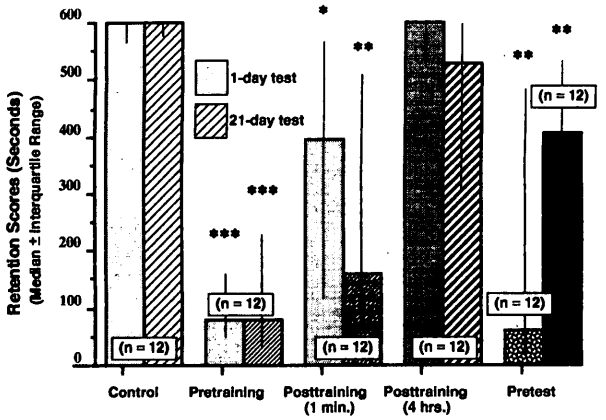

Fig. 1. Effects of pretraining, posttraining and pretest injections of buspirone on 1-day retention (the dot pattern bars) and 21-day retention (line pattern bars) in the inhibitory avoidance task. Rats in the control, pre- or posttraining injected groups received two tests on 1 day and 21 days after training, while the two pretest injection groups were tested either 1 or 21 days after training, but not both. Number of subjects in each group is denoted in the parentheses. ***p < 0.001, **p < 0.01, *p < 0.05 different from the correspondent controls.

posttraining treatment had a negligible effect. A Kruskal-Wallis one-way ANOVA revealed significant differences among various groups ($H'(4) = 29.7$, $p < 0.0001$). Paired comparisons by Mann-Whitney two-tail U-tests showed that rats given buspirone before training showed significant lower retention scores than the controls ($U = 2$, $p < 0.0001$). Rats receiving buspirone immediately after training also had significant lower retention scores than the controls ($U = 24$, $p < 0.005$) and also rats given buspirone 4 hrs after training ($U = 26$, $p < 0.01$). However, retention scores of the immediate posttraining group were significantly higher than those of the pretraining group ($U = 33$, $p < 0.05$). Buspirone injected before testing suppressed expression of memory: Retention performance of the pretest group was lower than that of the controls ($U = 22.5$, $p < 0.005$).

To examine whether buspirone given around training induced persisting effects, all rats except those in the pretest injection group were tested repeatedly on the 21st day after training. Another group of rats trained 21 days ago were tested simultaneously for the first time with all other groups. This new group received an injection of buspirone 30 min before the 21-day test. The 21-day retention scores are shown in Figure 1 (line hatched bars): The effects induced by pre- or posttraining buspirone injections observed in the 1-day tests persisted in the 21-day test. A Kruskal-Wallis one-way ANOVA revealed significant differences among the groups ($H'(4) = 25.6$, $p < 0.0001$). According to the Mann-Whitney two-tail U-tests, rats given buspirone before training showed significant lower retention scores than controls ($U = 7.5$, $p < 0.0001$). Rats receiving buspirone immediately after training also had significantly lower retention scores than both the controls ($U = 22.5$, $p < 0.005$) and rats receiving buspirone 4 hrs after training ($U = 32.5$, $p < 0.05$). Buspirone injected before the 21-day test suppressed memory expression: Retention scores of the pretest group were somewhat lower than those of the controls ($U = 22.5$, $p < 0.01$). A comparison between performance in the 1- and 21-day tests showed that pretest injections of buspirone seemed to be less effective in the 21-day test than in the 1-day test, although the difference only approached statistical significance ($U = 40.5$, $0.05 < p < 0.10$).

Effects of Pretraining Injections of Buspirone on Acquisition and Retention of An Active Avoidance Response

This experiment examined the effects of buspirone given before training on acquisition and retention in the active avoidance task. The number of avoidance responses made on the training and testing days are shown in Figure 2. Pretraining buspirone injections retarded acquisition as well as retention in the active avoidance task. The data were analyzed by a 2 × 2 (Drug × Day) repeated measure design ANOVA, with "Drug" as the between-subject variable and "Day" as the within-subject variable. The analysis revealed a significant "Drug" main effect ($F(1, 18) = 22.18$, $p < 0.0002$), suggesting that pretraining buspirone injections reduced the avoidance frequency on both training and testing days. The "Day" main effect was also significant ($F(1, 18) = 138.89$, $p < 0.0001$), suggesting that both the control and drug groups had significantly more avoidance responses on the testing day than on the training day. However, a significant

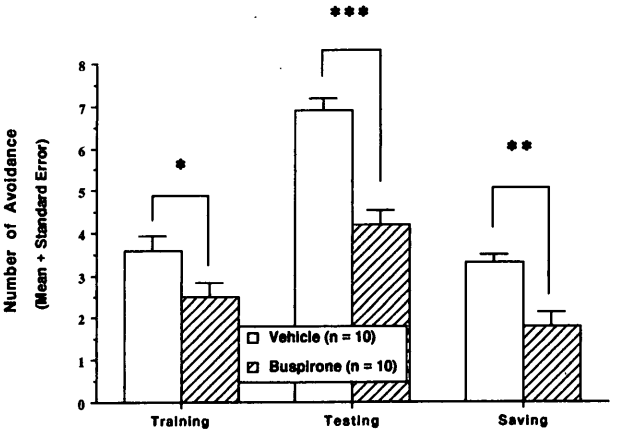

Fig. 2. Effects of pretraining injections of buspirone on the number of avoidance responses at training and 1-day testing in the active avoidance task. The saving score equals to the number of avoidance on the testing day minus that on the training day. ***p < 0.001, **p < 0.01, *p < 0.05.

"Drug × Day" interaction effect ($F(1, 18) = 14.22$, $p < 0.0014$) indicated that the controls showed more improvement from the training day to the testing day than the drug group. A saving score, which equals to the difference in the number of avoidance responses between the training day and the testing day, was used as an index of the memory strength after one day of training. The control group had significantly higher saving scores than the group given buspirone before training ($t(18) = 3.84$, $p < 0.002$).

Effects of Pretest Buspirone Injections on Expression of the Active Avoidance Memory

To reveal whether pretest injections of buspirone had any long-lasting effects on the memory trace, a complete within subject design was adopted to assess the effect of pretest injections of buspirone on retention in an active avoidance task. Briefly, a rat was tested repeatedly on 1 and 2 days as well as 21 and 22 days after training. In the 1/2 test session, half of the rats received vehicle and the other half received buspirone 30 min before the first day test, treatments were crossed over for the two halves on the second day test. The same counterbalance procedure was also applied to the 21/22 test session.

Results are shown in Figure 3. In both test sessions, the vehicle-treated rats made more avoidance responses than rats given pretest buspirone. The data were analyzed by a 2 × 2 × 2 (Order × Retention Interval × Drug) repeated measure design 3-way ANOVA, with "Order" as a between subject variable and the remaining two factors as the within subject variables. The analyses revealed that the "Order" main effect was not significant ($F(1, 18) < 1$). The "Drug" main effect was statistically significant

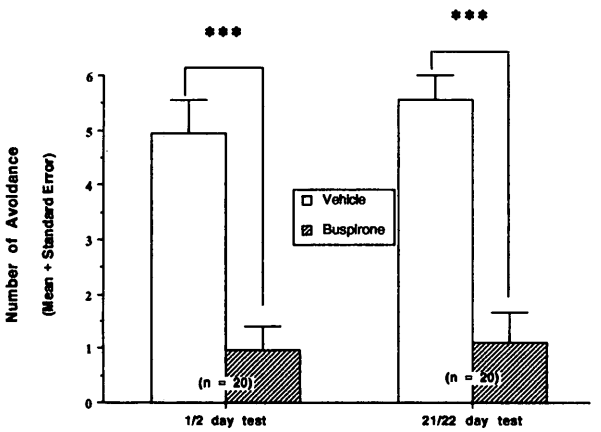

Fig. 3. Effects of pretest injections of buspirone on the number avoidance response in the 1/2-day and 21/22-day retention tests of the active avoidance task. Each subject was tested 4 times (1, 2, 21 and 22 days after training) and injected with vehicle in two of the tests and with buspirone in the other two. ***p < 0.001.

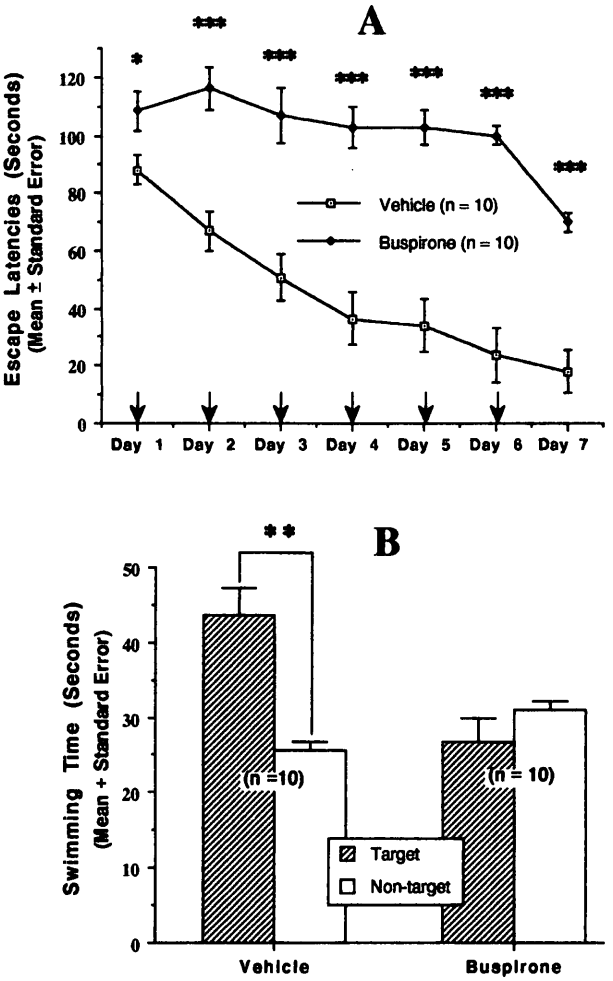

Fig. 4. Pretraining injections of buspirone impaired acquisition and retention in the Morris water maze. Panel A shows the effects on escape latencies in both the training and testing days. Arrows denote the days on which buspirone was injected before training. Panel B shows the effects on the mean searching time within the target and non-target quadrants in the probe test trial on the testing day. ***p < 0.001, **p < 0.01, *p < 0.05.

($F(1, 18) = 45.50$, $p < 0.0001$), but neither the "Retention Interval" main effect nor any interaction effect was significant, suggesting that regardless of the order of treatments, rats always had better performance after a vehicle injection than after a buspirone injection in both the 1/2 test and 21/22 test sessions.

Effects of Pretraining Buspirone Injections on Acquisition and Retention in the Morris Water Maze

Two groups of rats were trained on the Morris water maze for 6 days. One group of rats received buspirone 30 min before each daily training session, while the other group received vehicle. On the seventh day, both groups were tested with no injection. The performance is shown in Figure 4A. The control

group showed improvement over 6 days of training. Pretraining injections of buspirone severely impaired learning and yielded an almost flat acquisition curve. The differences in the daily performance between the control and the buspirone groups were analyzed by planned F-tests. The analysis revealed that the control group had significantly better performance than the buspirone group on each of the training day ($F(1, 18) = 4.71, 40.57, 28.38, 37.29, 47.17$, and 84.17; $p < 0.05$ for the Day 1 comparison, and $p < 0.0001$ for all other comparisons). Further, on the test day when neither group received injection, the control group still showed better performance than the group previously treated with buspirone ($F(1, 18) = 33.38$, $p < 0.0001$), suggesting that the treatment did induce a retention deficit.

The retention deficit was further assessed by a probe trial given on the 7th day. The data shown in Figure 4B indicated that the control animals searched more in the target quadrant than in the non-target quadrants, the difference between the mean searching time in non-target quadrants and that in the target quadrant was statistically significant ($t(9) = 14.21$, $p < 0.01$). On the other hand, rats previously receiving buspirone showed little preference between the target and non-target quadrants, the difference in the mean searching time was not statistically significant ($t(9) = 0.99$, $p > 0.1$).

Effects of Pretest Buspirone Injections on Memory Expression in the Morris Water Maze

Two groups of rats were trained, one was tested on 1 and 2 days after training, while the other was tested on 21 and 22 days after training. Within a two-day (1/2 or 21/22) test session, rats received injections of vehicle and buspirone on separate days, with the treatment order counterbalanced as described in the previous experiment. The escape latencies in the two test sessions are shown in Figure 5A. The figure shows that buspirone given before testing blocked memory expression in the water maze, but the difference between the control and buspirone groups was more conspicuous in the 1/2 test session than in the 21/22 test session.. The data were evaluated by a $2 \times 2 \times 2$ (Order × Retention Interval × Drug) repeated measure design 3-way ANOVA, with "Order" and "Retention Interval" as the between subject variables and "Drug" as the within subject variable. Only the "Drug" main effect was statistically significant ($F(1, 32) = 8.26$, $p < 0.01$). Paired comparisons by dependent-t tests indicated that in the 1/2 test session rats given buspirone took significantly longer time to find the hidden platform than the controls ($t(18) = 33$, $p < 0.005$). While the same trend also appeared in the 21/22 test session, the difference failed to reach

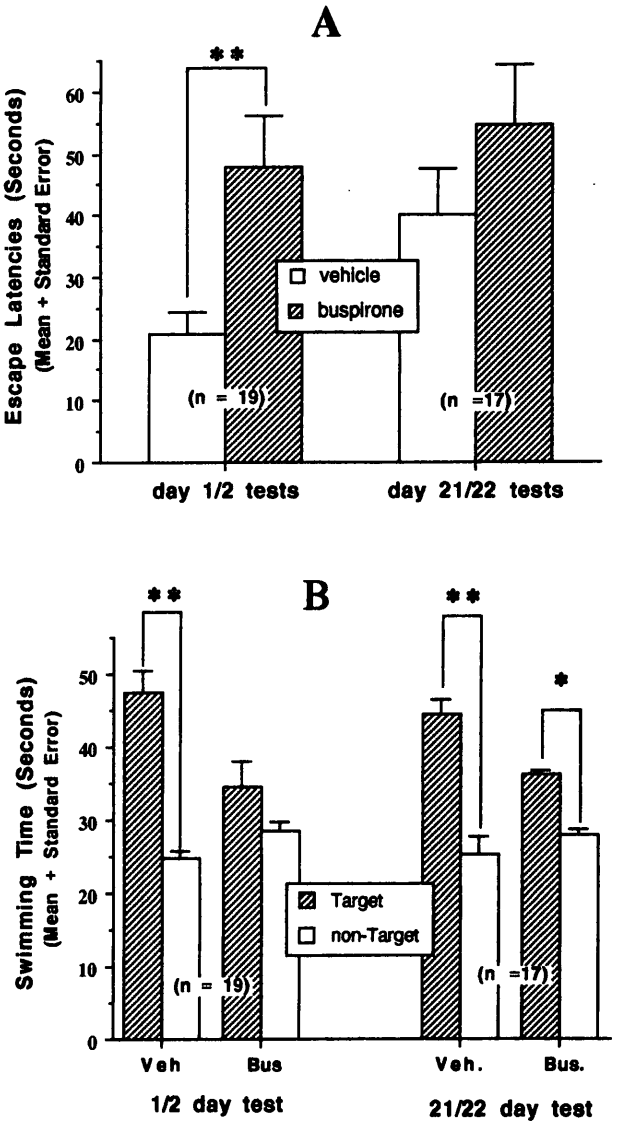

Fig. 5. Effects of pretest injections of buspirone on escape latencies in a regular test trial (Panel A) and on the mean searching time within the target and non-target quad-rants in a probe test trial (Panel B) on the 1/2-day or 21/22-day tests in the Morris water maze. $^{**}p < 0.01$, $^{*}p < 0.05$.

statistical significance ($t(16) = 1.2$, $p > 0.10$).

Figure 5B shows the effect of buspirone assessed by a probe test. In both test sessions, rats receiving vehicle spent more time in exploring the target quadrant than the non-target quadrants. For the buspirone groups, the time difference between searching the target and non-target quadrants was less pronounced. A preliminary analysis found no significant effect of the treatment order, thus the data were collapsed on the "Order" factor. Planned comparisons by t-tests showed that in the 1/2 test session, the difference between the mean searching time in non-target quadrants and that in the target one was statistically significant ($t(18) = 5.7$, $p < 0.0001$),

but that for the buspirone-treated rats was not ($t(18) = 1.3$, $p > 0.1$), implying that buspirone impaired performance effectively in the 1/2 test session. On the other hand, in the 21/22 test session, both the vehicle and buspirone groups spent more time to search the target quadrant than the non-target ones ($t(16) = 6.8$ and 2.4; $p < 0.0001$ and 0.03, respectively), indicating substantial memory for the target position in both groups.

Pretraining Injections of Buspirone Affected Processing of Affective Information in the Inhibitory Avoidance Task

The consistent results from the different tasks suggested that buspirone may compromise a component common to all the adopted tasks–processing of affective information. To pursue this possibility further, three groups of rats were subjected to training on the inhibitory avoidance task with a modified two-day procedure. On the first day, rats were allowed to explore the alley for 2 minutes without receiving any shock, supposedly from which the rat would learn the spatial configuration of the apparatus. On the second day, the rats were placed directly into the dark side of the alley and received a 1 mA/1 s shock to acquire affective significance of the dark compartment . One group received vehicle before each phase of training. A second group received buspirone before spatial training and vehicle before affective training. The third group received vehicle before spatial training and buspirone before affective training. Training commenced 5 min after the injection. Retention tested on the third day is shown in Figure 6. Rats given vehicle on both phases of training showed good retention that was comparable to previous results. Buspirone had a slight effect if injected before spatial training but had a profound effect if injected before affective training. A Kruskal-

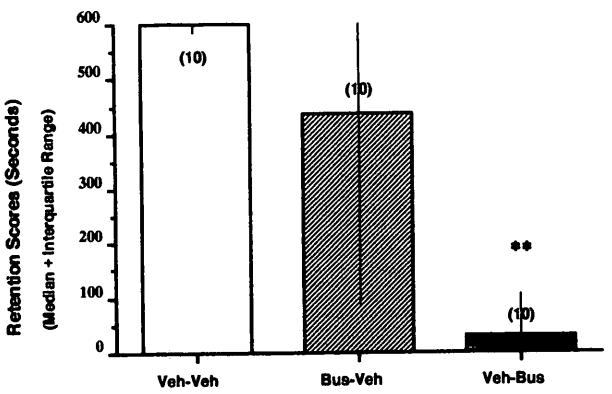

Fig. 6. Effects of injecting buspirone at the spatial training phase (denoted as Bus-Veh) or the affective training phase (denoted as Veh-Bus) on later retention. **p < 0.01, different from the control (denoted as Veh-Veh) group.

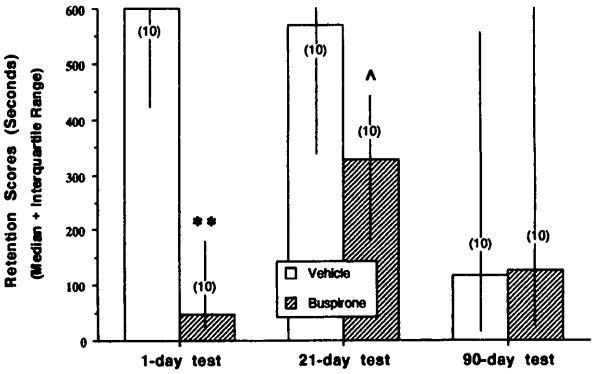

Fig. 7. Effects of pretest injections of buspirone on performing the inhibitory avoidance task in tests with 1, 21, or 90 days of retention intervals. **p < 0.01; ^p < 0.05 (one-tail) different from the correspondent Vehicle group.

Wallis one-way ANOVA revealed significant differences among the groups ($H'(2) = 10.65$, $p < 0.005$). Further paired comparisons indicated that the rats receiving buspirone at the affective training phase had retention significantly worse than the vehicle controls ($U = 14$, $p < 0.005$), but rats receiving buspirone at the spatial training phase were not significantly different from the vehicle controls ($U = 34$, $p > 0.1$).

Effects of Pretest Injections of Buspirone in Tests with Various Retention Intervals

Results from two above experiments showed that pretest buspirone administration appeared to be more potent in suppressing expression of a 1- or 2-day memory than a 21- or 22-day memory. To further confirm this finding, the length of retention intervals was formally manipulated and the effects of pretest injections of buspirone on memory retrieval were examined at various times after training. Six groups of rats trained on the inhibitory avoidance task were tested 1, 21 or 90 days after training. Prior to each test, one group of rats received vehicle and the other group received buspirone. The retention scores for various groups are shown in Figure 7. The results replicated previous findings: Pretest injections of buspirone induced a profound deficit of memory retrieval in the 1-day retention test. The buspirone-treated rats had performance significantly lower than the correspondent controls ($U = 9.5$, $p < 0.005$). The same treatment produced a less prominent effect in the 21-day test: The buspirone-treated rats did show substantial retention, but which was still significantly inferior to that of the correspondent controls ($U=27$, $p<0.05$ one tail). Rats given buspirone in the 21-day test showed retention scores significantly better than those treated alike in the 1-day test ($U = 8$, $p < 0.001$). In the 90-day test, retention performance

of the controls deteriorated greatly, and did not significantly differ from the low retention scores of the buspirone-treated group ($U = 49$, $p > 0.1$).

The State-Dependency of the Buspirone Effect

To assess whether state-dependency was involved in the observed effects, vehicle (Veh) or buspirone (Bus) was administered before both training and testing in this experiment. Four groups of rats were given one of the following pretraining/pretest treatments: Veh/Veh, Veh/Bus, Bus/Veh, Bus/Bus. The results shown in Figure 8 replicated previous findings, buspirone given before training or testing alone impaired retention. Moreover, buspirone given before both training and testing still produced poor retention performance. A Kruskal-Wallis one-way ANOVA revealed a significant difference among the groups ($H'(3) = 13.4$, $p < 0.005$). Further paired comparisons indicated that the control group had better retention performance than the groups given buspirone before testing, before training, or before both training and testing (Veh/Veh group vs. Veh/Bus, Bus/Veh & Bus/Bus groups, $U = 15$, 51 & 54, $p<0.001$, 0.05 & 0.05, respectively). The three latter groups did not differ among themselves.

Effects of Pretest Intra-Hippocampal Infusion of Buspirone on Retention Performance in the Inhibitory Avoidance Task

To investigate the central site on which buspirone might act to affect expression of memory, four groups of rats bearing indwelling cannulae in the dorsal hippocampus were trained on the inhibitory avoidance task. Two groups were tested 1 day after training and the other two were tested 21 days after training. In each test, rats received bilateral intra-hippocampal infusion of either vehicle or 5.0 μg buspirone 5 min prior to the testing. Results are shown in Figure 9, which indicated that pretest intra-hippocampal infusion of buspirone impaired performance significantly in the 1-day retention test but had no effect on performance in the 21-day test. The Mann-Whitney two-tail U-tests showed that in the 1-day retention test, the buspirone-treated rats had significantly lower scores than the control rats ($U = 45$, $p < 0.05$). On the other hand, the two groups in the 21-day test did not differ ($U = 40$, $p > 0.10$). The distribution of cannula tips in the hippocampus for the buspirone-infused rats is shown in Figure 10 based on the brain atlas by Paxinos and Watson (33).

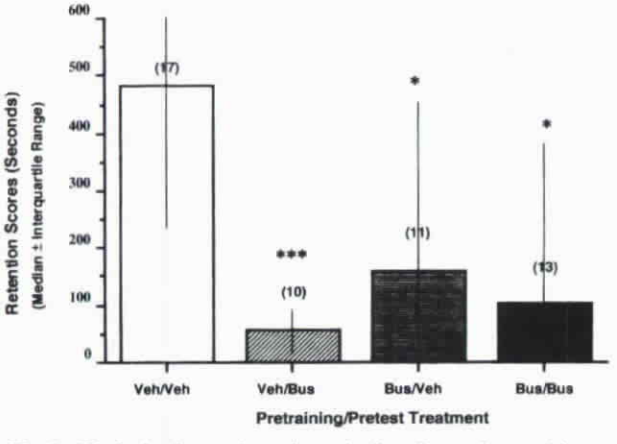

Fig. 8. The lack of state-dependency in the effects of pretraining or pretest injections of buspirone in the inhibitory avoidance task. ***p < 0.001, *p < 0.05 different from the Veh/Veh group. (The first abbreviation denotes the pretraining treatment and the second abbreviation denotes the pretest treatment).

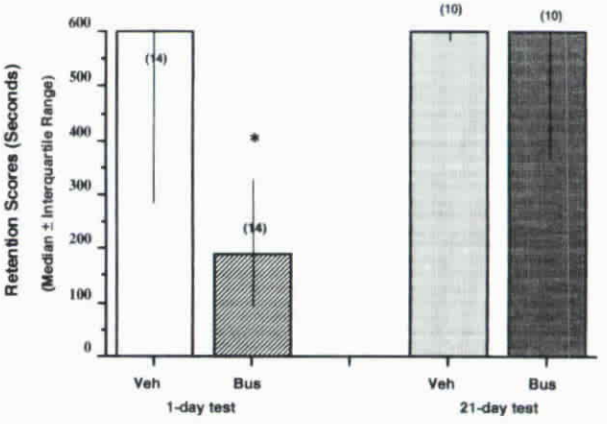

Fig. 9. Effect of pretest intra-hippocampal infusion of buspirone on performance in the 1- or 21-day retention test of the inhibitory avoidance task. *p < 0.05.

Discussion

This study obtained the following major results: In three different types of avoidance tasks, pretraining injections of buspirone impaired acquisition and retention, which may be related to disruption of memory processing for affective information induced by the drug. In the inhibitory avoidance task, posttraining buspirone injections caused a time-dependent retention deficit. Pretest injections of buspirone also suppressed performance in the 1- and 2-day retention tests of the three tasks, but the effectiveness decreased with prolongation of the retention intervals. The observed effects were not state-dependent. Finally, in the inhibitory avoidance task, pretest infusion of buspirone into the hippocampus impaired retention performance in the 1-day test but had little effect in the 21-day test.

Buspirone has been reported to exert biphasic effects on stress-induced immobility (31) and thus may have affected performance if given before training

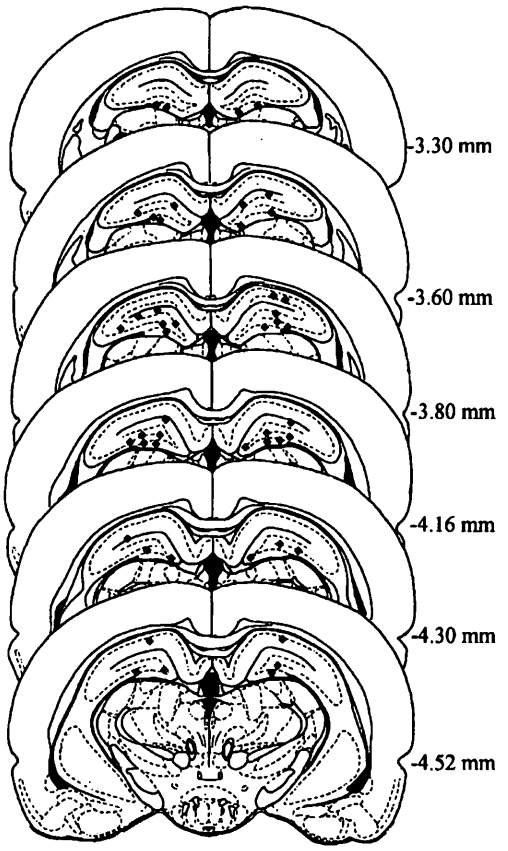

Fig. 10. Distribution of cannula tracts in the hippocampus of rats receiving posttraining infusion of buspirone depicted in a series of coronal plains from bregma -3.30 mm to bregma -4.52 mm based on the brain atlas by Paxinos and Watson (33). (Copyright 1997 by Academic Press. with permission.)

or testing. However, in view of similar effects of this drug in both the inhibitory and active avoidance tasks, which engage just the opposite patterns of behavior, neither increased nor decreased locomotion could easily account for the present results. Buspirone may have altered the pain sensation induced by electric shocks (5). Yet the present study showed that buspirone also impaired learning of a task not motivated by shocks. The flat acquisition curve generated by buspirone in the water maze was not due to performance suppression, otherwise rats such treated should have shown memory similar to that of the controls once the drug was withdrawn on the test day. The differential efficacy of pretest buspirone in suppressing expression of 1- and 21-day memory also renders the performance interpretation implausible. Thus, the whole profile of results can not be parsimoniously interpreted as buspirone-induced sensori-motor or motivational changes.

Previous evidence has shown that a response learned under a drug state may be less readily retrievable under a non-drug state and vice versa (32). Thus, the poor retention performance of rats given buspirone before training or testing might have resulted from a state-dependent effect. However, rats given buspirone before both training and testing did not showed any better retention than rats given buspirone on either occasion alone. Actually, our informal observation found that at a lower dose, buspirone given at both training and testing would aggravate rather than alleviate the otherwise mild deficit caused by giving the drug only at training or testing. Thus, state-dependent storage or retrieval of memory is not likely to contribute to the buspirone effect.

Our results indicate that in the inhibitory avoidance task, posttraining injections of 5.0 mg/kg buspirone caused a time-dependent retention deficit: The effect decreased as the training-treatment delay lengthened. Such data strongly support that buspirone affects the memory consolidation process per se. A previous study found that at a lower dose (2.5 mg/kg), buspirone given at the training phase did not affect memory (35). In that study, the ceiling score was set at 300 sec, which would not be able to detect the mild but significant deficit (medians 396.8 s in the buspirone group vs. 600 s in controls) found in this study. As the retention interval lengthened to 21 days, the effect of buspirone given after training became more apparent: In contrast to the lack of memory decay in the controls, retention deteriorated substantially in the buspirone-treated rats. Our results comply with the data that post-trial injections of 8-OH-DPAT also affected memory formation (8).

Pretest buspirone injections have been shown to impair retention. We extended the previous findings by showing that this drug given before testing left no permanent damage to the memory trace. In the active avoidance task or Morris water maze, rats treated with pretest buspirone and expressing poor performance in the first test showed normal retention when received pretest vehicle in the second test, as attested by the lack of any main or interactive effect involving the treatment order variable in all results. Our informal observation showed similar findings in the inhibitory avoidance task too. This temporary retention deficit is in contrast with the persistent or even aggravating deficit induced by pre- or posttraining injections of buspirone, as shown in a second test given 21 days later. Thus, buspirone given before testing compromised expression or retrieval processes of memory rather than disrupting the memory trace per se as pre- or posttraining buspirone did.

In both the inhibitory avoidance task and the Morris water maze, buspirone given shortly before a 1-day test induced a greater effect than given shortly before a 21-day test. In contrast, pretest injections of buspirone induced the same degree of effect on

retrieving the 1- and 21-day active avoidance memory. The reason for the discrepancy is unclear. It should be pointed out that in the active avoidance task, training and testing procedures are essentially the same and retention is assessed by the reacquisition saving score. Thus, the influence of buspirone on retrieval per se may be confounded by its pronounced effect on reacquisition, particularly after a prolonged retention interval. In addition, testing the same animal repeatedly in the 1/2 and the 21/22 test sessions may also contribute further to the confounding. This issue should be examined by a between-subject design in the future.

Little existing data address how and where in the brain buspirone may affect fear-related learning and memory. Buspirone induces a constellation of pharmacological effects. In addition to its role as a partial agonist on the 5-HT_{1A} receptor, buspirone also acts as a D_2 antagonist (41). Further, buspirone, after metabolized to 1-(2-pyrimidinyl)-peperazine, blocks central α_2 noradrenergic receptors and attenuates the antinociceptive effect caused by clonidine (5, 14). Certain evidence has suggested that the 5-HT_{1A} receptor are involved in the anxiolytic effect of buspirone (18, 39), while the D_2 receptor are related to the motor suppressant effect of this drug (9). In view of the effects of 8-OH-DPAT and ipsapirone on some unconditioned or conditioned fear responses, it is logical to assume that buspirone may also exert its effect on affective learning through the 5-HT_{1A} receptor. Yet a former study has shown that 8-OH-DPAT, ipsapirone and buspirone produced different profiles of effects in blocking the expression of conditioned fear in a potentiated startle paradigm (12). Research to clarify the exact receptor mechanism of buspirone in affecting learning and memory is now undertaken in this laboratory.

Our results indicated that intra-hippocampal infusion of buspirone effectively blocked memory expression in the inhibitory avoidance task. These results were consistent with the report that systemic injections of buspirone reduced hippocampal theta activity (45), which had been related to memory consolidation (21). On the basis of effects caused by 5-HT_{1A} agonists or antagonists in the Morris water maze, hippocampal 5-HT_{1A} receptors could be involved in spatial navigation (6, 7). The present study showed that buspirone also caused deficits in two other types of avoidance tasks less relying on spatial navigation in an open environment. Further, our results showed that buspirone was much more powerful in blocking memory when given at the affective training phase than when given at the spatial training phase. Thus, hippocampal 5-HT_{1A} receptors appear to be involved in affective processing common to all three tasks, although their non-affective functions are by no means ruled out. Our findings suggest that activation of the 5-HT_{1A} receptors inhibits the hippocampal anxiogenic function proposed by a previous theory (13, 16). The dorsal raphe nucleus was reported to be the most sensitive site for buspirone to affect stress-induced vocalization (37), but not to be involved in the effect of buspirone on conditioned fear potentiation of acoustic startle (12). Thus, different neural substrates may mediate the influences of buspirone on various innate or learned fear responses.

It is interesting that buspirone microinfused into the hippocampus was more effective in blocking expression of a 1-day memory than blocking that of a 21-day memory. Such results are in part parallel with our former findings that blocking the limbic structures such as the amygdala or hippocampus suppressed expression of recent memory, while blocking the medial prefrontal or insular cortex suppressed expression of remote memory in the inhibitory avoidance task (26, 28). Systemic buspirone injections prior to a 21-day test still induced a moderate impairing effect. In view of that in rats conditioned fear stimuli altered 5-HT release in the medial prefrontal cortex (44) which is involved in retrieving a remote affective memory (28), it is intriguing to conjecture whether pretest infusion of buspirone into the medial prefrontal cortex may affect expression of affective memory in the 21-day test. Our present findings nonetheless suggest that the change of memory traces along with time may be more extensive than previously conceived and thus raise questions on the adequacy of the conventional model concerning short-term and long-term memories (30).

It remains to be addressed whether buspirone also acts on the hippocampus to affect acquisition and memory formation in aversive tasks and whether the effect of buspirone is mediated exclusively by the hippocampus or also involves the amygdala, which has been extensively implicated in affective memory (25, 26, 27). Finally, it should be noted that an impairing influence on learning and memory has often been taken as one of the undesirable side effects of anxiolytics. However, in view of that conditioning prevails in our daily life through which various otherwise neutral stimuli become associated with fear, that anxiolytics are capable of preventing fear associations from formation or expression may instead positively contribute to the efficacy of these drugs in reducing fear and anxiety.

Acknowledgment

The present study was supported in part by a grant NSC-86-2413-H-002-012 from the National Science Council, ROC as well as funding from

Department of Psychology, National Taiwan University to K. C. Liang. The authors would like to thank Dr. Sigmund Hsiao for helpful discussion on this study and Mr. Dew-Yu Chen for his assistance in computer work. Special gratefulness is expressed to Mr. Wu-Yen Yu for his continual excellent service to the facilities of our physiological psychology laboratory.

References

1. Barrett, J.E., L. Zhang, S. Gleeson, and E.H. Gamble. Anxiolytic and antidepressant mechanisms of 5-HT_{1A} drugs in the pigeon: Contributions from behavioral studies. *Neurosci. Biobehav. Rev.* 18: 73-83, 1994.
2. Bass, E. W. Jr., L.W. Means, and B.A. McMillen. Buspirone impairs performance of a three-choice working memory water escape task in rats. *Brain Res. Bull.* 28: 455-461, 1992.
3. Beninger, R.J. The role of serotonin and dopamine in learning to avoid aversive stimuli. In: *Aversion, Avoidance, and Anxiety*, edited by T. Archer and L.-G. Nilsson, Hillsdale, NJ: Lawrence Erlbaum Associates, 1989, pp. 265-280.
4. Buhot, M.-C. Serotonin receptors in cognitive behaviors. *Curr. Opin. Neurobio.* 7: 243-254, 1997.
5. Cao, B.-J. and W.-P. Li. Antagonism of clonidine antinociception by buspirone and 1-(2-pyrimidinyl)-peperazine. *Eur. J. Pharmacol.* 259: 75-78, 1994.
6. Carli, M., R. Luschi, P. Garofalo, and R. Samanin. 8-OH-DPAT impairs spatial but not visual learning in a water maze by stimulating 5-HT_{1A} receptors in the hippocampus. *Behav. Brain Res.* 67: 67-72, 1995.
7. Carli, M., R. Luschi, and R. Samanin. (S)-WAY 100135, a 5-HT_{1A} receptor antagonist, prevents the impairment of spatial learning caused by intra-hippocampal scopolamine. *Eur. J. Pharmacol.* 283: 133-139, 1995.
8. Carli, M., S. Tranchina, and R. Samanin, 8-hydroxy-2-(di-n-propylamino)tetralin, a 5-HT_{1A} receptor agonist, impairs performance in a passive avoidance task. *Eur. J. Pharmacol.* 211: 227-234, 1992.
9. Cole, J.C. and R.J. Rodgers. Ethological evaluation of the effects of acute and chronic buspirone treatment in the murine elevated plus-maze test: comparison with haloperidol. *Psychopharmacol.* 114:288-296, 1994.
10. Coplan, J.D., S.I. Wolk, and D.F. Klein. Anxiety and serotonin1A receptor. In: Psychopharmacology: *The Fourth Generation of Progress,* edited by F.E. Bloom and D.J. Kupfer, New York, NY: Raven Press, 1995, pp. 1301-1320.
11. Curran, H.V. Benzodiazepines, memory and mood: a review. *Psychopharmacol.*, 105: 1-8, 1990.
12. Davis, M., J.V. Cassella, and J.H. Kehne. Serotonin does not mediate anxiolytic effects of buspirone in the fear-potentiated startle paradigm: comparison with 8-OH-DPAT and ipsapirone. *Psychopharmacol.* 94: 14-20, 1988.
13. Diaz-Granados, J.L., P.L. Greene, and A. Amsel. Selective activity enhancement and persistence in weanling rats after hippocampal X-irradiation in infancy: Possible relevance for ADHD. *Behav. Neural Biol.* 61: 251-259, 1994.
14. Giral, P., P. Soubrie, and A.J. Puech. Pharmacological evidence for the involvement of 1-(2-pyridinyl)-peiperzaine (1-PmP) in the interaction of buspirone or gepirone with noradrenergic systems. *Eur. J. of Pharmacol.* 134: 113-116, 1987.
15. Gray, J.A. The Neurobiology of Anxiety: An Inquiry into the Functions of the Septo-Hippocampal System. New York, NY: Oxford Univ. Press, 1982, .
16. Guimaraes, F.S., E.A. Del Bel, C.M. Padovan, S. Mendonca Netto, and R. Titze de Almeida. Hippocampal 5-HT receptors and consolidation of stressful memories. *Behav. Brain Res.* 58: 133-139, 1993.
17. Harder, J.A., C.J. Maclean, J.T. Alder, P.T. Francis, and R.M. Ridley. The 5-HT_{1A} antagonist, WAY100635, ameliorates the cognitive impairment induced by fornix transection in the marmoset. *Psychopharmacol.* 127: 245-25, 1996.
18. Heninger, G.R. Indoleamines: The role of serotonin in clinical disorders. In: *Psychopharmacology: The Fourth Generation of Progress*, edited by F.E. Bloom and D.J. Kupfer, New York, NY: Raven Press, 1995, pp. 471-481.
19. Kehne, J.H., J.V. Cassella, and M. Davis. Anxiolytic effects of buspirone and gepirone in the fear-potentiated startle paradigm. *Psychopharmacol.* 94: 8-13, 1988.
20. Kia, H.K., M.J. Brisoregueil, M.C. Miquel, G. Daval, M. Riad, S. El Mestikawy, M. Hamon and D. Verge. Immunocytochemical localization of 5-HT_{1A} receptors in the rat central nervous system. *J. Comp. Neurol.* 365: 289-305, 1996.
21. Landfield, P., R. Tusa, and J.L. McGaugh. Effects of posttrial hippocampal stimulation on memory storage and EEG activity. *Behav. Biol.* 8: 485-505, 1973.
22. Lee, E.H.Y., H.Y. Chen, W.R. Lin, W.H. Shiu, and K.C. Liang. Retention of an inhibitory avoidance response in rats is enhanced by fluoxetine and impaired by 8-OH-DPAT injected into the lateral septum. *Physiol. Behav.* 51: 681-688, 1992.
23. Lee, E.H.Y., C.P. Lee, H.I. Wang, and W.R. Lin. Hippocampal CRF, NE and NMDA system interactions in memory processing in the rat. *Synapse* 14: 144-153, 1993.
24. Liang, K.C., W. Hon, Y.-M. Tyan, and W.-L. Liao. Involvement of hippocampal NMDA and AMPA receptors in acquisition, formation and retrieval of spatial memory in the Morris water maze. *Chin. J. Physiol.* 37: 201-212, 1994.
25. Liang, K.C. and J.L. McGaugh. Lesions of the stria terminalis attenuate the amnestic effect of amygdaloid stimulation on avoidance responses. *Brain Res.* 274: 309-318, 1983.
26. Liang, K.C., S.-J. Hu, and S.C. Chang. Formation and retrieval of inhibitory avoidance memory: Differential roles of glutamate receptors in the amygdala and medial prefrontal cortex. *Chin. J. Physiol.* 39: 155-166, 1996.
27. Liang, K.C., W. Hon, and M. Davis. Pre-and post-training infusion of N-Methyl-D-aspartate receptor antagonists into the amygdala impair memory in an inhibitory avoidance task. *Behav. Neurosci.* 108: 241-253, 1994.
28. Liang, K.C. and W.-L. Liao. Pretest infusion of lidocaine into the insular or medial prefrontal cortex impairs retrieval of remote memory in an inhibitory avoidance task. *Soc. Neurosci. Abst.* 21: 1448, 1995.
29. McNaughton, N. and R.G.M. Morris. Buspirone produces a dose-related impairment in spatial navigation. Pharmacol. Biochem. Behav. 43: 167-171, 1992.
30. Nadel, L. and M. Moscovitch. Memory consolidation, retrograde amnesia and the hippocampal complex. *Curr. Opin. Neurobiol.* 7: 217-227, 1997.
31. Nishimura, H., M. Tanaka, A. Tsuda and Y. Gondoh. Atypical anxiolytic profile of buspirone and a related drug, SM-3997, in a modified forced swim test employed straw suspension. *Pharmacol. Biochem. Behav.* 46: 647-65, 1993.
32. Overton, D.A. Experimental methods for the study of state-dependent learning. *Fed. Proc.* 33: 1800-1813, 1974.
33. Paxinos, G. and C. Watson. *The Rat Brain In Stereotaxic Coordinates*. Third compact edition. San Diego, CA: Academic Press, 1997.
34. Pazos, A. and J.M. Palacios. Quantitative autoraiographic mapping of serotonin receptors in the rat brain. I. serotonin-1 receptors. *Brain Res.* 346: 205-230, 1985.
35. Rowan, M.J., W.K. Cullen, and B. Moulton. Buspirone impairment of performance of passive avoidance and spatial learning tasks in the rat. *Psychopharmacol.* 100: 393-398, 1990.

36. Santucci, A.C., P.J. Knott, and V. Haroutunian. Excessive serotonin release, not depletion, leads to memory impairments. *Eur. J. Pharmacol.* 295: 7-17, 1996.
37. Schreiber, R. and J. De Vry. Neuronal circuits involved in the anxiolytic effects of the 5-HT_{1A} receptor agonists 8-OH-DPAT, ipsapirone and buspirone in the rat. *Eur. J. Pharmacol.* 249: 341-353, 1993.
38. Sirvio, J., P. Riekkinen, Jr., P. Jakala, and P. J. Riekkinen. Experimental Studies on the role of serotonin in cognition. *Prog. Neurobiol.* 43: 363-379, 1994.
39. Takao, K. , T. Nagatani, K.-I. Kasahara, and S. Hashimoto. Role of the central serotonergic system in the anticonflict effect of d-AP159. *Pharmacol. Biochem. Behav.* 43: 503-508, 1992.
40. Treit, D., A. Robinson, S. Rotzinger, and C. Pesold. Anxiolytic effects of serotonergic interventions in the shock-probe burying test and the elevated plus-maze test. *Behav. Brain Res.* 54: 23-34, 1993.
41. Tunnicliff, G., A.S. Eison, and D.P. Taylor. Buspirone: Mechanisms and Clinical Aspects. San Diego, CA: Academic Press, 1991.
42. Vanderwolf, C.H. Near-total loss of learning and memory as a result of combined cholinergic and serotonergic blockade in the rat. *Behav. Brain Res.* 23: 43-57, 1987.
43. Wenk, G., D. Hughey, V. Boundy, A. Kim, L. Walker, and D. Olton. Neurotransmitters and memory: Role of cholinergic, serotonergic, and noradrenergic systems. *Behav. Neurosci.* 101: 325-332, 1987.
44. Yoshioka, M, M. Matsumoto, H. Togashi and H. Saito. Effects of conditioned fear stress on 5-HT release in the rat prefrontal cortex. Pharmacol. *Biochem. Behav.* 51: 515-519, 1995.
45. Zhu, X.O. and N. McNaughton. Minimal changes with long-term administration of anxiolytics on septal driving of hippocampal rhythmical slow activity. *Psychopharmacol.* 118: 93-100, 1995.