

Ethological Characterization and Pharmacological Validation of Elevated “I – maze” as Animal Model of Anxiety

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Abstract

Objective: The objective of the present study was to design a novel animal maze which can detect anxiety in mice and effect of different anxiogenic and anxiolytic treatments.

Methods: The maze was behaviorally validated by recording the behaviors of mice on the maze before and after administration of anxiolytic drug treatments like Diazepam (1 and 2 mg/kg), Gabapentin (10 and 20 mg/kg), Fluoxetine (5 and 10 mg/kg), Ondansetron (0.1 and 1 mg/kg) and anxiogenic treatments like caffeine (15 and 30 mg/kg) and exposure to immobilization stress. Ethological characterization was done by tracking behavioral pattern of mice on the maze.

Results: Diazepam significantly increased the percentage of time spent in the open areas (%TO) and the number of unprotected head dips (uHDIPS), and reduced the number of protected head dips (pHDIPS) and stretch attend postures (SAP) from close to open arm. Similarly, gabapentin significantly increased the %TO and uHDIPS, and reduced the pHDIPS and SAP from close to open arm. Fluoxetine significantly increased the %TO and uHDIPS, and SAP from close to open arm, but it did not have any significant effect on number of pHDIPS. The 5-HT₃ receptor antagonist, ondansetron did not produce any significant change in all the behaviors, observed, as compared to vehicle-treated control mice. On the other hand, the anxiogenic agent, caffeine and immobilization stress did produce a significant decrease in %TO and the number of uHDIPS, and significantly increased the number of pHDIPS and SAP from close to open arm.

Conclusion: The present data indicate that the novel “I - maze” design, pharmacological and ethological analysis provide a sensitive model for detection of anxiolytic/anxiogenic drug action.

Keywords: Anxiety; Diazepam; Gabapentin; Fluoxetine; Ondansetron; Caffeine; Elevated I-maze; Mice

Introduction

Human anxiety may be defined as a feeling of apprehension, uncertainty and tension stemming from the anticipation of an imagined or unreal threat [1]. Pharmacological evaluation of anti-anxiety agents is performed employing behavioral paradigms. An animal model of anxiety is an experimental paradigm that simulates specific symptoms of anxiety disorders. Studies on anxiety generally involve exposure of animal to a situation that has not been experienced earlier by animal. Most of these procedures expose an animal to a novel enclosure such as maze, open field or other test chamber [2]. In particular, the elevated plus-maze has been used and validated as a tool to assess anxiety in rodents, as well as to determine the efficacy of various anxiolytic compounds. In this paradigm, subjects are placed on the central platform of the elevated plus-maze at the start of the test, and the amount and distribution of time spent in the open and closed arms are measured. Increased time spent in the open arms is indicative of a low level of anxiety, whereas increased time spent in the closed arms is indicative of a high level of anxiety. Validation of this procedure has been confirmed by the effects of anxiolytic compounds. Pre-existing modifications in the design of elevated plus-maze are (a) elevated Zero-maze (EZM) [3] and (b) elevated T-maze (ETM) [4]. These mazes were proposed as animal models that avoid the inherent deficiency of central platform in EPM. It is argued by investigators that time spent on central platform compromises interpretation of time spent in open/close arms. Mice have been observed to spend 20-30% of the test period on central square [5]. The elevated T-maze was designed to test the conditioned and unconditioned fear in the single apparatus, whereas elevated zero-maze was utilized for detailed behavioral analysis including risk assessment by animal, in addition to

measure of anxiety. Though, these mazes, offer clear advantages over elevated plus-maze *viz.* avoidance of central platform (in case of both ETM and EZM) and uninterrupted exploration of the apparatus (in case of EZM), the design of I-maze facilitate (a) expedited exploration of maze because, as the animal reaches at the end of closed end, it spontaneously turn back and encounters a much clear view (option) of open space, present inherently between two closed arms (choices), as compared to all other mazes like EPM, ETM and EZM. (b) authors opine that I-maze offers a rather more robust measure of anxiety as compared to the designs of EPM, ETM and EZM, because, during the whole period of animal stay (300 sec) in the maze, the design of I-maze facilitate a clear view of all the portions of maze. Due to such structure of the maze, during its stay in any one arm (open or close), animal has always a clear vision of other arm, where it can enter as per its own preference. This feature adds to the reliability of the I-maze, because animal during its stay in open arm, still have a clear view of close arms, on both sides of its stay on the straight open platform of maze. In this situation, still, if the animal does not opt to enter close arm, then it may be taken as more robust measure of animal preference for open arm *i.e.* anxiolytic- like behavior of animal or drug action, rather than

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an artifact or an unexplained stay of animal in any one arm (out of animal's own confusion and retardation of decision making, which is an inherent feature of anxiety or due to lack of a clear view of another arm during its stay in one arm). (c) This further adds to the utility of I-maze to facilitate a significant interpretation of more subtle drug effects. (d) Further, similar to ETM and EZM, I-maze also avoids the central platform in its design.

Various categories of drugs like benzodiazepines, barbiturates, alcohol, tri-cyclic antidepressant, have been used for long time to treat anxiety. Apart from these established categories of drugs, there are certain categories, which have been explored for their anxiolytic potential. These include selective serotonin reuptake inhibitors (SSRIs) like paroxetine, citalopram and fluoxetine. This category has been considered as possible therapeutic replacements for some of traditional anxiolytics because they are found to have comparable anxiolytic effect to diazepam. Further, some anti-epileptic like tiagabin, gabapentin and pregabalin has been explored for anxiety treatment.

The present study was undertaken to examine the possible anti-anxiety activity of selected categories of drugs on proposed novel model of anxiety, named as "I-maze". A model is said to be pharmacologically and ethologically validated, if a model facilitates the expression of a significant pharmacological activity of selected drugs in mice, thereby, showing a clear behavioral change in mice, as a result of administration of selected drug(s).

Materials and Methods

Animals: Swiss albino mice (male; 20-25g) were used in this study. Animals were housed under standard laboratory conditions, maintained on a 12 h light and dark cycle. Animals had free access to standard food and water. Animals were acclimatized to laboratory conditions before the test.

Drugs: Diazepam (1 and 2 mg/kg), Gabapentin (10 and 20 mg/kg), Fluoxetine (5 and 10 mg/kg), Ondansetron (0.1 and 1 mg/kg) and Caffeine (15 and 30 mg/kg) were all dissolved in distilled water, which given alone served as control group employed in the present study. All drugs were administered intraperitoneally (i.p.) 30 min prior to behavioral testing.

Apparatus: The apparatus is a straight wooden passage, resembling to English letter "I" (Figure 1a). It consists of a 48 cm × 5 cm, straight passage, divided equally (16 cm each) into two enclosed areas (close arms) at both ends of the "maze" and an open area in the center of two enclosed ends (arms). Height of the walls of enclosed areas is 12 cm. Final dimensions of "I" maze are proposed to be 48 cm long × 5 cm wide with 12 cm high walls at both ends. Entire maze is elevated to the height of 25 cm. (Figure 1b)

Experimental procedure

Twelve groups of mice were employed in the present study. Each group consisted of ten mice. Stress was produced in mice by immobilizing them for 6 h (8 a.m.–2 p.m.) by taping all its four limbs and trunk on a wooden board. Mice subjected to immobilization were called as stressed mice. Vehicle, diazepam, gabapentin, fluoxetine, ondansetron, caffeine were administered in separate groups of mice, 30 min before subjecting them to behavioral testing. Mice were placed into the central open area of the maze, facing outside environment of the maze. All the parameters were observed and results documented from video recording of mice behavior on maze, by a blind observer. In all experiments, the test apparatus was cleaned with 5 % ethanol and

thoroughly dried between each test period. All the experiments were carried out between 9am – 4pm. The experimental protocols were approved by institutional animal ethics committee and were conducted as per CPCSEA guidelines on use and care of experimental animals. Following behavior parameters were studied in the mice:

Percentage time spent in open arm (% TO)

It denotes the time which is spent by animal in open area (open arm). It is calculated as percentage time spent in open arm. Animals were scored as in the open area, when all four paws of animal were in an open arm (Figure 2). It was determined as follows:

$$\% \text{ Time spent} = 100 \times \frac{\text{number of seconds spent on open arms}}{300 \text{ seconds (5 min observation time)}}$$

Unprotected Head Dips (uHDIPS)

It denotes scanning by animal over the sides of the maze downward towards the floor from unprotected area i.e. uncovered open arm. uHDIPS are counted as number of head dips from open arm (Figure 3).

Head dipping from close arm (pHDIPS)

It denotes scanning by animal over the sides of the maze downward towards the floor from protected area i.e. covered close arm. pHDIPS are counted as number of head dips from close arm (Figure 4).

Stretch Attend Posture (SAP)

It is characterized by a forward elongation of the body exhibited when the animal is either standing still or moving slowly forward (Figure 5).

All the above mentioned behavioral patterns are helpful to assess anxiety behavior in rodents, which has been shown by mice behavior on elevated I maze.

Descriptive ethological analysis

A four pattern ethogram was used. Pattern names, abbreviations and their descriptions are shown in Table 1. Frequency and duration of patterns were evaluated. Mean values were calculated by adding every individual absolute measure divided by the total number of animals. Head dipping were split into protected or unprotected behaviors, on the basis they occurred in the maze. Parameters evaluated were: percent time spent in open arm, stretched attends posture and head dipping (protected and unprotected). Operational criterion for entry was whole body with four paws of the animal.

Markovian analysis

A transition matrix of observed occurrences between patterns was used as the basis for the sequential analysis. This matrix showed the frequency with which a pattern followed the previous one. For Markovian analysis, the transition matrix was converted into a stochastic probability matrix, where the sum of rows was normalized to one [6]. This conversion was based on the fact that the probability that an animal would change its behavior from one element to any / all other was one. The stochastic matrix was represented by a non-discrete stationary Markov chain, where the most relevant intertransitions between patterns could be observed ($P > 0.1$).

Experimental groups

Vehicle- treated group (Group 1) - Mice were administered with vehicle and subjected to behavioral testing on elevated I maze.

Diazepam (1 mg/kg) treated group (Group 2) - Mice were administered diazepam (1 mg/kg, ip). After 30 min, mice were

subjected to behavioral testing on elevated I maze.

Diazepam (2 mg/kg) treated group (Group 3) - Mice were administered diazepam (2 mg/kg, ip). After 30 min, mice were subjected to behavioral testing on elevated I maze.

Gabapentin (10 mg/kg) treated group (Group 4) - Mice were administered gabapentin (10 mg/kg, ip). After 30 min, mice were subjected to behavioral testing on elevated I maze.

Gabapentin (20 mg/kg) treated group (Group 5) - Mice were administered gabapentin (20 mg/kg, ip). After 30 min, mice were subjected to behavioral testing on elevated I maze.

Fluoxetine (5 mg/kg) treated group (Group 6) - Mice were administered fluoxetine (5 mg/kg, ip). After 30 min, mice were subjected to behavioral testing on elevated I maze.

Fluoxetine (10 mg/kg) treated group (Group 7) - Mice were administered fluoxetine (10 mg/kg, ip). After 30 min, mice were subjected to behavioral testing on elevated I maze.

Ondansetron (0.1 mg/kg) treated group (Group 8) - Mice were administered ondansetron (0.1 mg/kg, ip). After 30 min, mice were subjected to behavioral testing on elevated I maze.

Ondansetron (1.0 mg/kg) treated group (Group 9) - Mice were administered ondansetron (1.0 mg/kg, ip). After 30 min, mice were subjected to behavioral testing on elevated I maze.

Caffeine (15 mg/kg) treated group (Group 10) - Mice were administered caffeine (15 mg/kg, ip). After 30 min, mice were subjected to behavioral testing on elevated I maze.

Caffeine (30 mg/kg) treated group (Group 11) - Mice were administered caffeine (30 mg/kg, ip). After 30 min, mice were subjected to behavioral testing on elevated I maze.

Stressed group (6 h immobilization) (Group 12) - Mice were subjected to the immobilization for 6 h. After 6 h immobilization, mice were set free for 10 minutes and then subjected to behavioral testing on elevated I maze.

Statistics

All the results are expressed as Mean \pm S.E.M. The data were analyzed by Student's t-test and one way ANOVA. $P < 0.05$ was considered as statistically significant.

Results

In the present model, a significant increase in %TO and uHDIPS and a significant decrease in pHDIPS and SAP, by selected treatments, as compared to that in vehicle- treated mice indicate an anxiolytic- like activity. On the other hand, a significant decrease in %TO and uHDIPS and a significant increase in pHDIPS and SAP, by selected treatments, as compared to that in vehicle- treated mice indicate an anxiogenic- like activity.

Effect of Diazepam

Diazepam (1 and 2 mg/kg) produced a significant increase in %TO, an increase in uHDIPS, a decrease in pHDIPS and a decrease in SAP, as compared to that in vehicle- treated mice (Figures 6-9). All these data suggest that diazepam show an anxiolytic- like activity on I maze.

Effect of Gabapentin

Gabapentin (10 and 20 mg/kg) produced a significant increase in



Figure 1 (a): Top view of I – Maze.

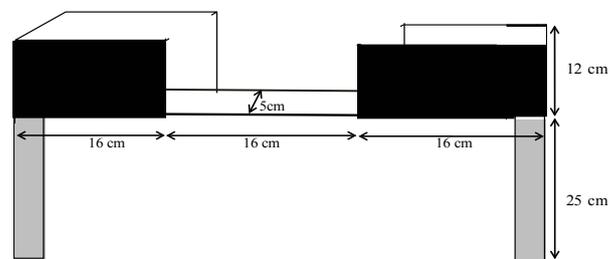


Figure 1(b): Sketch of Three Dimensional View of Novel I – Maze.



Figure 2: A mouse in open arm .



Figure 3: A mouse Unprotected Head Dipping.

%TO, an increase in uHDIPS, a decrease in pHDIPS and a decrease in SAP, as compared to that in vehicle- treated mice (Figures 10-13). All these data suggest that gabapentin show an anxiolytic- like activity on I maze.

Effect of Fluoxetine

Fluoxetine (5 and 10 mg/kg) produced a significant increase in %TO, an increase in uHDIPS, a decrease in SAP. However, Fluoxetine (5 and 10 mg/kg) did not produced any change in pHDIPS as compared to that in vehicle- treated control mice (Figures 14-17). All these data suggest that fluoxetine show an anxiolytic- like activity on I maze.

Effect of Ondansetron

Ondansetron (0.1 and 1 mg/kg) did not produced any significant change in all the parameters, as compared to vehicle- treated control group (Figure 18-21).

Effect of caffeine

Caffeine (15 and 30 mg/kg) produced a significant decrease in %TO, a significant decrease in uHDIPS, an increase in pHDIPS and an increase in SAP, as compared to that in vehicle- treated mice (Figures 22-25). All these data suggest that caffeine show an anxiogenic- like activity on I maze.

Effect of stress

Stress induced by immobilization of 6h produced a significant decrease in %TO, a significant decrease in uHDIPS, a significant increase in pHDIPS and a significant increase in SAP, as compared to vehicle- treated mice (Figures 26-29). All these data suggest that immobilization induced stress show anxiogenic- like activity on I maze.

Descriptive Ethological analysis

Mean frequency and duration of stretch attend posture, head dips (protected and unprotected) and percent time spent in open arm is shown in Table 2.

Markovian analysis

The transition matrix of observed occurrences is shown in Table 3. This transition matrix was transformed into a stochastic probability matrix (Table 4). The stochastic matrix was simplified and represented by Markov chain (Figure 30). Protected head dip is the central behaviour of the mouse. Stretched attend posture and unprotected head dip are linked to this central behaviour. Stochastic probability of occurrence of behaviour is 0.5 – 1, shown in the Figure 30.

Discussion

An experimental model of anxiety should be analogous to the human disorder in symptoms. A model is required to produce a behavioral change that (a) can be monitored, (b) should respond to standard clinical treatments and (c) should exhibit reproducibility in animal behavior [7]. An animal model should at least display three kinds of validity namely (a) face validity i.e. it should produce anxiety-like symptoms in animal, (b) construct validity i.e. its physical design should produce similar biochemical changes as observed in clinical anxiety and (c) predictive validity i.e. animal behavior on the maze should respond to standard therapeutic treatments [8]. The present maze studies in the present paper bears a physical design that is helpful in studying detailed anxiety- like behavioral changes. Further, "I-maze" provides an environment, which help to increase the sensitivity to, and facilitating interpretation of drug action. The novel apparatus do not

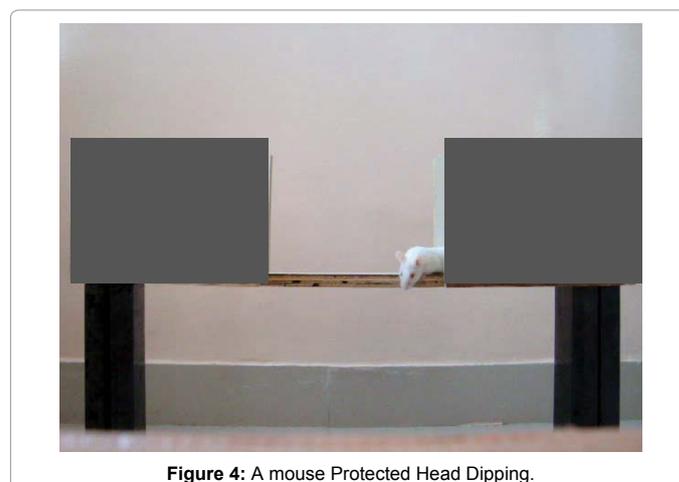


Figure 4: A mouse Protected Head Dipping.

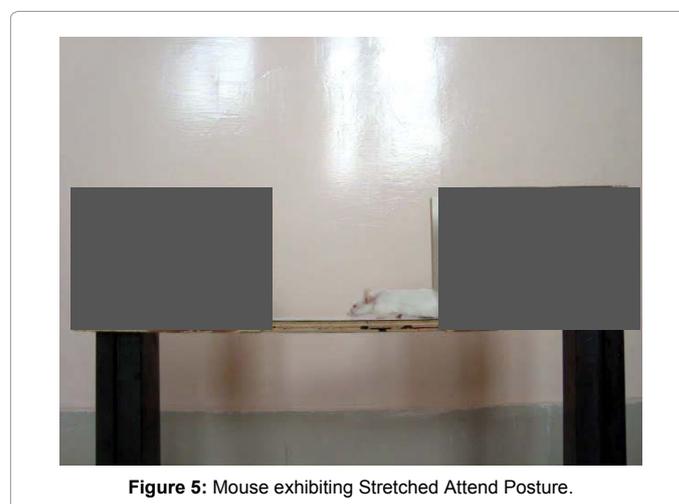


Figure 5: Mouse exhibiting Stretched Attend Posture.

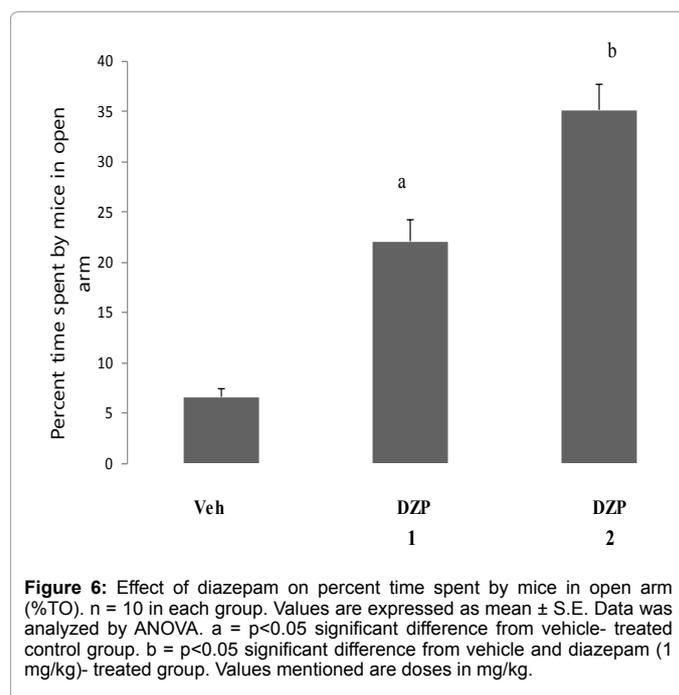
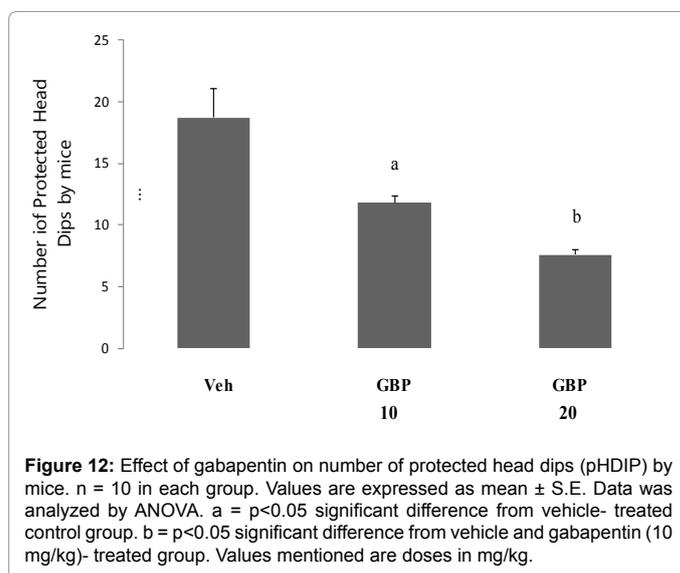
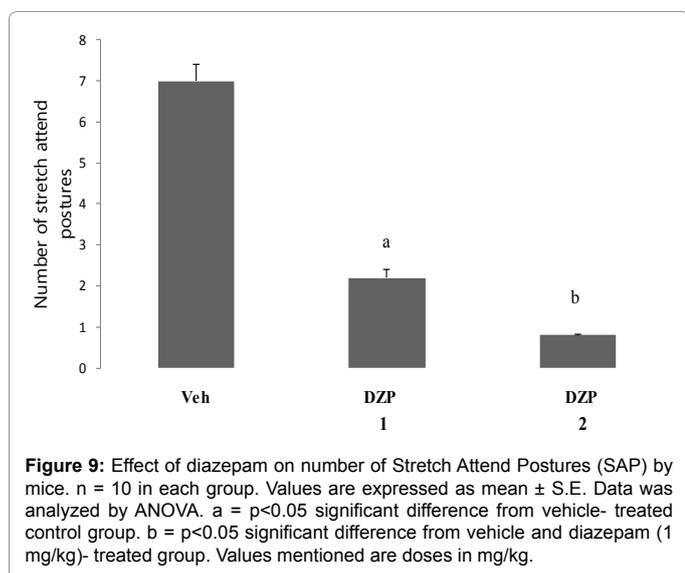
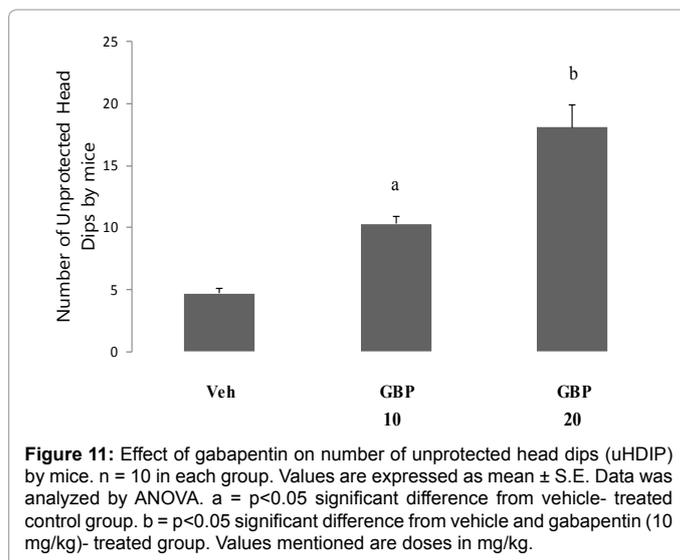
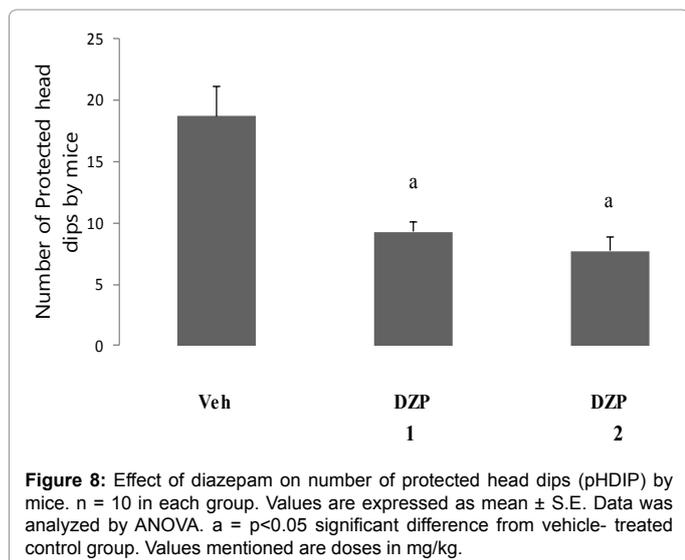
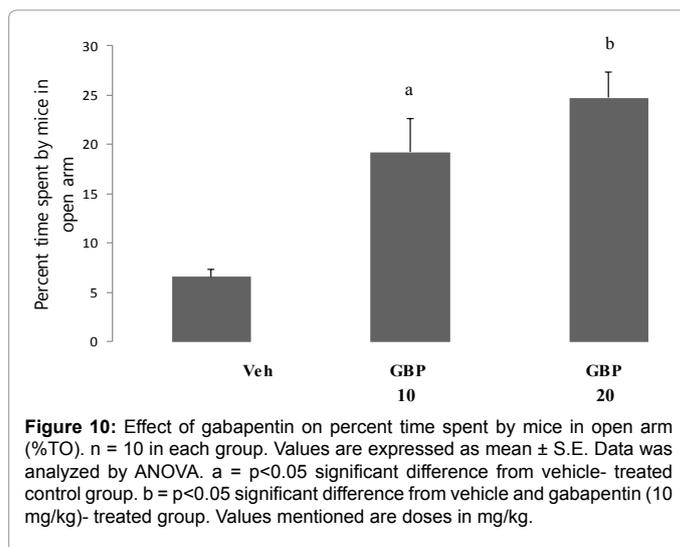
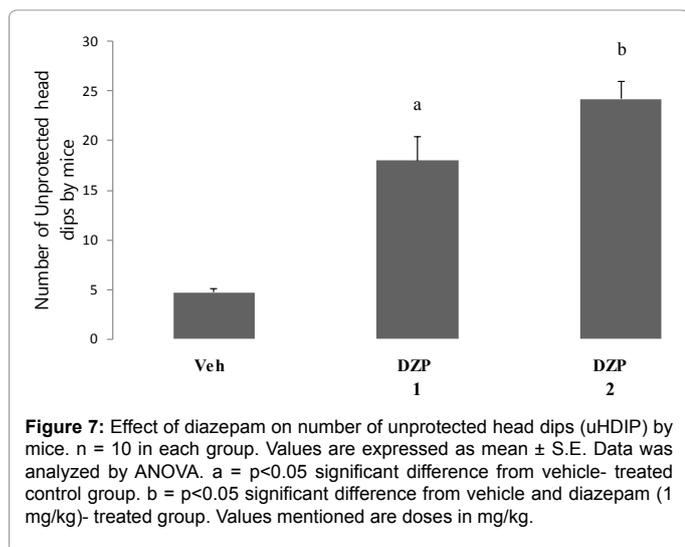
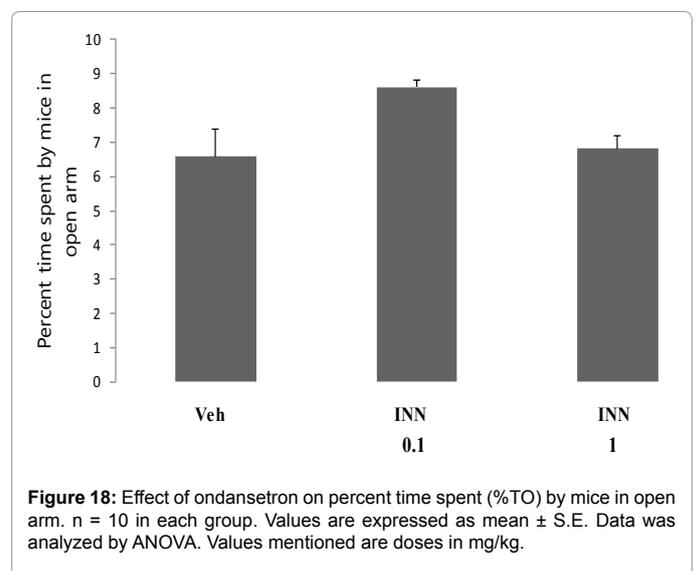
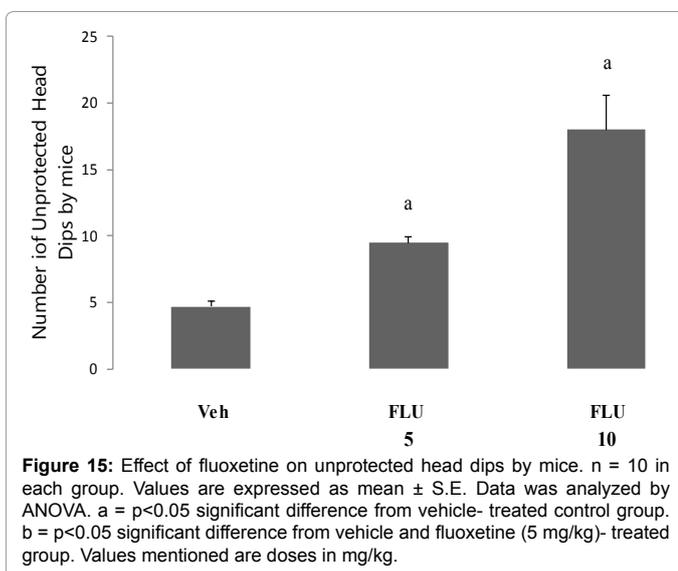
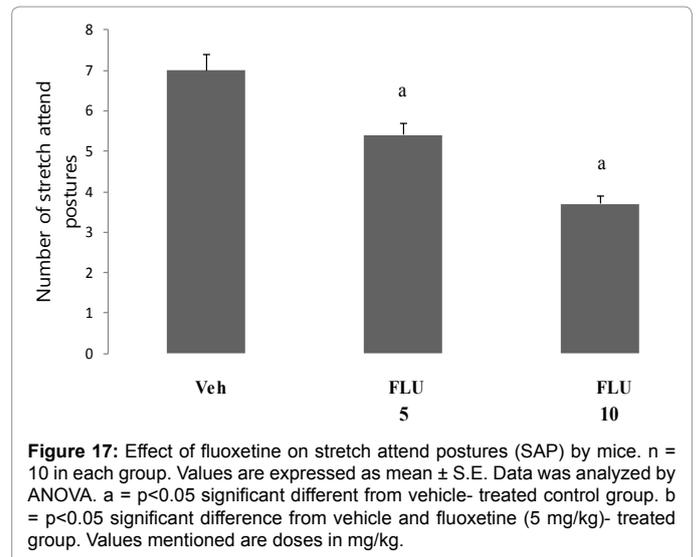
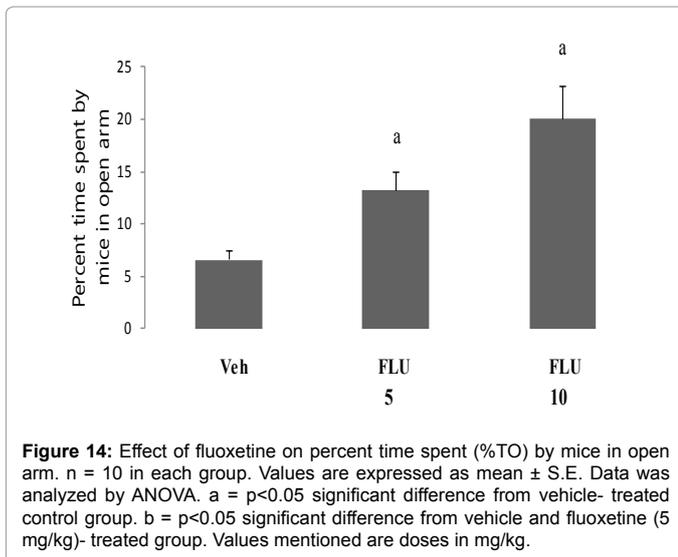
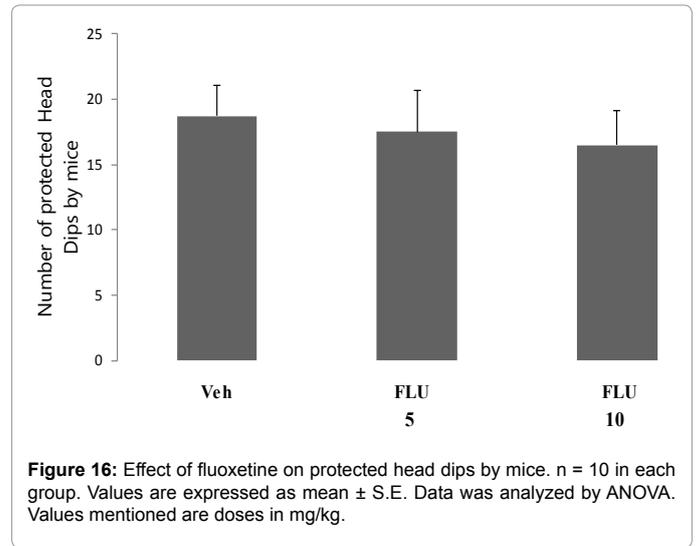
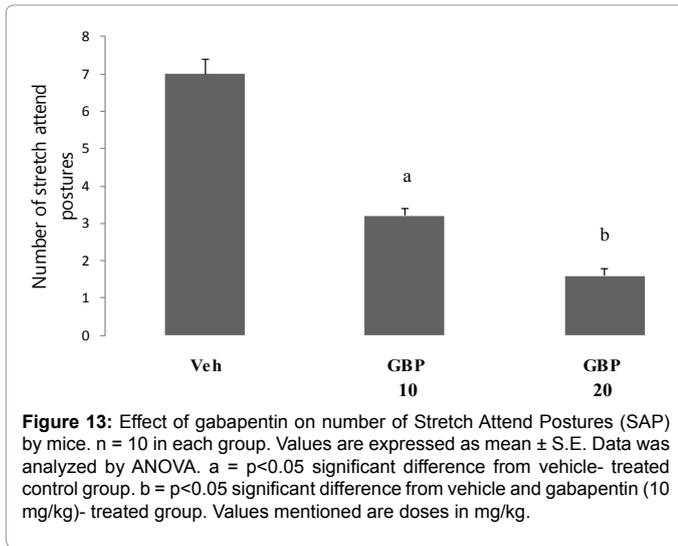
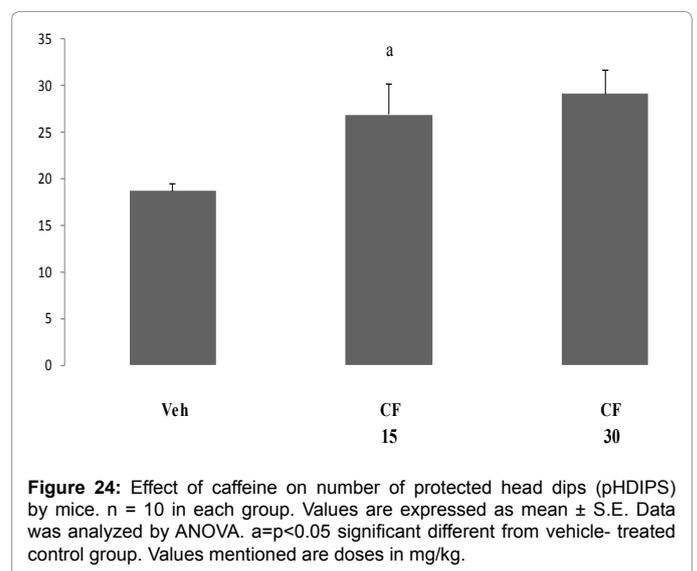
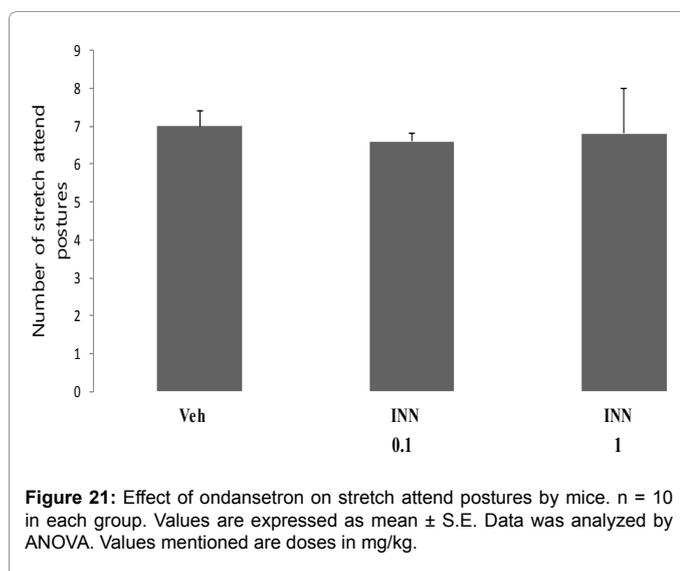
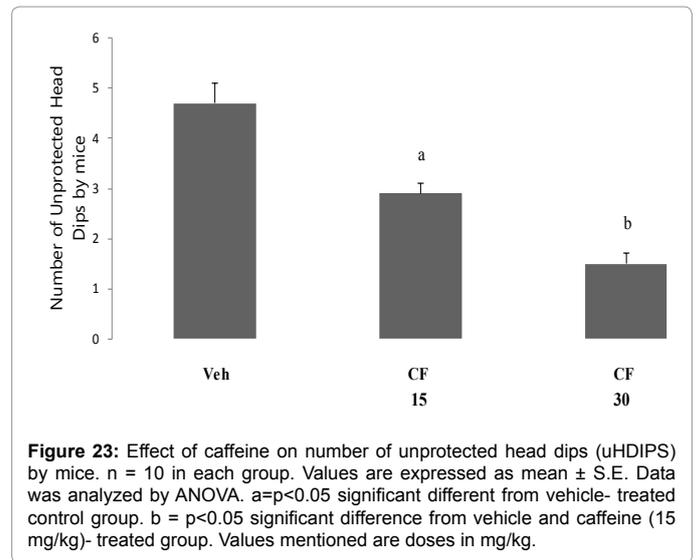
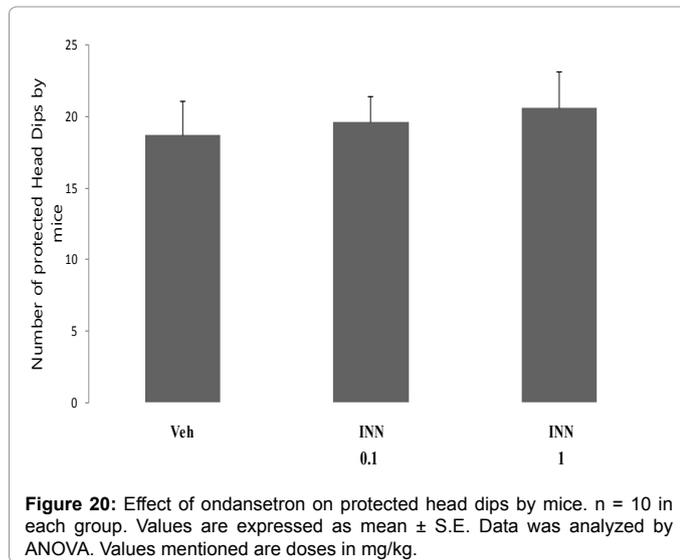
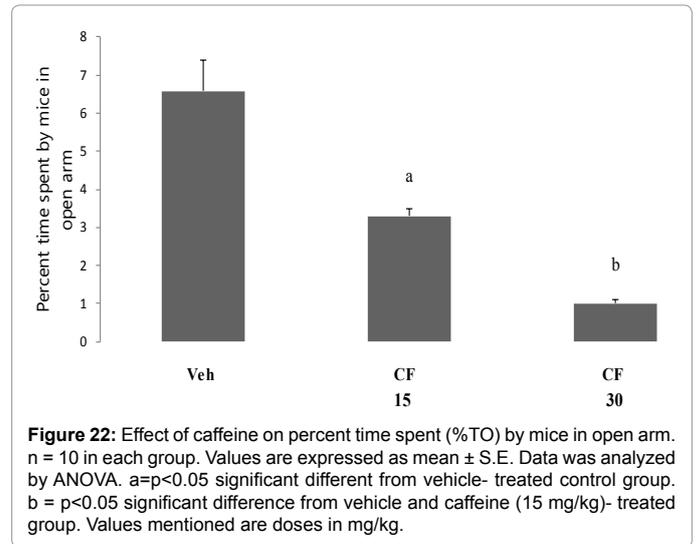
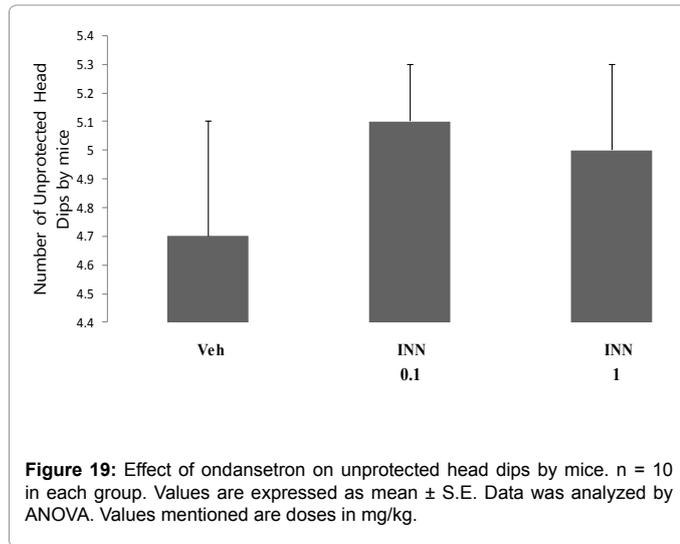
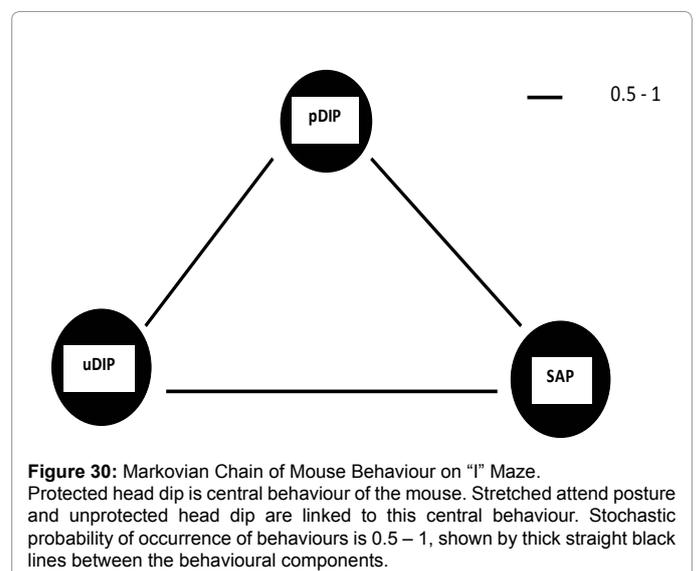
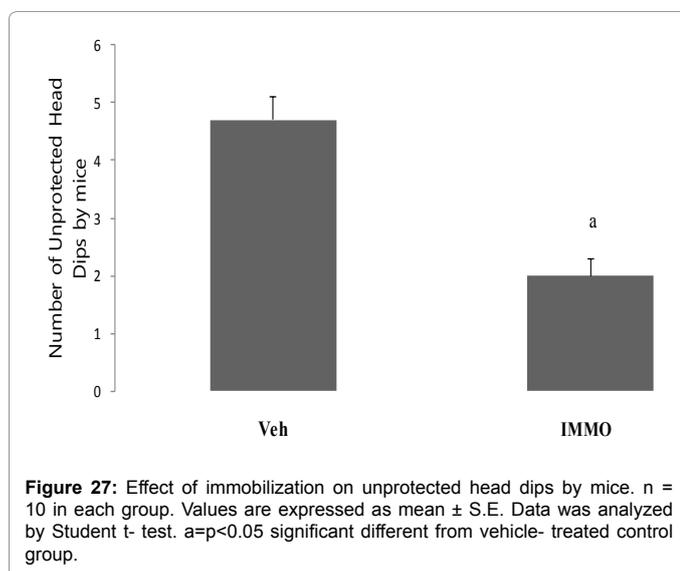
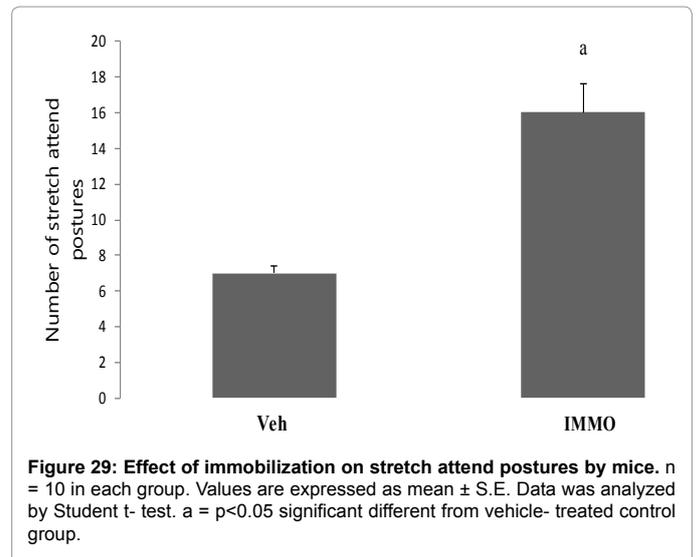
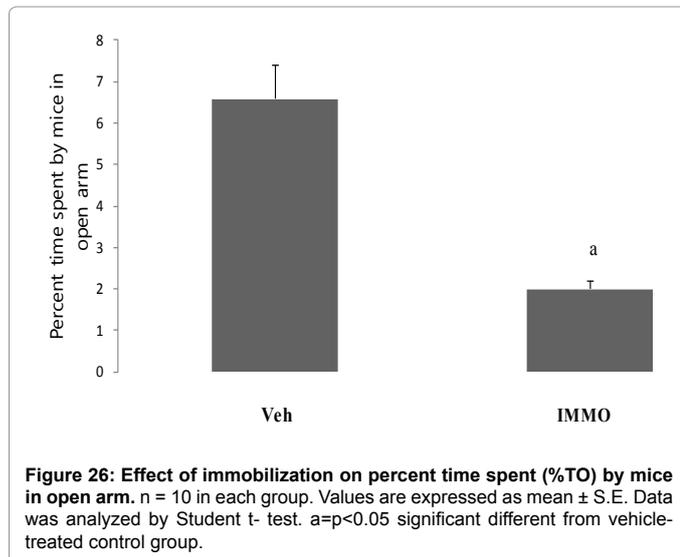
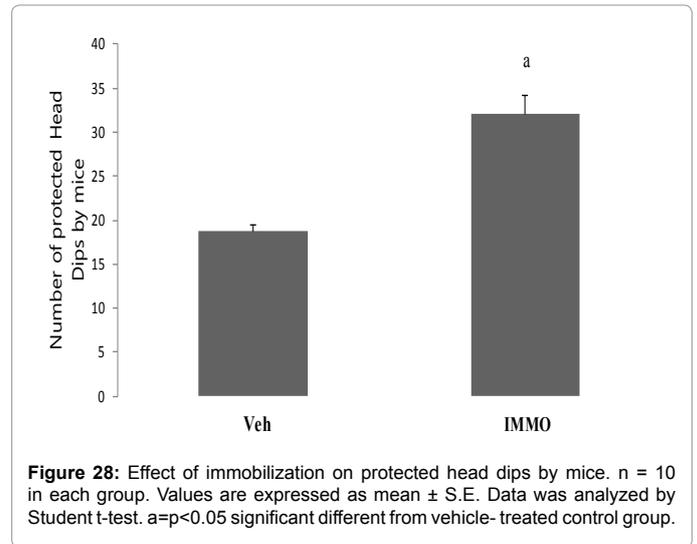
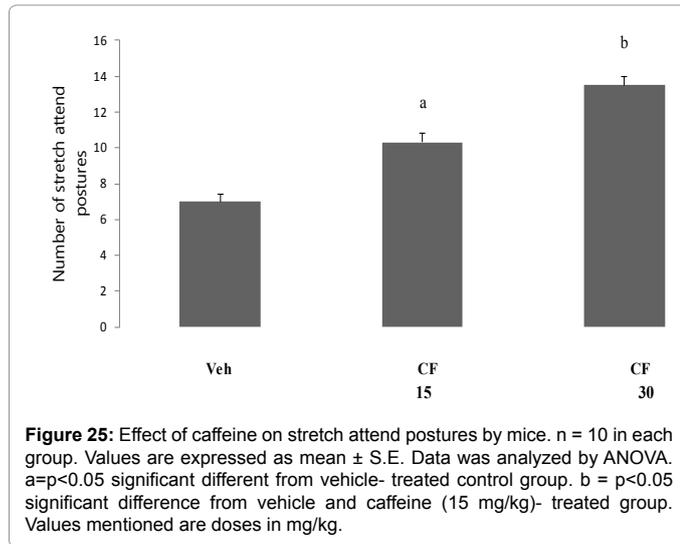


Figure 6: Effect of diazepam on percent time spent by mice in open arm (%TO). n = 10 in each group. Values are expressed as mean \pm S.E. Data was analyzed by ANOVA. a = p<0.05 significant difference from vehicle- treated control group. b = p<0.05 significant difference from vehicle and diazepam (1 mg/kg)- treated group. Values mentioned are doses in mg/kg.









Abbreviation	Pattern and Description
SAP	Stretched attend posture (forward elongation of the animal body and retraction to the original position)
pDIP	protected head dips (Scanning over the sides of the maze from closed arm towards the floor)
uDIP	unprotected head dips (Scanning over the sides of maze from open arm towards the floor)
% TO	Percent time spent in open arm

Table 1: Ethogram employed for mouse behaviour in the maze.

		VEHICLE	1 mg/kg	2 mg/kg
		SAP	6±0.2	3±0.2
	pDip	17±0.4	10±0.2	7±0.3
	uDip	5±0.1	16±0.4	22±0.4
	%TO	5±0.1	20±0.4	31±0.4
		VEHICLE	10 mg/kg	20 mg/kg
GABAPENTIN	SAP	8±0.4	3±0.2	2±0.2
	pDip	18±0.6	10±0.2	7±0.1
	uDip	5±0.2	10±0.2	18±0.5
	%TO	6±0.2	19±0.7	24±0.6
FLUOXETINE		VEHICLE	5 mg/kg	10 mg/kg
	SAP	7±0.3	6±0.2	4±0.1
	pDip	17±0.4	16±0.5	12±0.4
	uDip	4±0.1	10±0.1	16±0.7
	%TO	6±0.1	15±0.3	22±0.6
ONDANSETRON		VEHICLE	0.1 mg/kg	1 mg/kg
	SAP	7±0.3	6.8±0.1	7.20.8
	pDip	16±0.7	21±0.6	24±0.7
	uDip	4.7±2.1	5.1±1.4	5±1.7
	%TO	6.5±0.5	8.0±0.1	5.5±0.2
CAFFEINE		VEHICLE	15 mg/kg	30 mg/kg
	SAP	7±0.2	10±0.2	13±0.2
	pDip	17±0.1	22±0.6	29±0.5
	uDip	4.8±0.4	3±0.2	1.5±0.2
	%TO	6.5±0.6	3±0.2	1±0.1

Values are mentioned as mean ± S.E.M

Table 2: Ethological Analysis: frequency and duration of animal movement pattern

	Oe	pDIP	uDIP	SAP	RT
Oe	0	2	2	0	4
pDIP	0	1	0	1	2
uDIP	0	0	1	1	2
SAP	0	0	0	1	1
CT	0	3	3	3	-----

Oe - Open arm entry; pDIP - Protected head dip; uDIP - unprotected head dip; SAP - stretched attend posture; RT- Row Total; CT- Column Total

Table 3: Transition Matrix of Observed Patterns.

	Oe	pDIP	Udip	SAP
Oe	0	0.5	0.5	0
pDIP	0	0.5	0	0.5
uDIP	0	0	0.5	0.5
SAP	0	0	0	1

Oe - Open arm entry; pDIP - Protected head dip; uDIP - unprotected head dip; SAP - stretched attend posture. Probability values are higher than 0.1

Table 4: Stochastic Probability Matrix.

Component	Type of Principal Component	Positively Loaded Behaviour	Negatively Loaded Behaviour
I	Anxiety- like Behaviour	Percent time spent in open arm	pDip
		Unprotected head dip	-
II	Approach Avoidance Conflict	Stretch attend posture	-

Table 5: Principal components of the mouse behaviour on the maze.

possess central platform, as mice have been observed to spent 20-30% time on central square of elevated plus maze [5]. Removal of the central area further help to detect drug effects on time spent in the open area [3]. Design of I-maze covers maximum relevant features of anxiety – (a) open and close arms for exploration (b) No central platform; (c) clear demarcation between open and closed areas, that (i) contrasts the stretched attend postures (SAP) and (ii) provides clear differentiation between protected (close) and unprotected (open) environment to observe unprotected and protected head dips. Therefore, I-maze offers feasibility of detailed behavioral analysis by employing *viz.* (a) unprotected head dip (b) protected head dip and (c) stretch attend posture; the relevant features of human anxiety. The behaviors that are considered as experimentally reliable behaviors for studying anxiety in rodents are (a) time spent in open space, (b) Number of head dips from open area of the maze, (c) Number of head dips from closed area of the maze and (d) number of stretched attend postures by animal on the maze. These behaviors can be experimentally recorded reliably in a similar manner as adopted and reported by several investigators in anxiety research [9,10].

Present data indicate that the standard clinical therapeutic option for anxiety i.e., diazepam has produced a clear and consistent behavioral change in test animals in the I-maze. The proportion of time spent on the open area (one of the behavioral change recorded in the elevated plus-maze procedure or Zero maze) was increased by diazepam. A detailed behavioral analysis after administration of diazepam also indicated the similar significant increases in exploratory head dipping of animals from open space of the I-maze and decrease in stretched attend postures from the closed to the open arm. There is an evidence to support the view that a decrease in SAP is consistent with reduced anxiety [3,11-13]. This finding is in line with observations with the mouse elevated plus-maze, in that diazepam increased percent time spent by mice in open arms. Further, I-maze procedure does not involve measurement of number of entries in open or closed arm of maze, as this parameter is associated with conflicting opinions that it may not reflect a measure of anxiety, but more of locomotor activity. The dual nature of design of I maze facilitates not only detailed behavioral analysis but also serve to clearly indicate anxiogenic or anxiolytic behavior in mice, because it also facilitates the measurement of stretched attend postures in addition to percent time spent in arms.

Though, it is a generally accepted requirement to validate animal models of anxiety with benzodiazepines. But it is equally needful to search for novel classes of anxiolytics which are free from the numerous problems associated with chronic benzodiazepines. The effect of the 5-HT anxiolytic; ondansetron in the I-maze is important in view of the variable reports in the literature with this drug. In this category, ondansetron has given inconsistent results in the past literature including anxiolytic [14,15] and no effect [16,17]. In the present study too, data failed to indicate any significant effects of ondansetron. It is understood that an objective of a novel animal model is the detection of anxiolytics which produce their effects in a different manner to those of the benzodiazepines, probably acting via different underlying neurochemical mechanisms. Therefore, it would be acceptable to record behavioural profile that is achieved using non- benzodiazepines.

Among anticonvulsants, used in the treatment of anxiety are carbamazepine, valproate and gabapentin, pregabalin and tiagabin. Gabapentin has been found to increase GABA content after its administration [18]. It has also been used to validate elevated plus maze and zero maze, two effective models of anxiety [19]. In the present study too, gabapentin has significantly exerted antianxiety- like profile as it

has significantly increased time spent by mice in open arm of I-maze as well as increased the unprotected head dips and significantly decreased the protected head dips as well as stretch attend postures by mice.

SSRIs have been observed to be most effective for anxiety treatment [20]. Fluoxetine has also shown a significant antianxiety-like activity as it significantly increased time spent by mice in open arm of I-maze as well as increased the unprotected head dips and significantly stretch attend postures by mice. However, it failed to produce any significant change in number of protected head dips in mice as compared to vehicle- treated control mice. Observations with caffeine as an anxiogenic agent [21], further illustrate the utility of I-maze for detailed behavioral analysis of mice. Results with caffeine on I-maze indicate the anxiogenic- like activity, as evident by a significant decrease in behaviors, indicative of increase in anxiety like (a) time spent by mice in open area of the maze and (b) unprotected head dips and a significant increase in behaviors, indicative of anxiety like (a) protected head dips and (b) stretch attend postures.

The descriptive ethological study gave a good picture of mouse behavior, which is based on mean frequency and duration of exhibition of each observed behavior. The most exhibited behaviors were uHDIPS (unprotected head dips), pHDIPS (protected head dips) (Figures 3,4). Mice were placed on the maze for 5 min for analysis of behavior. pHDIPS (protected head dips) and SAP (stretched attend postures) are commonly used behavioral indicators of anxiety- like behavior. The present model is based on natural fear of mice to open environment and elevated platforms. Resultantly, mice have a tendency to avoid the open arm of I maze in the present study. This change in behavior usually measured for evaluating changes in anxiety state, e.g. after drug treatment.

Markovian sequential analysis gave a picture of the sequential structure of mouse behavior. In the present study, the directionality found in the mouse behavior is a feature of behavioral systems, and reliable changes in the sequential structure are of value for testing drugs. pHDIPS was shown to be central pattern, as expected in rodents. So, pHDIPS is the main behavior of mouse on the I maze. pHDIPS and SAP are important anxiety related behavior and linked to each other, which mice usually display in succession secure closed arm and central section of the maze. If there is CNS manipulation or a pharmacological treatment given to the rodents then changes occur in transitional probabilities between pDIP and other behavior response also changes in directionality between anxiety-related responses.

As for principal component analysis, two principal components were obtained in which four factors are present (Table 5), four factors occur through analyzing the correlation between them. Principal 1 component is consisting of anxiolysis including %TO and uHDIPS and pHDIPS. %TO and uHDIPS positively loaded on component 1, and contrast to pHDIPS, which is oppositely related which is loaded on negatively (i) the higher the value of % TO and uHDIPS, the lower the anxiety level, and (ii) the higher the value of pHDIPS, shows the stronger anxiety level. In the second component include SAP which is considered as anxiety related response but this is poorly loaded in component 1. Hence, this factor appears to reflect a different aspect of anxiety.

These results shows that %TO, uHDIPS and pHDIPS are the best behavior parameters for measuring anxiety in rodents placed on the I maze. %TO and uHDIPS values were indicated anxiolytic, while uHDIPS and SAP indicated the anxiety. It is well known that anxiolytic drugs diazepam, fluoxetine, gabapentine and 5-HT antagonist

ondansetron increase uHDIPS, %TO behavior and decrease SAP, caffeine is anxiogenic drug, that decrease uDDIPS, %TO and increase SAP.

The all results found that unprotected and protected behaviors have different profiles (uHDIPS and pHDIPS are inversely related), confirming that differentiate protected and unprotected behaviors. SAP is considered as anxiety – related pattern and loaded on an independent component to that of anxiolysis, which shows the approach-avoid conflict behavior.

Resultant observed advantages of "elevated I-maze"

- o I-maze avoids central platform of plus maze.
- o In plus maze, observations are based on animal's preference for any one arm, which is chosen after its movement from the arm in which the animal is present in that very time frame. On the other hand, I-maze offers an ever-ready vision of closed arms in front of its eye. This ever-ready vision helps animal to make a quick choice without spending even a single second of observation period (300 s). Straight vision and choice inherent in I-maze serve to avoid a time lapse spent by animal in any of the arm and a situation, where animal can voluntarily choose or decline a clear visible option.
- o Design of I-maze facilitate expedited exploration of maze because, as the animal reaches at the end of closed end, it spontaneously turn back and it encounters a much clear and wider view (option) of open space, present inherently between two closed arms (choices). This situation presented by I-maze further help animal to clearly make its choice between open or closed arm without being fascinated or intrigued by longer animal stay in closed as well as open arms of plus maze.
- o I-maze provides a visible clue to animal, besides providing a relatively "frank" open space, so that if, at all, animal is lost in its complex thoughts; arising out of integral confusion of anxiety disorder, as compared to the elevated plus maze or Zero maze, where other arms are not visible, when an animal is in any one arm (open or closed).
- o Further, authors opine that I-maze provides a robust measure of animal's preference for any of the two environments because I-maze tests the animal preference in relatively "frank" options.

Conclusion

In conclusion, preliminary evidence, suggest that the design of elevated I maze, facilitates expression of anxiety- like behavior of mice, which is clearly modified by selected anxiogenic and anxiolytic treatments, and represent a new and equally reliable model of anxiety for detection of different categories of anxiogenics and anxiolytics.

However, in order to establish the reliability and reproducibility of the present model, it is recommended that the maze be utilized by different laboratories so that results on animal behaviors are recorded under different laboratory, environmental and seasonal conditions. Further, the maze needs to be tested with agents and treatments that provide different mechanism of anxiogenic and anxiolytic stimuli to animal under behavioral observations.

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