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Working Memory Limit of Young Male Wistar Rats in Return T Maze Delayed Spontaneous Task Alternation

Joy Karmakar[1*], Juhi Gupta[1]
[1]Department of Physiology, Andaman & Nicobar Islands Institute of Medical Sciences, Port Blair, India

Original Research Article

***Corresponding author**
Joy Karmakar

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Abstract: In rat maze training tasks, the working memory is a short term memory of remembering a previously visited area or reward to solve the task which lasts for a single trial. 10 young (age = 4 months) male Wistar rats were selected to perform delayed spontaneous alternation task in Return T Maze which involves working memory. For each rat, 8 trials are given per day for 15 sec restrain, 30 sec restrain, 45 sec restrain, 60 sec restrain, 75 sec restrain, 90 sec restrain, 105 sec restrain, 120 sec restrain respectively for 10 days (Each day 8 restrain trials x 10 days = 80 trials for each rat). We did not find any preference for the right or left turn during the first spontaneous free choice which excludes the possibilities of presence of attractful cues on a particular side as well as innate nature of a particular rat to turn on the same side repeatedly. Our result shows the range of working memory in between 45-75 seconds, however the rat can continue to solve the task beyond that limit either by simple chance or orientation by their reference memory which is developed from repeated exposure on the maze. This result will help to conduct different maze task which involves working memory.
Keywords: Working Memory, Return T Maze, Delayed Alternation Task.

INTRODUCTION

Working Memory is defined as a type of short-term memory that involves active manipulation by the individual [1]. For humans, working memory example is holding on to an item which can be compared with the presented items or simply remembering a group of digits or words. For rats, this working memory can be a short term memory for an object, stimulus, or location that is used within a testing session, but not typically between sessions [2]. The concept of human working memory is worked out very well in the rats by developing episodic memory tasks is mazes [3].

Working Memory is very much distinguishable from Reference Memory. For most often, Reference Memory is the memory of general principle or rules [4]. In maze training of the rats, reference memory is developed after repeated trials. In contrast, working memory is a representation of a cue over a delay period in which the cue is not present, to make a subsequent response [5]. Working memory functions only for a single trial that can be forgotten on subsequent trial.

T maze has been widely used for assessing Working Memory of laboratory rats [6]. Delayed alternation task in T maze involves working memory as the only subserving system where a rat is exposed to a sample task which is swapped after a given delay [6]. For animals like rat, this kind of alternation behaviour is spontaneous [7]. When given free choice, they less likely repeat a given behaviour for which they have already been rewarded because of their curious nature of novelty findings [8].

AIMS & OBJECTIVES

In this study, we investigated the working memory limit (maximum duration) of laboratory Wistar rats for spontaneous delayed alternation task in Return T Maze.

MATERIALS & METHODS

(a) Subjects

10 young (4 months of age) male Wistar rats were selected for this study. The animals were housed in standard polypropylene cages (max. three to four rats per cage) and maintained in 24±2°C and relatively low humidity with 12:12 hours day and night cycle. All the rats were provided with commercially available rat pellet diet (Animal diet, Provimi) and water ad-libitum. The guidelines of committee for the purpose of control

and supervision of experiments on animals (CPCSEA), Govt. of India were followed and prior permission was sought from the institutional animal ethics committee, IPGME&R, Kolkata, India for conducting the study.

(b) Materials

Return T Maze

The maze **(*Fig. 1*)** is made up of transparent acrylic boards. It is rectangular in shape with 60 inch length and 40 inch width. The height of the wall is 8 inch. The stem of the T is in the centre. Two arms (20 inch length) of the T are located at one end of the stem and are acting as alternating choice arms. The other end of the T is acting as Start compartment or arm. The Right and Left T arms are connected to the Start arm by means of a return alley which provides direct return of the rat to the start arm without being removed from the maze manually and placed in the start arm. The start arm or compartment is guarded with 3 manually operating gates: one start gate separating it from the stem of the T and two return gates providing selected entry from the return alley. These three gates are used for restraining the rat for a given period or delay. The arms of the T are also provided with arm gates which prevents the rat from re-entering the stem of the T once arm choice is made.

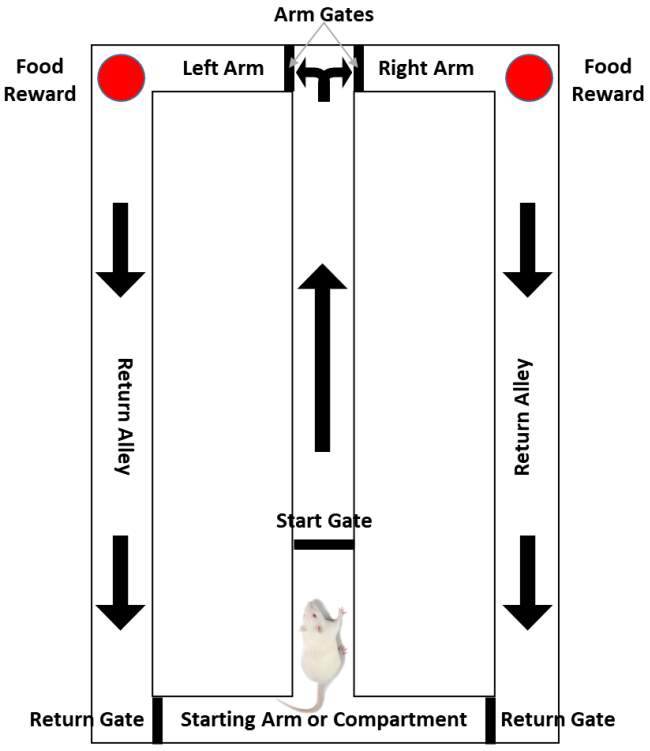

Fig-1: Schematic diagram of Return T Maze

Food Rewards

Sprouted grams are placed over a circular food tray on the corners of the right and left arms of T maze. For each trial, one sprouted gram is placed on the food tray to maintain optimum appetite for the next successive trials.

(c) Methods

Rats were kept on fast overnight (for 6-8 hours) to maintain adequate appetite taking pre-cautions not to lose the weight more than 20% of ad-libitum body weight.

Acclimation

Rats in home cage are placed in testing room for at least 1hr before testing to minimize effects of stress.

Habituation

Rats were first habituated in the Return T maze for 3 consecutive days and were allowed open access to both arms of T maze including return alleys.

Testing protocols [14]

- Food rewards are placed on each arm of the T maze.
- Left and right arm gates are opened while start gate is closed and animal is placed in start arm.
- Start gate is opened quietly.
- When all four paws enter one arm, that sided arm gate is closed and arm entry is recorded as R or L.
- Rat is allowed to eat the food reward (Sprouted grams).
- Start arm return gate connected to the same sided return alley is opened. Opposite side return gate as well as start gate remain closed.

- When rat reach the start arm, the return gate is closed and rat is restrained for a given time duration mentioned below.
- After restrain, the start gate is opened again. Left and right arm gates are also opened.
- When all four paws enter one arm, that sided arm gate is closed. Arm entry is recorded and a score of 1 or 0 is given whether the animal has entered the opposite arm (the correct choice) or the same previously visited arm (the wrong choice as the food rewards are already collected).
- Rat is returned to the home cage and stayed for at least 20 minutes for the next trial.
- For each rats, 8 trials are given per day for 15 sec restrain, 30 sec restrain, 45 sec restrain, 60 sec restrain, 75 sec restrain, 90 sec restrain, 105 sec restrain, 120 sec restrain respectively.
- Trials are given for 10 days (Each day 8 restrain trials x 10 days = 80 trials for each rat).

Testing parameters

- First spontaneous choice: Right or Left turn (Percent)
- Delayed alternation response as a score of 1 or 0 after,
  - ✓ 15 sec restrain
  - ✓ 30 sec restrain
  - ✓ 45 sec restrain
  - ✓ 60 sec restrain
  - ✓ 75 sec restrain
  - ✓ 90 sec restrain
  - ✓ 105 sec restrain
  - ✓ 120 sec restrain
- For each restrain trials, 10 days cumulative score is calculated (which can maximum be 10 x 1 =10)

Statistical Analysis: Analysis of the data is performed in Graph Pad Prism v 5.03.

RESULTS

Analysis of the spontaneous first choice **(Fig. 2)** revealed that most of the rats have no preference for Right or Left turn. Unpaired T test analysis between Right Turn and Left Turn shows the p value of 0.6837 which is not significant with a 't' score of 0.414 (Spontaneous Right Turn has a mean of 39.5 ± 5.401 and Spontaneous Left Turn has a mean of 40.5 ± 5.401).

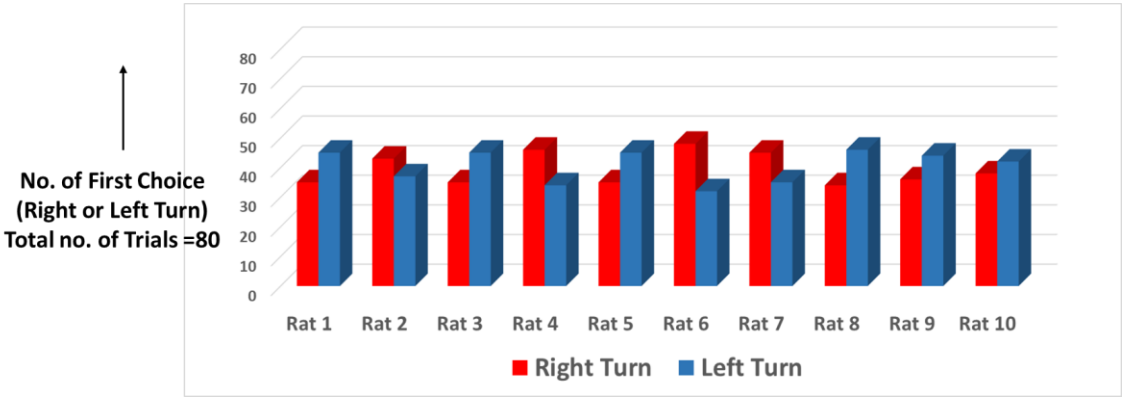

Fig-2: Spontaneous First Choice: Right or Left Preference

Cumulative delayed alternation response as a score of 1 or 0 for the correct and wrong response shows that score gradually decreases when the restrain time is increased **(Fig. 3)**.

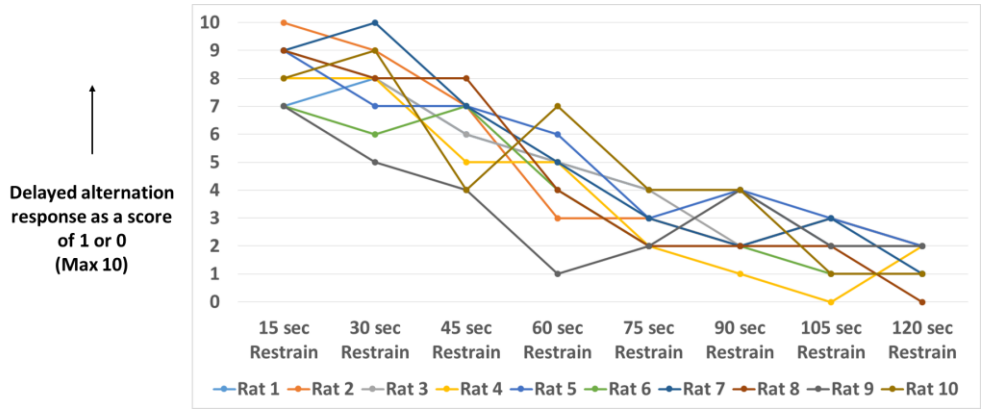

Fig-3: Delayed Alternation Response as a score of 1 or 0 for the correct and wrong response

Further analysis of delayed alternation response **(Table 1 and Fig. 4)** by one way ANOVA between the restrain group shows the p value <0.0001 which is highly significant (F = 48.12 and R square = 0.8239). Bartlett's test for equal variance in ANOVA shows that the group variance is minimum (p value 0.2663).

Table-1: One way ANOVA group analysis

	15 sec Restrain	30 sec Restrain	45 sec Restrain	60 sec Restrain	75 sec Restrain	90 sec Restrain	105 sec Restrain	120 sec Restrain
Number of values	10	10	10	10	10	10	10	10
Minimum	7	5	4	1	2	1	0	0
25% Percentile	7	6.75	4.75	3.75	2	2	1	1
Median	8.5	8	6.5	5	3	2	2	2
75% Percentile	9	9	7	5.25	4	4	3	2
Maximum	10	10	8	7	4	4	3	2
Mean	8.3	7.8	6.1	4.5	2.9	2.7	2	1.5
Std. Deviation	1.059	1.476	1.37	1.65	0.8756	1.16	1.054	0.7071
Std. Error	0.335	0.4667	0.4333	0.5217	0.2769	0.3667	0.3333	0.2236
Lower 95% CI	7.542	6.744	5.12	3.32	2.274	1.871	1.246	0.9942
Upper 95% CI	9.058	8.856	7.08	5.68	3.526	3.529	2.754	2.006

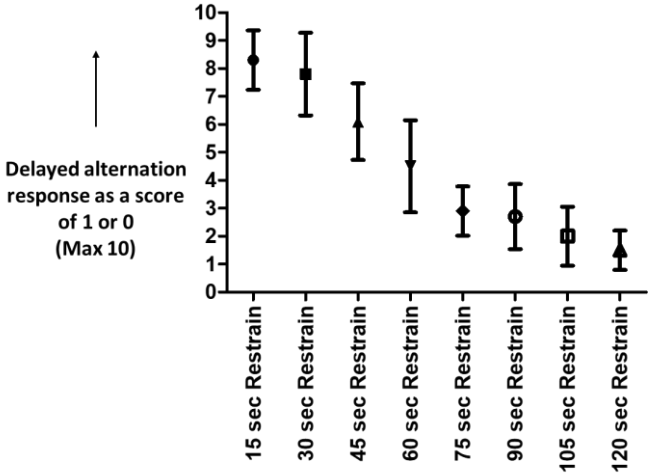

Fig-4: Group presentation of Delayed Alternation Response as a score of 1 or 0 for the correct and wrong response

Tukey's multiple comparison test **(Table 2)** between the adjacent groups shows that the difference is maximum between 30 sec restrain and 45 sec restrain with mean difference 1.700 & studentized range (q) 4.46 followed by 45 sec restrain and 60 sec restrain with mean difference 1.600 & studentized range (q) 4.198 and between 60 sec restrain and 75 sec restrain with mean difference 1.600 & studentized range (q) 4.198.

Table-2: Tukey's Multiple Comparison Test between the adjacent groups

Tukey's Multiple Comparison Test	Mean Diff.	q
15 sec Restrain vs 30 sec Restrain	0.5	1.312
30 sec Restrain vs 45 sec Restrain	1.7	4.46
45 sec Restrain vs 60 sec Restrain	1.6	4.198
60 sec Restrain vs 75 sec Restrain	1.6	4.198
75 sec Restrain vs 90 sec Restrain	0.2	0.5247
90 sec Restrain vs 105 sec Restrain	0.7	1.837
105 sec Restrain vs 120 sec Restrain	0.5	1.312

DISCUSSION

Rat is a foraging animal. They are attracted to new unexplored area due to their curious nature [8, 17]. In the maze task, they work out well by remembering the previously explored place or reward. To find a new reward or explore new area, they nicely hold the representation of their previous experience which is a type of working memory [11].

Tolman was one of the first experimenters on T maze where he described a pronounced tendency of rat to alternate the arm choices on repeated trials [6]. Most of the time the rat uses an allocentric (world based) approach to solve the T-maze by remembering the location of the most recently visited arm based on its spatial relationship with extra-maze landmarks [12]. Alternatively, the rat might use a response strategy—remembering which turn it has made (e.g. left), and make the opposite turn on the subsequent trial [12].

In our study, we did not find any preference for the right or left turn during the first spontaneous free choice for the arm entry. This excludes the possibilities of presence of attractful cues on a particular side as well as innate nature of a particular rat to turn on the same side repeatedly. This result also excludes the chance of remembrance of previous trial experience during the inter-trial period (which is at least 20 minutes in this study) when the rat is returned to the home cage, which may influence the behaviour pattern on the subsequent trials.

Delayed alternation response focuses on the developing tasks involving working memory to see how long a rat could remember a stimulus that was not present [9]. Different strain of rats have different limits of working memory and the working memory also varies depending upon the task spatial vs non-spatial. Working memory is a short lasting memory that decays over time [1, 2]. The duration of working memory in a laboratory rat may extend to 60 minutes depending upon the task [15, 16]. Suzuki et al. show that rats rely on the spatial relationships between extra-maze landmarks when solving the task, as rearrangement of these landmarks during the delay period resulted in chance performance following the delay [17].

However, we found that the working memory of the young male wistar rats is significantly declined over 45-75 sec restrain. After 1 minutes (= 60 sec), the score becomes less than 50% (mean score 4.5 ± 1.65) of the maximum score (=10). And after 2 minutes (= 120 sec), the score becomes significantly low (mean score 1.5 ± 0.7071). Here, lies the chance factor of randomly scoring the correct response which is ambiguous to previous choice made. In this regard, Pontecorvo et al suggest that delayed operational task leaves open the opportunities that the rat can bridge the delay period by postural orientation by their reference memory (memory of general principle or rule).

This result will help to conduct different maze task which involves working memory. Also, understanding working memory will help us to better understand pathophysiologic basis of many neurological disorders like Alzheimer's disease. The concept of working memory (in human it is actually problem solving memory) can also be applied in cognitive field like education and training programme.

CONCLUSION

We conclude that the working memory in young (age = 4 months) male Wistar rats in T maze spontaneous delayed alternation task lies in between 45-75 seconds, however the rat can continue to solve the task beyond that limit either by simple chance or orientation by their reference memory which is developed from repeated exposure on the maze.

Conflict of Interest: None.

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