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Research Article

Use of TSD (Time Space Determinant) Maze, CSS or CLOSE Maze to Measure Spatial Navigation, Working Memory, Reference Memory, Memory Errors, Locomotors Activity and Anxiety Like Behavior in Rats

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ABSTRACT

The Open field maze was one of the utmost frequently used platforms for assessment of behavioral profile of model animals. Initially an open-field use was defined in Hall and Ballechey's 1932 paper, "a study of the rat's behavior in a field: A contribution to method in comparative psychology". Open Field Test (OFT) was a modest apparatus used in the evaluation of anxiety, exploration, and locomotion activity. TSD (Time Space Determinant) maze, CSS or CLOSE maze was recently developed in our laboratory aimed to find the cells involved in earlier food seeking in rats given food stimulus in short and long pathway. However, it demonstrates relatively optimal test that provide various information of behavior ranging from motor activity, emotionality, navigation, cognition and memory of tested animal. Open Field Test (OFT) was used in the evaluation of limited behavioral profile anxiety, exploration, and locomotion activity. However, a range of optimal behavioral tests as motor activity, anxiety, emotionality, spatial navigation, cognition, memory and memory errors evaluated *via* TSD maze equipped with video tracking software. A TSD maze comprises of a wall enclosed area that is of adequate height to prevent the escaping of rats. The TSD maze was recently used platform to measure in animal model especially in rats. It is relatively optimal test for evaluation of behavioral profile in rats. This procedure will readily become useful to investigate the effect of different pharmacological compound on learning and memory as well as anxiolytic and anxiogenic effects.

Keywords: TSD; Emotionality; Navigation; Anxiolytic; Anxiogenic

INTRODUCTION

The Open Field Maze (OFM) was well described apparatus developed in 1934 to measure the emotionality in rodent's models. It has gained importance to measure behavior profile in animal psychology [1,2]. Various shortcoming confronted while application of OFM these include time, duration of light and interference of foreign object. Variations in protocol of experiment which are necessary for wide range of application create difficulties in comparison of studies.

TSD (Time Space Determinant) maze, CSS or CLOSE maze was recently developed in our laboratory aimed to find the cells involved in earlier food seeking in rats given food stimulus in short and long pathway. He named these cells TSD (time space determinant cells) and maze used was given name as TSD maze. CSS denote its shape. CLOSE refer to its close 11 and 12 path.

It has attained the status of most widely used maze for measuring psychological behavior of animal. It provides easy and fast analysis of well-defined behavior require a little training to test rodent and no special training for researcher conducting the test. These properties will lead to wide spread use of TSD maze in research ranging laboratory rodent to wild rats. The reason from its superiority is that physiological and psychological concepts essential for these tests are straight forward and well comprehensible.

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MATERIALS AND METHODS

A TSD maze comprises of a wall enclosed area that is of adequate height to prevent the escaping of rats. The shape of maze comprised of long and short zig zag in CSS shape with enough area, depending on the size of the testing animal. Several parameters can be recorded in the TSD maze with maximum parameters including various kinds of locomotors activities. Most commonly ambulation behavior is studied but others such as rearing or latency can also be recorded.

Mostly bare maze is used in analysis of rodent behavior. Thus, the addition of rats, either of given pathway, adds the capability to see how the rats navigate in food stimulus in in less time and space arena. Significant parameters when rodents are accessible are typically thigmotaxis, fecal boli, rearing, emotionality and anxiety like behavior. Many tests of anxiety related behavior are established on the body activity of testing animal and locomotion. However, interpretation of emotionality behavior from non emotional, such as motor activity, has been matter of investigation. As the TSD maze was initially designed, two parameters of behavior were assumed, spatial navigation and time space navigation. However, studies have shown that these two measures provide unrelated supporting conclusion as emotionality is multidimensional in rodents. However, there are differences in the research about these parameters' emotionality or anxiety in rodent models. Investigator conclusively relates results from analysis of TSD maze with other procedure of anxiety while comparing rodent models (Figure 1).

Figure 1: TSD maze, CSS maze or close maze.

Preparation of room for testing and close maze apparatus

- Use a two unit TSD maze comprising of two zig zag short and long pathways was used for this analysis. Long zig zag pathway measured 391.1 cm (length) × 10 cm (width) × 15 cm (height) and was made from high density glass and short pathway was measure 313.9 cm. Total length of both pathways was 705 cm
- TSD maze was consisting of a door of 5 cm in diameter with additional windows in roof of pathways at the end of both pathways.
- Maze floors were texture for traction throughout ambulation however maze walls were kept smooth. Maze pathways were fully empty for the performing the test. In concern of the rest of this protocol, both pathways of the maze mention above will be used to represent the TSD maze.
- Prior to use wipe the both pathways before tests with a 95% ethanol and eradicate any scent secreted by the previous testing rat.
- Waited some time before testing rat to evaporate ethanol completely. This may take 5 min-10 min between each testing session.
- The analysis was manually performed due to unavailability of video tracking camera and software.
- As experimenter, be sure that there should be enough space in the room to be entirely imperceptible by the rodent being tested in the maze so that rat's behavior may not have influenced.

Administration of the TSD maze test

- Bring the rat from housing room into their testing room in steel cages. Prior to starting the test allow them to acclimate to the procedure room for at least 30 min.
- Gently grasping rat by its tail remove it from the cage and place the rat in the front door of TSD maze while simultaneously observing the behavior of rats and carry stop watch for time measurement. Normally the rats move instantly to the boundary walls of the maze and the timing of release and food navigation of the rat should have recorded to measure this movement.
- Allow the testing rat to move freely and continuous throughout the respective pathway of the maze for a 15 min period during this time, the observer track the distance and time.
- Pick up the rat gently at end of the test period, remove it from the maze and return it to its steel cage.
- Before cleaning the maze manually count the fecal boli found in maze.Wipe up all spots of urination after removing all fecal pellets and spray the floor and walls of the maze pathway with 95% ethanol. The ethanol solution should be completely dried before testing next rat.
- Repeat this method for all rats.

The TSD maze is one of the most recently used protocols for studying behavior of animals. During the TSD maze performance multiple important conventional and ethological measures are composed and evaluated. These data obtained from TSD maze allowed the investigator to analyze behaviors such as locomotor activity to rearing, anxiety, thigmotaxis, object recognition, spatial navigation, learning and memory. However, there are some shortcomings in use of TSD maze. One confusing matter is the multiple static variables that must be manipulated while performing tests. For example, time, novel object inclusion and lighting conditions. In spite of these problems, the TSD maze considered as one of the most efficient technique in rat's behavior research.

Here, seven features of TSD or CLOSE maze are readily characterized while behavioral study using this procedure.

- During entire testing session the measurement of distance (cm) covered by rats.

- Thigmotaxis or wall hugging behavior it is measure of anxiety and characterize by the time rats remain adjacent to wall of TSD maze for time duration of 15 minutes.
- Counting the number of fecal pellets in each pathway after the removal of rats.
- Emotionality defecation is a negatively associated with emotionality in rodents and can be used to specify levels of anxiety in the rats.
- Spatial navigation depends upon path preference by rats when food stimulus is baited in ends of both l1 and l2 short and long pathway. Short pathway preference is indication of normal function of hippocampal grid cells, place cells, head direction cells and specially TSD cells. Working memory and reference memory.
- Anxiety, thigmotaxis or wall hugging behavior it is measure of anxiety and characterize by the time rats remain adjacent to wall of TSD maze for time duration of 15 minutes.
- Time space navigation specially depends upon the function of TSD cells.

Evaluation of working and reference memory and errors in TSD or CSS maze

Working memory can be demarcated as a memory for an object, recognition, or location that is used within a testing period, but not usually between the periods. It is variant from reference memory which is demarcated a memory that would typically be attained with rehearsal training and would sustain from days to months. The reference memory is mostly the memory for the 'rules' of a given chore. For instance, when testing object press a bar receive a food object or a water maze established with a hidden platform or entrances into the food containing pathway of the TSD CSS maze. Moreover, working memory enable the testing object to remember which pathway it had visited in a testing period.

Testing animal adaptation period

The rats were presented two periods of adaptation on two succeeding days before the learning process commences. The testing rats were allowed to walk around the food baited pathway of the maze for 15 min during the testing time. The testing rats were explored the TSD maze baited with food stimulus first in long pathway, then food baited in short pathway and at the end food baited at both pathways and path acquisition in each case was recorded. Following the adaptation period, the acquisition process was ongoing.

Testing animal acquisition career

During the testing animal acquisition career or (learning session), the rats were assumed three trials of acquisition per day until the rats achieved the learning criteria. The learning criteria were confronted as follows. The trial was sustained for 15 min and the training was continuous until the rats achieved the criteria of 80% correct choice; *i.e.*, at least four correct entries out of five. The duration of this session varies depending upon condition of research procedure the maze was washed with ethanol (70%) at start of trial session and thereafter one path was baited with food stimulus. For first trial the rat was kept in central box and was permitted to choose any pathway. When a rat reached the end of pathway and ate the bait reward, the path choice was noted. Only the first approach to the baited pathway was documented as a correct choice and the maze pathway. For second trial the pathway was rebaited and entries of rat in baited pathway were recorded. Entrances into the path containing no food stimulus were recorded as Reference Memory Errors (RME). For third trial the both pathways were baited with food stimulus and path entries of rat was recorded reentries into baited pathway when both pathways are baited referred as WME. For fourth trial the both pathways were baited and choice of short pathway was recorded as correctness of TSN. Each rat was assumed four trials per day and obtained data from the four trials were averaged and included in analysis of final data. The performance pattern of rats was recorded by the percentage of the correct choices, RME and TSN (Time Space Navigation) in TSD maze, COSE maze or CSS maze.

RESULTS

Demonstrative results

Effect of Buprofezin on spontaneous behavior and impact of atropine: Spontaneous behavior testing was performed in TSD maze for 15 minutes on adult male rats. Total locomotion activity and rearing mean was measured in all testing rats. Results have shown the rats receiving acute exposure of buprofezin exhibited significant decrease in locomotion and rearing activity and pre-treated atropine rats represent no significant difference compared to control reveals reversal of Buprofezin toxicity by atropine. Decrease number of rearing represent the anxiety and depression behavior in buprofezin treated rats (Figure 2).

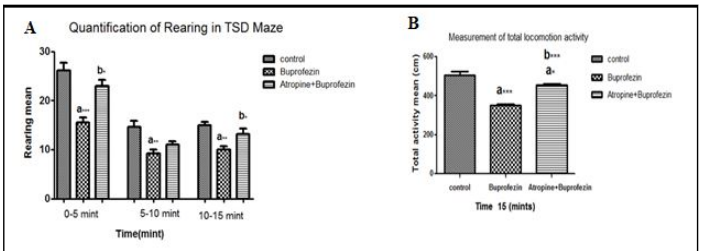

Figure 2: A) Demonstrated the significant protective effect of pre-treated atropine against Buprofezin induced anxiety (less no of rearing) in TSD maze for 15 minutes' duration. Note: P value=*p<0.05, **P<0.01***p<0.001. B) Represent the substantial reversal of total locomotion activity by pre-treated atropine caused by acute Buprofezin exposure in 705 cm long TSD maze. The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is demonstrated as mean ± SEM. Note: P value=*p<0.05, ***p<0.001. a=significant different from control; b=significant different from pre-treated atropine.

Buprofezin induced impairment in working memory, reference memory and spatial navigation was counteracted by atropine

Correct choice of path during acquisition career: Results were obtained by using one way anova followed by Tukey post hoc multiple comparison test. Our finding has revealed a significant loss of correct path choice during acquisition session in Buprofezin exposed rats compared to control. Rats were subjected to five trials for each and percentage of correct choice

was calculated. At first day the control group was unable to reach 80% correct choice of path. Second day after continues trial rats ultimately obtained 80% correct choice of path. Impairment of correct path choice was counteracted by pretreated atropine.

Correct choice of path during navigation session: In time space navigation session, the rats were trained to obtained food stimulus from shorter pathway to reach maximum 10% of correct choice although food is baited in both pathways. We have found less than 10% of correct choice of control rats at first day and Buprofezin treated rats showed significantly less correctness compared to control. Moreover, pre-treated atropine has reversed the effect. On second day control rats achieved the criteria of 10% correctness after five trials of training. Buprofezin again decreases the choice correctness. These finding suggest the deterioration and apoptosis of TSD, grid cells, speed cells and hippocampal place cells following the acute exposure of buprofezin (Figure 3).

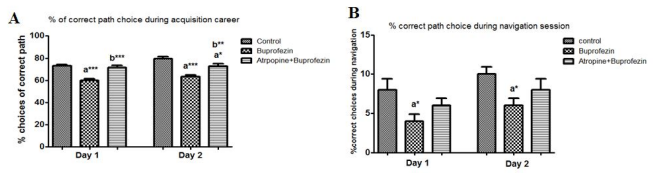

Figure 3: A) Showed the % of correct path choice during acquisition session and reversal of Buprofezin induced incorrect path choices by pre-treated atropine in adult male rats. Pre-treated atropine has shown to increase % of correct path choice. Note: P value=*p<0.05, **P<0.01***p<0.001. B) Represent the reduced %of correct path choice during navigation on day 1 and its counteraction by pre-treated atropine. Correctness of path selection is increased with training on day 2. The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Values are presented as mean ± SEM. Note: P value=*p<0.05; a=significant different from control; b=Significant different from pre-treated atropine.

Working memory and reference memory error during acquisition are attenuated by pre-treated atropine.

Working memory error: Working memory is memory of object stimulus or recognition of location used in testing session. So if rat enter in food baited path it is working memory correctness and if it reenters into baited pathway when both pathways are baited it is referred as working memory error. On first day of training the Working Memory Errors (WME) were greater in buprofezin treated rats compared to second day. Results have suggested that continuous training alleviate the incidence of working memory error and pre-treated atropine significantly attenuate the working memory errors.

Reference memory error: Reference memory is memory for rule of given condition. For example, acquisition of baited path provides food to rats. Entries of rat into pathway with no food stimulus are referred as reference memory error. So results have suggested that Reference Memory Error (RME) during acquisition session changed days after training. Buprofezin treated group exhibit more reference memory error compared to control group. On second day (RME) was comparatively more from day two to onward the memory errors reduced continuously. However, there was no substantial difference found in control and pre-treated atropine group. This demonstrates the reversal effect of pre-treated atropine on reference memory errors (Figure 4).

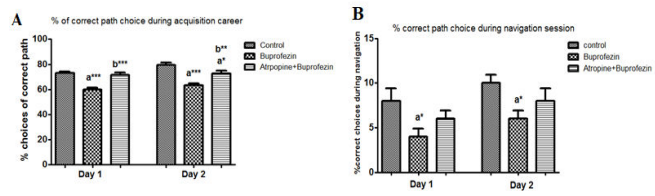

Figure 4: A) Revealed the significant reduction of Working Memory Error (WME) during acquisition session with pre-treated atropine induced by acute exposure of Buprofezin. P value *p<0.05, **P<0.01***p<0.001. B) Describe the attenuation of reference memory errors (RME) caused by acute exposure of Buprofezin with pre-administration of atropine. Memory errors reduced continuously from day two to onwards. The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Values are presented as mean ± SEM. P value=*p<0.05, ***p<0.001; a=significant different from control; b=Significant different from pre-treated atropine.

DISCUSSION

A variety of conventional and ethological behavior paradigms e.g., locomotion activity, anxiety and emotional behavior were performed by Open Field Maze (OFM) [3-5]. Various shortcoming are confronted while application of OFM these include time, duration of light and interference of foreign object. Variations in protocol of experiment which are necessary for wide range of application create difficulties in comparison of studies. Three parameters are rapidly characterized by using open field maze including total distance covered in given time cores of testing. Thigmotaxis for measure of anxiety and number of fecal pallets as more defecation is index of low emotionality in rats [6]. However, TDS maze. CSS or close maze is multifunctional in which most of behavioral analysis can be performed with maximum accuracy. Some of parameters rapidly characterize in TSD are:

- Total locomotion activity in 15 minutes' time duration.
- Thigmotaxis emotionality.
- Anxiety.
- Time space navigation.
- Novel object cognition.
- Spatial navigation.
- Working and reference memories.
- Working and reference memories errors in chemical exposed, KO or disease rats.

There is no variation in setup design and protocol so data from different studies can be rapidly compared. As maze is close there is little probability of foreign object interference, light and time. It infers that today's TSD maze is best technique of choice for behavioral analysis and drugs studies. It is specifically used to analyze the effect of toxicant, pesticides and acute drug exposure on specific type of memory.

It should be noticeable that some investigators have inferred high activity or increase exploratory behavior is a measure of low emotionality however others perceive that exploratory behavior doesn't depend on emotionality. However, in our experiment by using TSD maze we have conceived that exploratory behavior doesn't depends on emotionality.

Rearing behavior can define as standing of testing animal in a vertical upright position on both hind paws. It is measured an exploratory behavior and used as index of anxiety in both the elevated plus maze and OFM [7]. Some studies specify increased rearing is in associated with increased anxiety levels in rat while others suggest decreased in rearing is indication of increased anxiety [8,9]. Thus, rearing can distinguish anxiety linked behaviors from other ambulatory behavior. It has been suggested that anxiety analysis in rats is much more complex than using a single parameter in only one maze. Thus, rearing and anxiety behavior can be studied well by using numerous trials in a single test in TSD maze.

Thigmotaxis behavior is perceived largely in rodents and is associated to anxiety like behaviors. Irrespective of the principal cause, thigmotaxis is an essential anxiety linked behavior and often recognized as the initial point for further precise anxiety tests. In OFM maze thigmotaxis is used to measure anxiolytic, anxiogenic and even non pharmacological actions. Anxiety linked drugs such as chlordiazepoxide and diazepam have revealed substantial effects on rat's behavior in the OFM however dopamine agonists have revealed that dopamine receptors like D_1 and D_2 cause anxiogenic like effects due to high dopaminergic transmissions [10]. However, we have suggested that more thigmotaxis is associated with increased anxiety in TSD maze.

In the OFM Maze there were significant strain differences in response to rat anxiety like behavior. It was also clearly reported that highly emotional rats showed more defecation [11]. Recently it has been reported that defecations may definitely be a valuable index of emotional anxiety related behaviors in short testing periods as accomplished here as compared to long testing (30 min) where minor variation in responses are found [12] in OFM. Our finding also reported as the high emotionality is associated with increased defecation for short observation (15 min) in TSD maze.

Previously reported that in OFM maze the behavior of mice depends on their tactile sensations. Therefore, any damage to or shortage of whiskers of rats may results a substantial decrease in anxiety linked behavior measure as the mice fail to contact with maze walls and reached earlier at terminal of maze. Recent study on rat model also agreed with these finding that loss of whiskers sensation results earlier approach of rats at terminal of TSD maze arm and interfere in measure of anxiety linked behavior [13].

We debate the use of the TSD maze as it is associated with motor locomotion of the rat being tested, other behaviors tests can also be performed in TSD such as memory and novel object recognition. Time in the TSD maze with a novel object recognition can range from 15 min to 30 minutes and depends on type of memory being analyzed. Due to flexibility and simplicity of the TSD maze in the novel object recognition permits for short or long term memory testing, and can be used to evaluate the effect of acute drug administration on a particular stage of memory formation. Briefly, the TSD maze test is an optimum measure of performance.

CONCLUSION

Animal model have been proved valuable for researcher to give answer of question concerning the mechanism of behavior. The TSD maze was recently used platform to measure in animal model especially in rats. It is relatively optimal test for evaluation of behavioral profile in rats. As it concerns to rodent model this method permits the study of different laboratory and wild strain of rats. This procedure will readily become useful to investigate the effect of different pharmacological compound on learning and memory as well as anxiolytic and anxiogenic effects.

Ethical approval

The study was conducted in Koc University graduate school of health sciences. All methods were performed in accordance with relevant guidelines and regulations of "local ethics committee for animal experiments of Koc university." The animals were kept in the Koc University, Animal Research Facility (KUARF) of Centre for Translational Medicine (KUTTAM). The study was approved by the committee with Approval No. (2022-10).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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