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EVALUATION OF ALLOCENTRIC SPATIAL LEARNING IN RATS USING A NOVEL "ALTERNATED DUAL TASK"

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Abstract : Allocentric spatial learning can be assessed using popular spontaneous alternation behaviour (SAB) tested with T-maze, and also using radial arm maze (RAM) tasks. But the SAB testing has been reported to have lack of validity as a measure of retention, especially when used as a measure of short term memory. A more complex dual alternated task was designed to clarify whether increasing novelty and alternation factors in a task will increase or decrease the short term and long term memory in rats. Rats were made to learn both T-maze spontaneous alternation task and RAM task alternatively. Another group of rats were made to learn both the task separately without any alternation. And control group of rats were assigned to learn only one type of task. It was found that the group of rats performing "alternated dual task" could acquire the tasks more easily than the control groups and non alternated dual task groups. This enhancement of acquisition was associated only with the complex task (RAM task) among the dual tasks. More over their retention (memory) ability was very significantly enhanced for both the tasks in dual tasks. It can be concluded that, the principle of "alternated dual task" can be made use when a complex task has to be acquired and learned faster by rats; as alternation with simple task enhances the ability of rats to learn and memorize a complex task more efficiently.

Key words : spontaneous alternation behaviour, T-maze, allocentric spatial learning, radial arm maze

INTRODUCTION

Animals who are moving in space may compute their current position by path integration, that is, by detecting movement -generated or idiothetic cues; or they may use allothetic cues generated by combinations of environmental land marks (1, 2). It has been suggested that allocentric spatial impairments reflect the role of the hippocampus system in detecting and controlling the animal's movements through space (3, 4). Also it was shown that hippocampal – system lesions typically disrupt allocentric (defined with respect to external land marks) spatial learning but leave egocentric (defined with respect to rat's body axis) learning intact (5–7).

Allocentric spatial learning can be assessed using T-maze spontaneous alternation task (8) and also using Radial arm maze (RAM) task (9). In this study we have used both T-maze spontaneous

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alternation task and RAM task. The evaluation of allocentric spatial learning was done by making rats to learn both the task alternatively as well as learning them separately. The influence of one behavioural task on another one depending on its complexity was investigated in the present study.

When tested in a T-maze rats tend to alternate their choices spontaneously (10). Rats alternate even when no choice has been made on the first trial; thus, if put directly into the goal box at the end of one arm of a previously explored T-maze, and then given a choice between the two maze arms, they will tend to choose the arm leading to the other goal box (11). In general there is a tendency to go to the part of the environment that has been least recently explored (12). In its simplest form, spontaneous alternation behaviour was first described nearly 85 years ago (13), the phenomenon has been ascribed to the operation of a variety of mechanisms including Hullian reactive inhibition (14), stimulus satiation (15), action decrement (16), curiosity (17), habituation to novelty (18), foraging strategies (19) and spatial working memory (20).

The value of spontaneous alternation behaviour as a measure of retention has been questioned especially when used as a measure of short term memory (21). To get validity for retention test, we attempted to design a new task by introducing more complexity to the alternation task. A simple task like T-maze task was made to be learned by the rats in alternation to a complex task like RAM task. We named this particular task as “alternated dual task”. In the present study we determined whether learning a simple task (T-maze) along with a complex task (RAM), elongates or reduces the acquisition time for either of the task, and also whether it has any influence on retention (memory) could be observed.

MATERIALS AND METHODS

Subjects

A total of 36 male Wistar albino rats were used for this study. They were housed in groups, in propylene cages in an acclimatized (25–27°C) room and were maintained on a 12 hr light/dark cycle. There was free access to food and water until they aged 60 days at the beginning of the experiment. They were randomly grouped as T-maze spontaneous alternation task group (TM), radial arm maze task group (RAM), T-maze radial arm maze alternated dual task group (TRAM (A)), T-maze radial arm maze non alternated dual task group (TRAM (NA)), radial arm maze T-maze alternated dual task group (RAMT (A)) and radial arm maze T-maze non alternated dual task group (RAMT (NA)) with 6 rats in each group.

Apparatus

T-maze

The T-maze was made of wood with smooth polished surface. It consists of a stem (35×12 cm), a choice area (12×12 cm) and two arms (35×12 cm); at the end of each arm contain a food well. The sidewalls are 40 cm high. The choice area was separated from the arms by a sliding door (8).

Radial arm maze

Radial arm maze was made of Plexiglas, consists of eight equally spaced arms radiating from an octagonal central platform. Each arm was having a length of 56.2 cm, width of 7.9 cm and height of 10 cm. The entire maze was elevated 80 cm above the floor for easy locating of spatial cues by rats.

Experimental design

All the behavioural experiments were carried out in three phases viz; orientation and training session, learning performance test (acquisition test) and memory performance test (retention test). The rats were semi starved for 48 hrs before the start of behavioural experiments. The body weight was maintained at 85% of the original body weight, through out one session of behavioural experiment. Behavioural experiments were conducted in the same room, with the same allocentric cues, such as doors, windows, posters and the experimenter. Experimenter always maintained same position throughout the whole of the experiment.

The following behavioural experiments were included.

T-maze spontaneous alternation task (TM group)

This was analogous to non-matching to sample task (22), where the rat was rewarded only if the current choice doesn't match the previous one. In the orientation phase, the starved rats were allowed to spend 10 minutes/day for three days in the T-maze and trained to collect food pellet from the food wells.

During the acquisition test, all the rats were given six trials/day with an inter trial interval of one hour. Each trial consists of four sample and choice run. In the sample run, the rat was placed at the start end of the T-maze stem. Allowed to move towards one arm and collect the food pellet, while keeping the sliding door of other arm closed. In the choice run, the rat was placed at the start end of stem and both arms were kept open. If the rat visits the same arm as that of sample run, it was recorded as error and the rat was not rewarded with food. Instead, if the rat visits the alternate arm, it was recorded as correct score and the rat was allowed to eat food pellet (reward) in the food well. There was an interval of 30s between each run.

Score was given for alternate selection of arm during choice run and a maximum score of '4' can be obtained per trial. The acquisition test was continued until the rats attained the learning criteria of obtaining '4' correct score without any error for three consecutive trials.

Ten days after the last day of acquisition of the task, the rats were subjected to retention test. The test was conducted similar to acquisition test and was continued until the rats attained the learning criteria. A memory score was also calculated by taking the difference between number of trials required for acquisition test and number of trials for retention test.

Radial arm maze task (RAM group)

Orientation phase was for three days, where the starved rats were allowed to familiarize themselves with the radial maze. Prior to each acquisition trial, all the eight arms were baited with food pellets. The rat was placed in the center of the maze and allowed to freely explore the maze. The rats were required to take the food pellet from each arm without making a reentry into the arm already visited. The trial was terminated when the animal takes the food reward from all the eight arms or after 10 minutes if all the eight arms were not visited. A correct score was given when the rat visits an arm and collect the food reward, and a maximum score of '8' can be attained per trial. When a rat reenters an already visited arm or doesn't enter an arm, it was taken as error.

The acquisition test was continued until the rats attained learning criteria of obtaining a correct score ≥7, and an error ≤1, for three consecutive trials. Six trials/day was given with an inter trial interval of one hour.

Ten days after the last day of acquisition of the task, the rats were subjected to retention test. It was continued until the learning criteria were attained. The memory score was calculated as described for T-maze spontaneous alternation task.

T-maze radial arm maze alternated dual task (TRAM (A) group)

This group of rats was given dual task, i.e. T-maze spontaneous alternation task and radial arm maze task. The procedures for both the tasks were same as described above. Here the rat was given the T-maze trial first then followed by RAM trial. The task was alternatively given with six trials (3 T-maze trials and 3 RAM trials) per day. The acquisition and retention test was continued until the rats attained learning criteria for both tasks separately. Memory score was also calculated separately for both tasks.

T-maze radial arm maze non alternated dual task (TRAM (A) group)

This group of rats was also given dual task, but the tasks were learned separately without alternating, i.e. the rats learned T-maze task first and after attaining the learning criteria, the RAM task was learned. 10 days after acquisition of both task retention test was carried out similar to acquisition test. Memory score was also calculated separately for both tasks.

Radial arm maze T-maze alternated dual task (RAMT (A) group)

The experimental design here was similar to TRAM (A) group, except that the first trial given was RAM task which was alternatively followed by T-maze task.

Radial arm maze T-maze non alternated dual task (RAMT (NA) group)

This group of rats had the similar setup as TRAM (NA) group, except that the first task learned was RAM task followed by T-maze task.

Statistical analysis

Statistical analyses for behavioural studies were analyzed by one-way analysis of variance (ANOVA) and followed by Tukey – Kramer multiple comparisons test. Significance was accepted at $P<0.05$. Means±SD are reported.

RESULTS

RAM group and TM group was taken as control group. The performance of radial arm maze task and T-maze task during acquisition and retention by various groups were compared with respective control groups.

Acquisition

Radial arm maze task

As elucidated in Table I; the no: of trials required for acquisition of RAM task was similar in control and non-alternated dual task groups [TRAM (NA) and RAMT (NA)], and there was no significant difference. Among the non-alternated dual task groups also no significant difference were observed. But, alternated dual task groups took significantly less [TRAM (A) – $P<0.05$ & RAMT (A) – $P<0.01$] no: of trials than the control groups.

Acquisition of RAM task in alternated dual task groups not only showed significant difference to control groups, but also to non-

TABLE I: Table showing the average number of trials required for acquisition and retention.

Groups		*Avg. number of trials required for*	
		Acquisition	*Retention*[@]
RAM Group (Control)		21±2.2804	16±1.789
TM Group (Control)		16.33±2.1602*	11.5±1.871*
TRAM (Alternating) Group	**RAM**	16.16±2.3166[#]	8.33±1.966[###]
	TM	15.66±1.9664[NS]*	8.5±1.378[NS]*
TRAM (Non Alternating) Group	**RAM**	21.83±1.941[NS#]	16.33±1.862[NS#]
	TM	16±1.414***	10.66±1.751***
RAMT (Alternating) Group	**RAM**	15.83±2.317[##]	8.16±1.169[###]
	TM	16±2.366[NS]*	8.66±1.751[NS]*
RAMT (Non Alternating) Group	**RAM**	20.83±3.251[NS#]	15.83±2.483[NS#]
	TM	15.16±2.137***	10.83±1.472**

Results are mean±SD.

[@]No: of trials for acquisition is significantly different from no: of trials for retention in all groups (level of significance shown in text)

[NS]*not significant, *P<0.05, **P<0.01, ***P<0.001 TM task vs. RAM task within each group.

[NS#]not significant, [#]P<0.05, [##]P<0.01, [###]P<0.001 RAM task of each group vs. respective RAM control.

alternated dual task groups [TRAM (A) vs. TRAM (NA) – P<0.001, TRAM (A) vs. RAMT (NA) – P<0.05, RAMT (A) vs. TRAM (NA) – P<0.001, RAMT (A) vs. RAMT (NA) – P<0.01]. In addition, no significant difference was observed between TRAM (A) and RAMT (A) groups for no: of trials taken for learning RAM task.

T-maze task

No: of trials required for acquisition of TM task was almost similar in every group. None of them showed any significant difference with each other.

Comparison of acquisition of RAM and TM task within each group

As the values in Table I depict, the no: of trials required for acquisition of RAM task is significantly more (P<0.05) than TM task in control groups. But within the alternated dual task groups there was no significant difference, both the RAM and TM task took almost same no: of trials in both the groups.

Within the non-alternated dual task groups, significant difference (both groups P<0.001) were observed for no: of trials for learning RAM task and TM task. Their acquisition was similar to control groups.

Retention

No: of trials required during retention was significantly less compared to acquisition within all groups. Control groups and non-alternated dual task groups showed only moderate significant difference [RAM control group – P<0.01, TM control group – P<0.05, RAM task in TRAM (NA) – P<0.01, TM task in TRAM (NA) – P<0.01, RAM task in RAMT (NA) – P<0.01, and TM task in RAMT (NA) – P<0.05]. But the alternated dual task groups showed very high significant difference (all P<0.001) between the no: of trials for acquisition and no: of trials for retention.

Radial arm maze task

Retention of RAM task in alternated dual task groups took significantly less no: of trials than retention of RAM task in control groups and non-alternated dual task groups [all P<0.001]. There was no significant difference among the alternated dual task groups. Also, no significant difference was shown among the non-alternated dual task groups, and in addition they did not show much difference from control groups.

T-maze task

No: of trials required for retention of TM

task in alternated dual task groups was slightly less than in other groups, but no significant difference were observed among any groups.

Comparison of retention of RAM and TM task within each group

Similar to acquisition, significant difference between no: of trials for RAM and TM task within a group was observed only for control (P<0.05) and non-alternated dual task groups [TRAM (NA) – P<0.001, RAMT (NA) – P<0.01]. And no significant differences were observed for alternated dual task groups.

Memory score

RAM task

As seen in the Fig. 1, the memory scores of RAM task in alternated dual task groups were significantly different from control [TRAM (A) – P<0.001, RAMT (A) – P<0.01] and non-alternated dual task groups [TRAM (A) vs. TRAM (NA) – P<0.01, TRAM (A) vs. RAMT (NA) – P<0.001, RAMT (A) vs. TRAM (NA) – P<0.05, RAMT (A) vs. RAMT (NA) – P<0.01]. No significant difference were observed between RAM tasks of alternated dual task groups, between RAM tasks of non-alternated dual task groups and between control and non-alternated dual task groups.

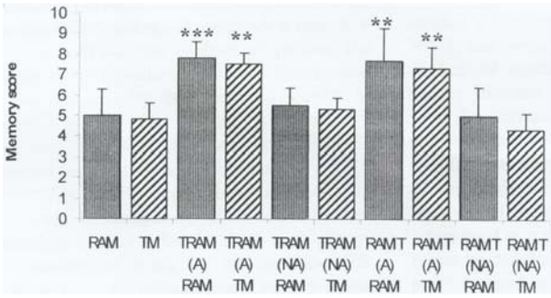

Fig. 1 : Bar graph showing memory scores (no: of trials for acquisition minus no: of trials for retention) of each group.

**P<0.01, ***P<0.001 compared with respective controls.

TM task

Memory scores of TM task also showed similar pattern as that of RAM task. Significant difference were observed between alternated dual task groups and control (both P<0.01) and also with non-alternated dual task groups [TRAM (A) vs. TRAM (NA) – P<0.05, TRAM (A) vs. RAMT (NA) – P<0.001, RAMT (A) vs. TRAM (NA) – P<0.05, RAMT (A) vs. RAMT (NA) – P<0.001]. No significant difference were observed between TM tasks of alternated dual task groups, between TM tasks of non-alternated dual task groups and between control and non-alternated dual task groups.

Comparison of memory score of RAM and TM task within each group

Interestingly, no significant differences were observed for memory scores of RAM task and TM task within any groups.

DISCUSSION

Number of trials required for acquisition and retention of TM task – control group was significantly less than RAM task – control group. This gave the basis for considering TM task as a simple task and RAM task as a complex one.

In the alternated groups [TRAM (A) and RAMT (A)] the number of trials required for retention and acquisition of TM task was similar to RAM task, in contrast to control and non alternated groups [TRAM (NA) and RAMT (NA)]. This indicates that the rats learning ability has increased when the task

was alternated. The probable reasons for this may be novel learning conditions given alternately. As stated by Dember WN and Fowler H in 1958 (10) rats tend to choose the environment that has been least recently explored. This could have increased the curiosity, and curiosity tends to increase the ability of alternation behavioural tasks (17). It was pointed out that rats prefer (over repeated trials) a path leading to a goal box containing complex stimuli over a blind alley or an empty goal box respectively (23). So when rats were alternated between T-maze and RAM their learning ability enhanced. Also when rats were learning both the task without alternation, the novelty drive hypothesis (23) was lacking and be the reason why they behaved similar to control groups for having a significant difference between the number of trials required for TM task and RAM task.

All the groups showed significant difference between acquisition and retention. Retention of task after 10 days took always less number of trials for all the rats. This prompted us to calculate a memory score which gave a clear idea about memory capacity of rats. Only the alternated groups showed a significantly higher memory score, in contrast to the non alternated group. It can be assumed that the alternated groups had a novelty drive and also a higher load on short term memory and working memory compared to non alternated groups. As the short term memory load was more a better long term memory formation may be possible. In 1968 Atkinson-Shiffrin model (24) described the structure of memory and mentioned the need for rehearsal to transfer short term memory to long term memory. Recently also it was shown that regular rehearsal helps in consolidation of long term memory (25). In our study alternated groups received regular rehearsal for complex task intermittent with simple task, whereas non-alternated groups received continuous rehearsal for a particular task. So a better retention capacity is possible in alternated groups, as indicated over here by an increased memory score in this group.

Langlais and Savage in 1995 provided evidence for the fact that high levels of spontaneous alternation are consistent with good spatial memory performance (26). Here in our experiment also as the complexity in alternation increased by using alternated dual task, the spatial memory performance also enhanced.

Spontaneous alternation has been labeled a hippocampal – dependent task (27–30). It has assumed considerable popularity in studies of spatial memory as a quick and simple measure of retention that avoids the need for extensive training and the use of conventional reinforcers (21). Even though spontaneous alternation can be a useful index of responsiveness to novelty, its value as a measure of retention is less certain especially when used as a measure of short term memory. It can be concluded that when “alternated dual task” is used, a complex task can be learned easily and acquired faster. Moreover, the long term memory for the complex task learned is better, and that the task learned faster is not easily forgotten. So this principle of “alternated dual task” can be made use when a complex task is to be learned by a rat within a short period of time.

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