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LEARNING AND MEMORY IN SWISS MICE FOLLOWING TREATMENT WITH BROMAZEPAM

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ABSTRACT: The study investigated the effect of bromazepam on learning and memory using eighteen (18) healthy Swiss mice of both sexes weighing 16 – 25g. The animals were divided into three (3) groups consisting of six (6) animals each. Group 1 served as the control group, group 2 as the stressed control group while group 3 were administered bromazepam. All the animals were tested for learning and memory performance using Novel object recognition task and Morris water maze test. The results obtained from the Novel object recognition task showed that there was a significant decrease ($p<0.05$) in total object approach in acquisition trial of bromazepam group when compared to the acquisition trial of stressed control group. There was a significant decrease ($P<0.05$) in retention trial of bromazepam group when compared to retention trial in the control and stressed control group. There was a significant decrease ($p<0.05$) in total duration exploring objects in acquisition trial of bromazepam group when compared to the acquisition trial of the stressed control group. There was a significant increase ($p<0.05$) in total duration exploring objects in retention trial of stressed control group when compared to the retention trial of the control group. There was a significant decrease ($P<0.05$) in the index of habituation of bromazepam group when compared to the control group. The index of discrimination showed a significant increase ($p<0.05$) in bromazepam group when compared to the stressed control group and a significant decrease ($p<0.05$) in bromazepam group when compared to the control group. In the Morris water maze test, Day 1 – 3 were for acquisition training, day 4 – 6 reversal training, day 7 the probe trial day and day 8 the visible platform day. During acquisition training in the Morris water maze test, there was no significant difference in Swim latencies in day 1 and 2. However in day 3, there was a significant increase ($p<0.05$) in swim latency of bromazepam group compared to stressed control group and a significant decrease ($P<0.05$) in swim latency of stressed control group compared to the control group. During reversal training in day 1, 2 and 3, there was no significant difference in swim latency

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among the three groups. Results for the retention quadrant in the probe trials showed a significant decrease ($p<0.01$) in bromazepam group when compared to the control group. From the results, it can be suggested that bromazepam impairs learning and memory functions.

KEYWORDS: Bromazepam, Swiss mice, learning, memory.

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1.INTRODUCTION

Learning is one of the most important mental functions of humans. It is an adopted change in individual behavior resulting from experience. It relies on acquisition of different kinds of knowledge supported by perceived information. The mechanisms of learning and remembering seem to depend on relatively enduring changes in the nervous system. Its goal is the increasing of individual and group experience[1].Obviously, learning and memory are closely related; for something to be remembered, it must first be learned.Memory is the faculty of the mind by which information is encoded, stored and retrieved and it is related to the limbic systems [2]. Often, memory is understood as an informational processing system with explicit and implicit functioning that is made up of sensory processor; short-term (or working) memory, and long-term memory [3]. The word “memory” has three primary definitions first; memory is the location where information is kept as in a store house or memory store. Second, memory can refer to anything that holds the contents of experience as in a memory trace or engram. Finally, memory is the mental process used to learn, store or retrieve information of all sorts [4].The terms learning and memory are used in specific ways in experimental psychology. In general, memory refers to the storage of information and the processes used to retrieve it. Learning is a term that has greater association with studies of conditioning that is more likely to involve animals. Bromazepam is a benzodiazepine drug that is being used to treat anxiety and panic states. It may also be used as a premedicant prior to minor surgery. Prolonged use of bromazepam causes tolerance and may lead to both physical and psychological dependence on the drug [5].Bromazepam is reported to be metabolized by a hepatic enzyme belonging to the cytochrome P450 family of enzymes [6]. Much of the current knowledge of memory has come from studying memory disorders which can result from extensive damage to the regions of the medial temporary lobe [7]. Benzodiazepines, such as bromazepam which has a half-life of 8-20 hours with plasma concentration occurring approximately 1 hours after its oral administration [8], have been used in the pharmacological treatment of anxiety since the early 60’s [9]. The benzodiazepine family of depressants is used therapeutically to produce sedation, induce

sleep, relieve anxiety and muscle spasms, and to prevent seizures. In general, benzodiazepines act as hypnotics in high doses, anxiolytics in moderate doses and sedatives in low doses. Their mechanism of action on the Central Nervous System is believed to be related to their ability to enhance the activity of gamma aminobutyric acid (GABA) which is a major inhibitory neurotransmitter [10-13].Effects of oral doses of bromazepam on memory, psychomotor activity, reaction time and vigilance performance have been widely demonstrated [14-18]. It has been observed that bromazepan deteriorates the ability of the individual to detect relevant information in the environment [19-22].

2. MATERIALS AND METHODS

Experimental animals

Eighteen (18) mice weighing about 16-25g were used for this study. The mice were purchased from the department of pharmacology, University of Calabar, Calabar and were kept under standard conditions in the animal house, Department of Physiology, University of Calabar, Calabar. The mice were kept in plastic cages and were given free access to rodent chow and water.The animals were acclimatized under standard conditions and were kept in 12 hours light/dark cycle for 7 days before conducting the experiment. This was done in order to enable the animals get familiar with the new environment.

Experimental protocol

The animals were randomly selected and assigned into three groups. Group one was used as control group, group two was used as stressed control group, and group three was the test group that were given bromazepam at a dose of 3mg/kg body weight dissolved in 3mls of distilled water. Each group of control, stressed control and test had six animals making a total of eighteen animals that were used for this study. The administration was carried out between 9am – 11am each day and lasted for a period of 9 days. During this period, the animals were tested with Novel object recognition task (NORT) and Morris Water Maze (MWM) to ascertain the effect of Bromazepam on learning and memory.

Novel Object Recognition Task (NORT)

The NORT evaluates the animals' ability to recognize a novel object in the environment. Prior to testing, all mice were habituated to the apparatus for 5-min beforehand. The Mice were carried to the test room in their home cages and run individually. They were moved from their home cage to the testing apparatus and back using a small container. After each 5-min trial, the mice were returned to their home cages and the apparatus was cleaned with methylated spirit and permitted to dry between trials. Two pairs of identical objects were used. Two trials (acquisition and recognition) were conducted on the same day, separated by a retention period of 5-min.. During the first trial, two identical objects (O1 and O2) were placed in diagonal corners opposite each other in the open field. Objects were secured to the floor of the apparatus with reusable adhesive. The mouse was

scooped up from its home cage in a yogurt container and placed in the middle of the open field arena. Each mouse was allowed to explore the arena and objects for 5-min. At the end of the trial the mouse was removed from the apparatus using the yogurt container and returned to its home cage. After a 5-min inter-trial interval (retention period) the mouse was returned to the test apparatus (trial 2). The arena now contained the familiar object (O1 or O2 from trial 1) in one of the two locations in trial 1 and a new object (N) that replaces O1 or O2. The same behaviors recorded for trial 1 were recorded for 5-min for trial 2.

The behaviors scored using the Open field [23] include:

1. Line Crossing: frequency with which the mouse crossed one of the grid lines with all four paws.
2. Rearing: frequency with which the mouse stood on their hind legs in the maze.
3. Rearing Against a Wall: frequency with which the mouse stood on their hind legs against a wall of the open field.
4. Stretch Attend Postures: frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position.
5. Grooming: frequency and duration of time the animal spent licking or scratching itself while stationery.
6. Approaches to each object: directing the nose to the object at a distance of < 1 cm and/or touching it with the nose.
7. Time spent with Each Object: Sniffing or climbing the object.

Sitting on the object is not considered as an exploratory behavior.

Morris Water Maze (MWM)

The Morris water maze that was used for the study was modified for mice [24]. Morris water maze is constructed from a circular polypropylene pool that measures 110cm in diameter and 20cm in depth. The pool was filled to the depth of 140cm (0.5cm over the escape platform). The water was left 24 hours to assume room temperature and was made opaque by the addition of a non-toxic chalk. The pool was divided into four quadrants; North-West, North-East, South-East, and South-West. Boundaries of the quadrant were marked on the edges of the pool with masking tape and labeled north, south, east and west.An escape platform made of a cylinder (13.5cm x 9cm) in diameter filled with cement to make firm was suspended and hidden 0.5cm beneath the pool. The MWM is an experimental test protocol that lasted for eight days as follows:

Day 1 - Acquisition day 1
Day 2 - Acquisition day 2
Day 3 - Acquisition day 3
Day 4 - Reversal day 1
Day 5 - Reversal day 2
Day 6 - Reversal day3

Day 7 - Probe trial day

Day 8 - Visible platform day.

Acquisition and Reversal trainings were done with the escape platform hidden 0.5cm below the opaque water (in the North-East quadrant during acquisition training and the South-west quadrant during the reversal training). During the probe trial, there was no escape platform so that visuo-spatial memory can be accessed. On the visible platform day, the platform was moved to another quadrant of the pool and a visible top is added to the platform.During the acquisition training (Day 1 – 3), the platform was placed (and hidden 0.5cm below) in the centre of the North east quadrant. Each mouse was given a maximum of 60 seconds to locate the hidden platform within the allotted time. It was then allowed at least 10 seconds on the platform to view extra maze cues after which it was removed from the pool using a small container and the swim latency (i.e the time it took the animal to locate and climb the escape platform was recorded.If the animals could not locate the platform after 60 seconds then it is directed to the platform using a small container and allowed for 10 seconds before it is taken out of the pool. It is important that all the animals be removed from the pool only after they may have climbed the escape platform so as to let the animals associate climbing of the platform with escape from the pool. When the animal is removed from the pool, it is usually placed in a holding cage where their body is dried using tissue before being returned to their home cages.During the reversal training (day 4 – 6), the location of the escape platform was changed to the south-west quadrant. The mouse was again assigned appropriate start locations and the same procedure as in acquisition training was repeated.On the probe trial day (day 7), visuo-spatial memory status of all the animals were accessed on this day, the platform was taken out of the pool. All the animals received only one trial from any one of all the four start locations (from the North pole) and allowed to explore the maze for 60 seconds. Here, the quadrant duration (i.e the number of times the animals spent on each quadrant) was recorded. At the completion of the trials, the animals were then scooped out of the maze using a small container and placed in its appropriate holding cage to dry and then returned to their home cages.It is believed that animals with good visuo-spatial memory will spend more time in the quadrants where the escape platform was located. On the visible platform day (day 8), the platform is placed in a new quadrant or location (north-west quadrant) but this time made visible through the attachment of a colorful detachable flag to the top of the platform. The same procedure as in acquisition and reversal training were repeated as each of the animals received and completed four trials.

Statistical Analysis

Data obtained from the study was expressed as mean ± SEM following one-way analysis of variance (ANOVA) and statistical comparison among the groups was performed with Turkey multiple comparison test using SPSS, version 17. 0. $P<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

Comparison of total object approaches during the acquisition and retention trials of the novel object recognition task in stressed mice treated with Bromazepam

The mean ± SEM of total approach trial 1 during the acquisition trials of the novel object recognition task in control, stressed control and bromazepam groups were 16.8 ± 1.38, 23.6 ± 1.03 and 17.9 ± 1.52 respectively while the mean ± SEM of total approach trial 2 during the retention trial of the novel object recognition task in control, stressed control and bromazepam groups were 12.2 ± 0.75, 23.0 ± 0.84 and 14.3 ± 1.01 respectively. There was a significant decrease (P<0.05) in retention trial of the control and bromazepam groups when compared to their acquisition trial. There was a significant increase (p<0.05) in acquisition trial of stressed control when compared to the acquisition trial of control group. There was a significant decrease (p<0.05) in acquisition trial of bromazepam group when compared to the acquisition trial of stressed control group. There was a significant increase (p<0.05) in retention trial of stressed control group when compared to the retention trial of the control group. There was a significant decrease (p<0.05) in retention trial of bromazepam group when compared to the retention trial of stressed control group (Figure 1).

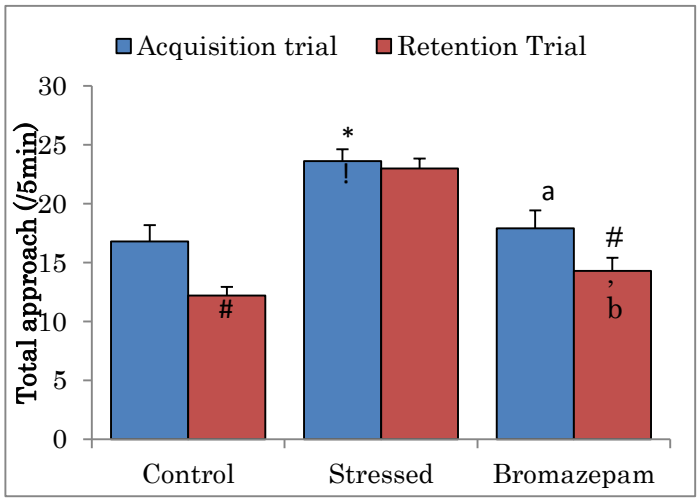

Fig. 1: Comparison of total object approaches during the acquisition and retention trials of the novel object recognition task in stressed mice treated with bromazepamValues are mean ± SEM, n=6.

- significant at p< 0.05 vs acquisition trial;

* - significant at p< 0.05 vs control during the acquisition trial;

a – significant at p< 0.05 vs stressed group during the acquisition trial;

! – significant at p< 0.05 vs control during retention trial;

b – significant at p< 0.05 vs stressed group during retention trial.

Comparison of total duration (sec) of exploring objects during the acquisition and retention trials of the novel object recognition task in stressed mice treated with bromazepam

The mean ± SEM of total duration trial 1 of control, stressed control and bromazepam groups were 38.23 ± 2.04, 54.2 ± 8.76 and 31.4 ± 2.68 respectively while the mean ± SEM of total duration of

trial 2 of control, stressed control and bromazepam groups were 28.7 ± 2.58, 55.6 ± 8.87 and 34.0 ± 2.45 respectively. There was a significant decrease ($p<0.05$) in retention trial of the control group when compared to its acquisition trial. There was a significant increase ($p<0.05$) in acquisition trial of the stressed control group when compared to the acquisition trial of the control group. There was a significant decrease ($p<0.05$) in acquisition trial of bromazepam group when compared to the acquisition trial of the stressed control group. There was a significant increase ($p<0.05$) in retention trial of stressed control group when compared to the retention trial of the control group. There was a significant decrease ($p<0.05$) in retention trial of bromazepam group when compared to the stressed control group (Figure. 2).

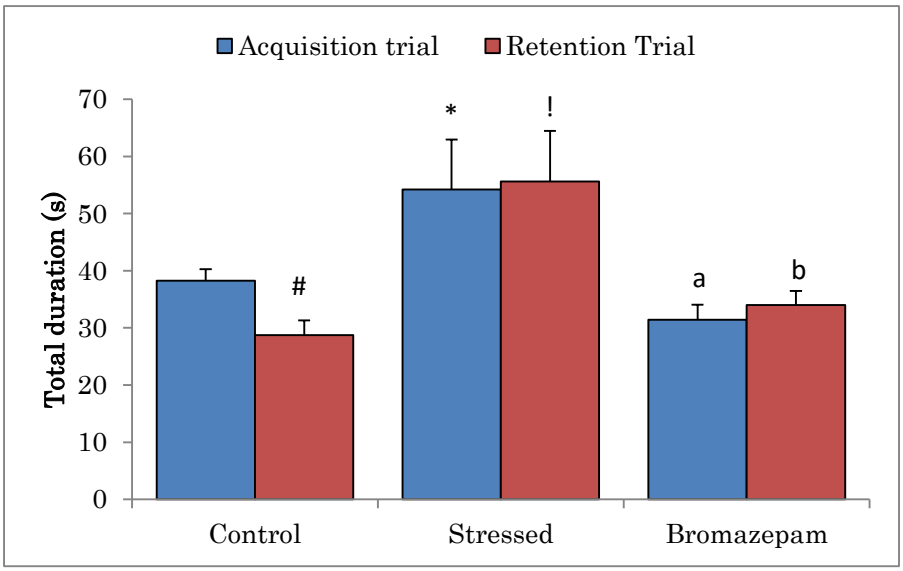

Fig. 2: Comparison of total duration exploring objects during the acquisition and retention trials of the novel object recognition task in stressed mice treated with bromazepam

Values are mean ± SEM, n=6.

- significant at $p< 0.05$ vs acquisition trial;

* - significant at $p< 0.05$ vs control during the acquisition trial;

a – significant at $p< 0.05$ vs stressed group during the acquisition trial;

! – significant at $p< 0.05$ vs control during retention trial;

b – significant at $p< 0.05$ vs stressed group during retention trial.

Comparison of index of habituation (h) in the novel object recognition task in stressed mice treated with Bromazepam.

The mean ± SEM of habitation index of control, stressed control and bromazepam groups were 11.49 ± 2.58, 1.4 ± 1.29 and 2.6 ± 1.85 respectively. There was a significant decrease ($p<0.01$) in H in stressed control group when compared to the control group. There was also a significant decrease ($p<0.05$) in H, in bromazepam group when compared to control group (Figure 3).

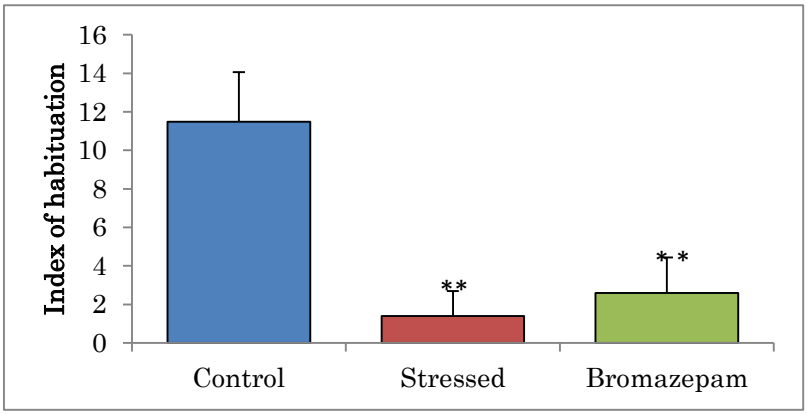

Fig 3: Comparison of index of habituation in the novel object recognition task in stressed mice treated with bromazepam. Values are mean ± SEM, n=6.

** - significant at p< 0.01 vs control

Comparison of index of discrimination (d) in novel object recognition task in stressed mice treated with Bromazepam.

The mean ± SEM of D for control, stressed control and bromazepam groups were 0.36 ± 0.06, 0.036 ± 0.02 and 0.19 ± 0.45 respectively. There was a significant decrease ($p<0.05$) in D in bromazepam group when compared to the control group. There was a significant decrease ($p<0.01$) in D in stressed control group when compared to the control group. There was a significant increase ($p<0.05$) in D in bromazepam group when compared to the stressed control group (Figure. 4).

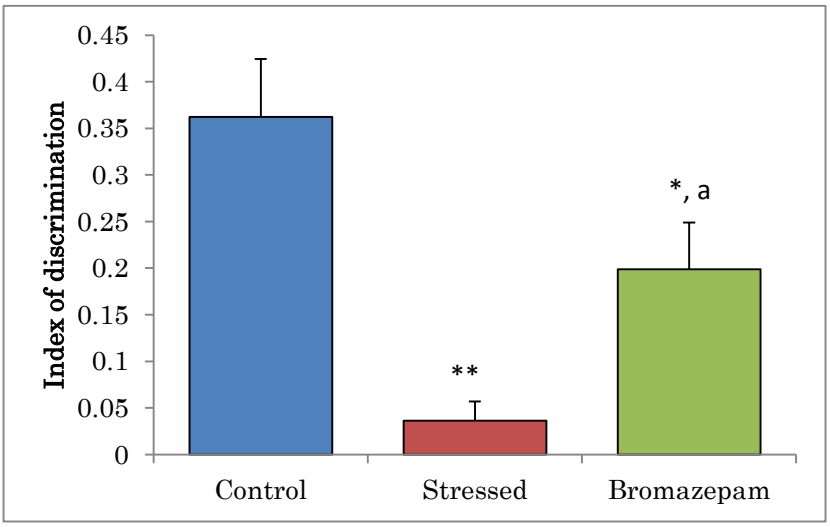

Fig 4: Comparison of index of discrimination in the novel object recognition task in stressed mice treated with bromazepam

Values are mean ± SEM, n=6.

* - significant at p< 0.05 vs control;

**significant at P< 0.01 vs control,

a – significant at p< 0.05 vs stressed group of mice.

Learning curves showing comparison of swim latencies during acquisition training of Morris water maze test in stressed mice treated with Bromazepam

The mean ± SEM of swim latencies values (Secs) during acquisition training in day 1 for control, stressed control and bromazepam groups were 48.8 ± 1.91, 51.95 ± 2.06 and 55.7 ± 1.27 respectively. The mean ± SEM of swim latencies values (Secs) during acquisition training in day 2 for control, stressed control and bromazepam groups were 42.65 ± 3.89, 37.85 ± 2.45 and 50.9 ± 1.83 respectively. There was no significant differences in swim latencies in day 1 and 2 among the group.The mean ± SEM of swim latencies values (secs) during acquisition training in day 3 in control, stressed control and bromazepam groups were 37.3 ± 1.61, 20.85± 4.25 and 43.7 ±3.40 respectively. However, there was a significant decrease ($p<0.05$) in stressed control group when compared to the control group and there was also a significant decrease ($p<0.05$) in stressed control group when compared to bromazepam group (Figure 5).

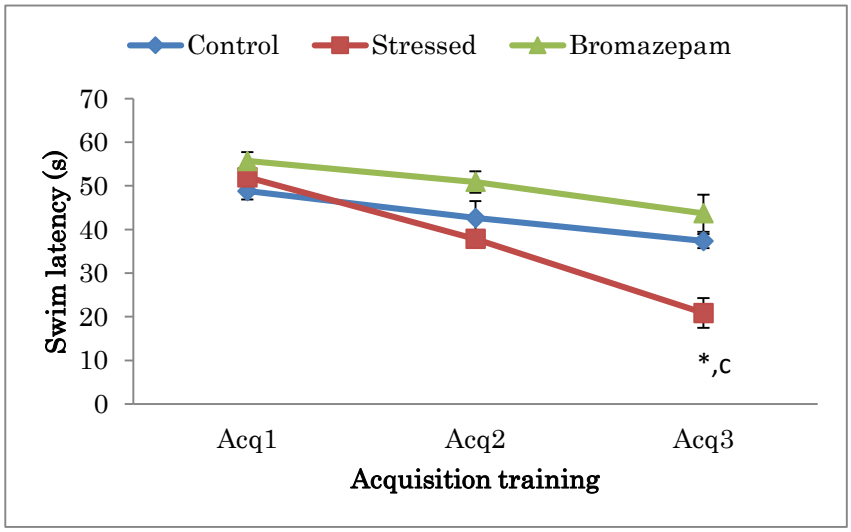

Fig 5: Learning curves showing comparison of swim latencies during the acquisition training of the Morris water maze test in stressed mice treated with bromazepam

Values are mean ± SEM, n=6.

* - significant at $p< 0.05$ vs control;

c – significant at $p< 0.05$ vsbromazepam group of mice.

Learning curves showing comparison of swim latencies during the reversal training of the Morris water maze test in stressed mice treated with bromazepam

The mean ± SEM of swim latencies values (secs) during reversal training in day 1 for control, stressed control and bromazepam groups were 32.15 ± 4.49, 34.9 ± 5.03 and 37.25 ± 1.29 respectively. The mean ± SEM of swim latencies values (secs) during reversal training in day 2 for control, stressed control and bromazepam groups were 27.25 ± 7.56, 26.6 ± 8.21 and 30.1 ± 3.02 respectively. The mean ± SEM of swim latencies values (secs) during reversal training in day 3 for control, stressed control and bromazepam groups were 20.45 ± 8.06, 16.3 ± 3.24 and 22.3 ± 4.16

respectively. There was no significant difference in swim latencies during the reversal training in day 1, 2 and 3 among the groups (Figure 6).

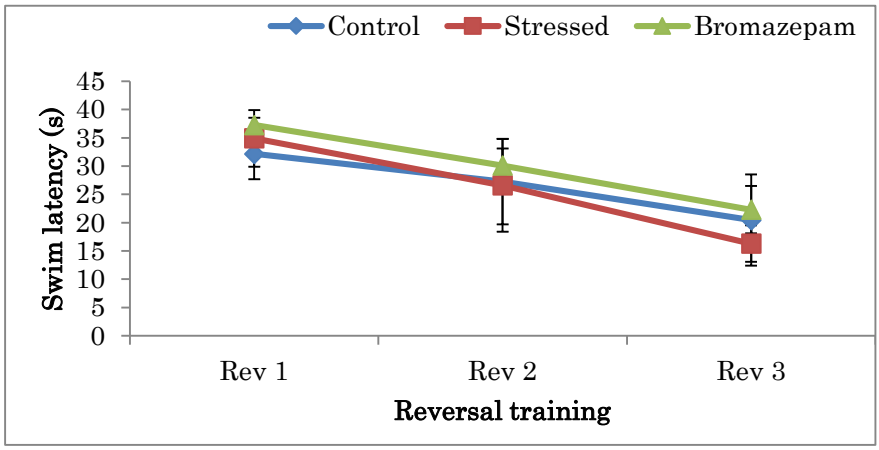

Fig. 6: Learning curves showing comparison of swim latencies during the reversal training of the Morris water maze test in stressed mice treated with bromazepam Values are mean ± SEM, n=6

Comparison of swim latencies in the visible platform task of the morris water maze test in stressed mice treated with bromazepam

The mean ± SEM of retention quadrant duration in the probe trial of the Morris water maze in control, stressed control and Bromazepam groups were 35.6 ± 0.50, 22.8 ± 1.29 and 26.5 ± 2.62 respectively there was a significant decrease ($p<0.01$) in stressed control group and Bromazepam group when compared to the control group (Figure 7)

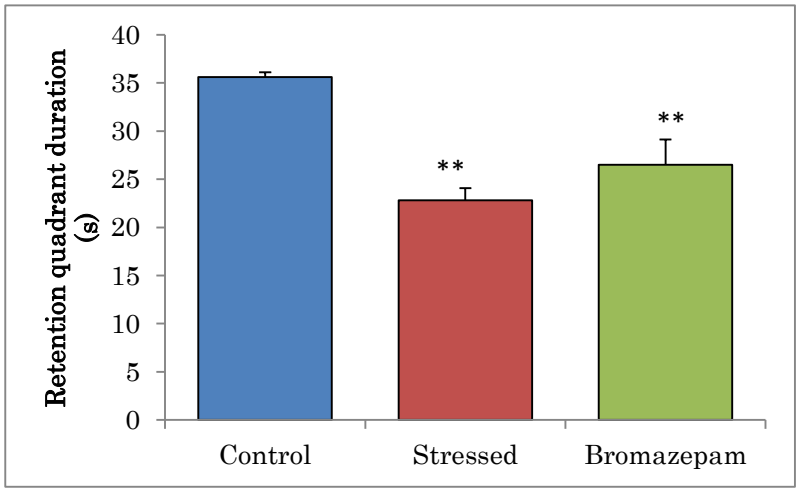

Fig. 7: Comparison of retention quadrant duration in the probe trial of the Morris water maze test in stressed mice treated with bromazepam

Values are mean ± SEM,

n=6. ** - significant at p< 0.01 vs control.

Comparison of swim latencies in the visible platform task of the Morris water maze test in stressed mice treated with bromazepam

The mean ± SEM of swim latencies values (secs) in the visible platform task of the Morris water maze test in control, stressed control and Bromazepam groups were 22.15 ± 5.27, 25.5 ± 3.58 and 32.33 ± 4.78 respectively. There was no significant difference among the groups (Figure 8).

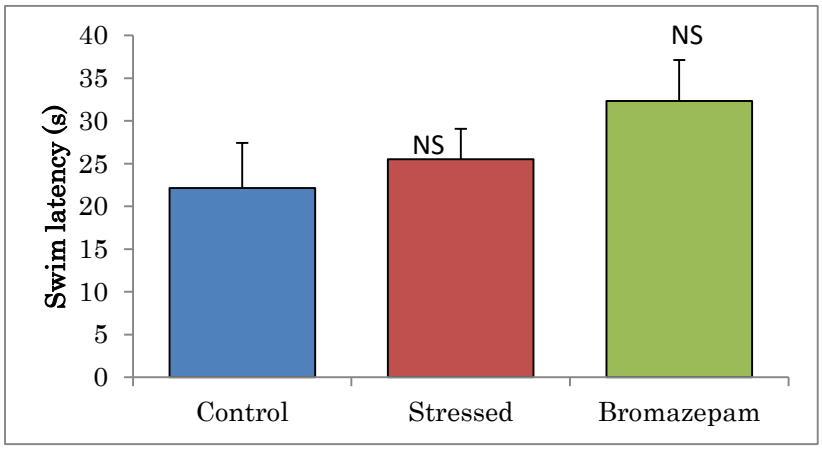

Fig. 8: Comparison of swim latencies in the visible platform task of the Morris water maze test in stressed mice treated with bromazepam

Values are mean ± SEM, n=6. NS - Not significant vs control.

DISCUSSION

Novel object recognition task

In this study, when comparing the total object approaches and duration during the acquisition and retention trials of the novel object recognition task, it was observed that the bromazepam group approached less frequently and spent less time with the novel object than the familiar one compared to the control group. When animals are exposed to a familiar and a novel object, they approach frequently and spend more time exploring the novel than the familiar one [25].Thus, it is pertinent to say that the control group learnt better than the bromazepam group and this could be as a result of the adverse effect of bromazepam on the central nervous system [16]When comparing the index of habituation which is the decrease of a response to a repeated eliciting stimulus, it was observed that bromazepam group had a lower habituation index compared to the control group. This suggests that the control group had a better learning and memory than the bromazepam group, this result is in consonance with [26] who observed that bromazepam impaired learning significantly among human volunteers. In comparing the discrimination index which is a measure of item quality whenever the purpose of a test is to produce a spread of scores reflecting differences in achievement, so that distinctions may be made among the performances, bromzepam group had a lower discrimination index compared to the control group, and a higher discrimination index compared to the stressed control group. This implies that bromazepam group learnt less when compared to the control group but showed a significant learning and memory ability when compared to the stressed control group.

Morris Water maze

The results obtained from the test showed that during the acquisition trial which lasted for three (3) days, the swim latency was longer in bromazepam group when compared to the control and the stressed control group. The stressed control group had a shorter swim latency compared to the control group. The stressed control group showed a stronger relationship between the number of trials and the swim latencies. This is because the shorter the swim latency, the better the learning and memory processes. During the reversal trainings, there was no significant difference among the three (3) group of mice. During the probe trial day i.e. day seven (7) there was a significant decrease in retention quadrant duration of bromazepam group when compared to the control group. There was also a decrease in retention quadrant duration of the stressed control group compared to the control group. This suggests that there was a significant decrease in learning and memory in both the bromazepam and stressed control group when compared to the control group.The visible platform expresses the visual abilities of the animals. On day eight (8) which was the visible platform day, there was no significant difference among any of the groups. This suggests that all three (3) groups had almost equal visual acuity.

CONCLUSION

Result from the study suggests that, at the dose given which was 3mg/kg body weight, bromazepam impaired learning and memory functions significantly.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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