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Research article

REGULAR REHEARSAL HELPS IN CONSOLIDATION OF LONG TERM MEMORY

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ABSTRACT
Memory, one of the most complex functions of the brain comprises of multiple components such as perception, registration, consolidation, storage, retrieval and decay. The present study was undertaken to evaluate the impact of different training sessions on the retention capacity of rats. The capacity of retention of learnt task was measured using exteroceptive behavioral models such as Hexagonal swimming pool apparatus, Hebb-Williams maze and Elevated plus-maze. A total of 150 rats divided into fifteen groups were employed in the present study. The animals were subjected to different training sessions during first three days. The ability to retain the learned task was tested after single, sub-acute, acute, sub-chronic and chronic exposure to above exteroceptive memory models in separate groups of animals. The memory score of all animals was recorded after 72 h, 192 h and 432 h of their last training trial. Rats of single exposure group did not show any effect on memory. Sub-acute training group animals showed improved memory up to 72 h only, where as in acute and sub-chronic training groups this memory improvement was extended up to 192 h. The rats, which were subjected to chronic exposures showed a significant improvement in retention capacity that lasted up to a period of eighteen days. These observations suggest that repeated rehearsals at regular intervals are probably necessary for consolidation of long-term memory. It was observed that sub-acute, acute and sub-chronic exposures, improved the retrieval ability of rats but this memory improving effect was short lived. Thus, rehearsal or training plays a crucial role in enhancing one's capacity of retaining the learnt information.

KEY WORDS: Training, retention, plus-maze, rehearsal.

INTRODUCTION

Memory, one of the most complex functions of the brain comprises of multiple components such as perception, registration, consolidation, storage, retrieval and decay (Dosher and Ma, 1998; Nesca and Koulack, 1994). Memory has been classified into several types depending upon the duration for which the information can be recalled. We have sensory memory lasting for few seconds (Schweickert, 1993), short-term memory, lasting for few hours (Baddeley and Wilson, 1985) and long-term memory, where in the information is stored for several years or even for life time (Baddeley., 1988; Nader et al., 2000). It's a known fact that environmental factors, emotional factors, educational and cultural background, all play an important role in building human memory. Attention, concentration, motivation and training influence the memory positively where as distraction, interference and shock affect memory adversely. Short-term memories disappear very rapidly (Peterson and Peterson, 1959) whereas; skill memory could be improved after several days of practice (Poldrack and Gabrieli, 2001). Recently, we have observed that regular swimming helps in improving memory in rats (Parle et al., 2005). Working memory reflects problem solving and reasoning ability of an individual (Fry and Hale, 1996; Kyllonen and Christal, 1990). The notion that working memory capacity is a fixed property of an individual (Kyllonen and Christal, 1990) is challenged by Klingberg et al. (2002) and Olesen et al. (2004). Rehearsals influence the memory of normal and retarded adolescents (Brown et al., 1973). In the light of above, the present study was undertaken to evaluate the impact of rehearsals on the rats' capacity of retaining the learnt task employing various behavioral models.

METHODS

Subjects

Wistar rats of either sex, weighing around 200g were employed in the present study. They were exposed to alternate light and dark cycles of 12 h each and had free access to food and water. They were procured from the disease free animal house of CCS Haryana Agriculture University Hisar (India). The animals were acclimatized to the laboratory conditions for at least five days prior to the behavioral test. The experiments were conducted between 9.00 h to 17.30 h, on all the days. Experimental protocol was approved by the institutional animals ethics committee (IAEC). Care of the animals was taken as per the guidelines of CPESEA, Ministry of Forests and Environment, Government of India (Reg. No.436).

Laboratory models

Retention capacity of animals was assessed by employing different behavioral models: Hexagonal Swimming Pool, Hebb- Williams Maze and Elevated Plus Maze.

Hexagonal Swimming Pool: A specially designed hexagonal swimming pool (with each side of hexagon 75 cm, diagonal length of 150 cm and depth around 60 cm) was employed for swimming task. A hidden platform was provided to the animals as the only means of escape from water. The rigid square (11 X 11 cm), and 29 cm long platform was placed 1 cm below the water surface. The pool was filled with water up to a height of 30 cm, which was made opaque by adding non- toxic white color to it so that there is no visible cue to animals regarding the spatial location of the platform. The starting point for placing the animals in the swimming pool was just opposite to the hidden platform. The time taken in seconds by the animal to swim from the starting point to the hidden platform was taken as its escape latency time (ELT). Animals were allowed to explore the platform for additional 20 seconds. ELT of each animal was recorded separately. A decrease in ELT on subsequent, exposures indicated successful retention of the learned task. Utmost care was taken not to disturb any object in the laboratory so as not to provide any clue (Morris, 1984; Morris et al., 1988).

Hebb-Williams Maze: It is an incentive based exteroceptive behavioral model useful for measuring spatial and working memory of rats (Parle and Singh, 2004). It consists of mainly three components. Animal chamber (Start Box), which is attached to the middle chamber (Exploratory area) and a reward chamber at the other end of the maze in which the reward (Food) is kept. All the three components are provided with guillotine removable doors. 12 h fasted rats were employed in the study. Each rat was placed in animal chamber (Start Box) and door was opened to facilitate the entry of the animal into the next chamber. The door of start box was closed immediately after the animal moved into the next chamber so as to prevent its back entry. Time taken in seconds by the animal to reach reward chamber (TRC) from start box was noted for each animal. Each animal was allowed to explore the maze for additional 20 seconds, with all the doors opened before returning to its home cage. A fall in TRC on subsequent maze exposures was taken as an index of successful retention.

Elevated Plus-Maze: Elevated plus-maze was used as yet another exteroceptive behavioral model to evaluate memory in rats. The procedure, technique and end point for testing memory was followed as per the parameters described by investigators working in the area of psychopharmacology (Dhingra et al, 2004; Itoh et al, 1990; Parle and Dhingra, 2003; Reddy and Kulkarni, 1998). Briefly, the elevated plus maze apparatus for rats consisted of a central platform (10 X 10 cm) connected to two open arms (50 cm X 10 cm) and two covered (enclosed) arms (50 cm X 40 cm X 10 cm) and the maze was elevated to a height of 50cm from the floor (Parle and Singh, 2004). In order to record transfer latency (TL), each rat was placed at the end of an open arm facing away from the central platform. Transfer latency was defined as the time in seconds taken by the animal to move into one of the enclosed arms with all its four legs. A fall in TL on subsequent, plus- maze exposures was taken as an index of successful retention.

Experimental protocol

A total of 150 rats were employed in the present study. These rats were divided into 15 groups as under:

Swimming task groups

Group I (Single exposure group): Served as a control group of rats. The animals were exposed to the swimming task using a specially designed hexagonal swimming pool on first day only and just once. ELT of each animal was recorded separately. Retention of the learned task by these animals was tested by recording their ELT after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) after their first exposure.

Group II (Sub-acute training group): Rats were subjected to three additional swimming trials on first day after first exposure and were retired on the same day. Animals were given an inter trial interval of 20 minutes. ELT of each animal was recorded separately. Retention of the learned task by these animals was tested by recording their ELT after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial.

Group III (Acute training group): Rats were subjected to a total of nine acute swimming trials in addition to first exposure on first day itself. ELT of each animal was recorded separately. These trials were conducted in two phases, first phase consisted of four consecutive training trials with 20 minutes inter trial interval. The second phase consisted of five consecutive training trials like first phase. A rest of 2 h was given to the animals between these 2 phases. Retention of the learned task by these animals was tested by recording their ELT after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial.

Group IV (Sub- chronic training group): Rats were subjected to a total of six swimming trials i.e. 3 consecutive trials on first and 3 consecutive trials on second day following the first swim exposure. ELT of each animal was recorded separately. Retention of the learned task by these animals was tested by recording their ELT after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial.

Group V (Chronic training group): Rats were subjected to a total of 9 swimming exposures in addition to first swim exposure. They received 3 consecutive trials on first day followed by 3 consecutive trials each on 2nd day (after 24 h) and third day (after 48 h). ELT of each animal was recorded separately. Retention of the learned task by these animals was tested by recording their ELT after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial.

Hebb-Williams maze groups

Group VI (Single exposure group): Served as control group of rats. The animals were exposed to Hebb-Williams maze on first day once only and TRC was recorded separately for each animal. Retention of the learned task by these animals was tested by recording their TRC after 72h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their retirement after single exposure to Hebb-Williams maze.

Group VII (Sub- acute training group): Rats were subjected to three consecutive training trials on Hebb-Williams maze, following first exposure on first day and were retired on the same day. TRC was recorded for each animal separately. The trials were conducted consecutively with an inter-trial interval of 20 minutes. Retention of the learned task by these animals was tested by recording their TRC after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial.

Group VIII (Acute training group): Rats were subjected to a total of nine acute training trials in addition to their first exposure on Hebb-Williams maze, on first day itself. TRC of each animal was measured separately. The trials were conducted consecutively with an inter-trial interval of 20 minutes. Retention of the learned task by these animals was tested by recording their TRC after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial.

Group IX (Sub- chronic training group): Rats were subjected to a total of six training trials in addition to their first exposure on Hebb-Williams maze. Rats received 3 consecutive training trials on first day and 3 consecutive training trials on second day (after 24 h) following their first exposure to Hebb-Williams maze. The rats were given an inter-trial interval of 20 minutes. TRC of each animal was recorded separately. Retention of the learned task by these animals was tested by recording their TRC after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial.

Group X (Chronic training group): Rats were subjected to a total of 9 training trials in addition to their first exposure on Hebb-Williams maze. They received 3 consecutive trials on first day followed by 3 consecutive trials each on 2nd day (after 24 h) and third day (after 48 h). Animals were given an inter-trial interval of 20 minutes. TRC of each animal was recorded separately. Retention of the learned task by these animals was tested by recording their TRC after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial.

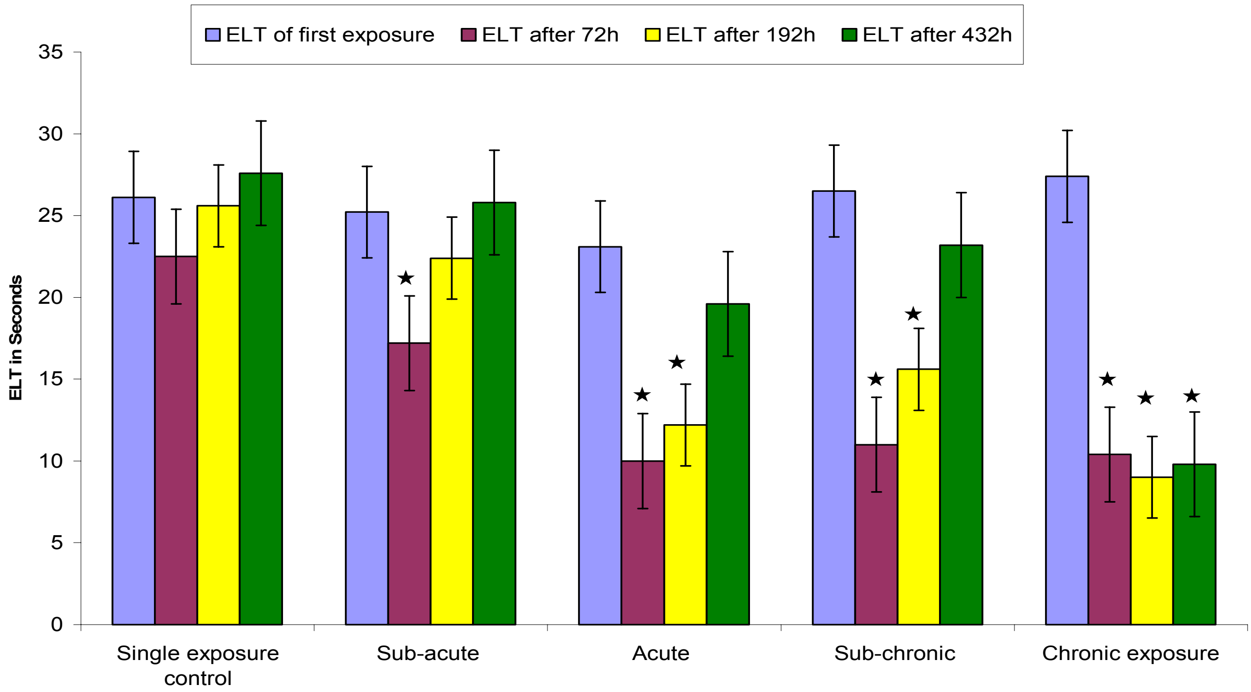

Figure 1. Effect of single, sub-acute, acute, sub-chronic and chronic exposure on ELT of rats using Hexagonal swimming pool. Values are means ± S.E.M. ★ denotes $p < 0.001$ when compared with first exposure group.

Elevated plus maze groups

Group XI (Single exposure group): Served as a control group of rats. The animals were exposed to plus maze apparatus on first day, only once and TL was measured. Retention of the learned task by these animals was tested by recording their TL after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their retirement after single exposure to plus maze.

Group XII (Sub- acute training group): Rats were subjected to 3 additional consecutive trials on first day after their first exposure to plus maze and were retired on the same day. TL of each animal was measured separately. Animals were given an inter-trial interval of 20 minutes. Retention of the learned task by these animals was tested by recording their TL after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial on plus maze.

Group XIII (Acute training group): Rats were subjected to a total of 9 acute training trials in addition to their first exposure on plus maze, on first day itself. These trials were conducted consecutively with an inter-trial interval of 20 minutes. TL of each animal was recorded separately. Retention of the learned task by these animals was tested by recording their TL after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial on plus maze.

Group XIV (Sub- chronic training group): Rats were subjected to a total of 6 training trials on plus maze. Animals received 3 consecutive training trials on first day and 3 consecutive training trials on 2nd day (after 24 h) following their first exposure to elevated plus-maze. Animals were given an inter-trial interval of 20 minutes. Retention of the learned task by these animals was tested by recording their TL after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial on plus maze.

Group XV (Chronic training group): Rats were subjected to a total of 9 training trials in addition to their first exposure on Plus-maze. They received 3 consecutive trials on first day followed by 3 consecutive trials each on 2nd day (after 24 h) and 3rd day (after 48 h). They were given an inter-trial interval of 20 minutes. TL of each animal was recorded separately. Retention of the learned task by these animals was tested by recording their TL after 72 h (on 4th day), 192 h (on 9th day) and 432 h (on 19th day) respectively of their last training trial on plus maze.

Statistical analysis

Repeated Measures ANOVA followed by Tukey test was applied for the statistical analysis of the data in order to account for the inter-subject variability and to facilitate the comparisons of within group as well as inter-group differences. P < 0.05 was considered as statistically significant.

RESULTS

Effect of single exposure to swimming on escape latency time (ELT) of rats using Hexagonal swimming pool: Animals of group I did not show any significant (p = 0.198) change in their ELT values, when measured after 72 h, 192 h and 432 h as compared to ELT of their first exposure (Figure 1).

Effect of sub acute swimming exposure on ELT of rats using Hexagonal swimming pool: The animals of group II, which were subjected to three consecutive swimming trials showed a significant decrease in ELT (p = 0.016), when measured after 72 h of their last training trial as compared to ELT of their first exposure. On the other hand, these animals did not show any significant change in their ELT values, when tested after 192 h and 432 h of their last training (Figure 1).

Effect of acute swimming exposure on ELT of rats using Hexagonal swimming pool: Group III animals, which received nine acute swimming trials on the same day showed a significant (p < 0.001) decrease in their ELT values, when measured after 72 h and 192 h of their last training exposure (Figure 1).

Effect of sub chronic swimming trials on ELT of rats using Hexagonal swimming pool: Animals of group IV, which received sub chronic swimming trials (i. e. total of six trials on 1st & 2nd day) showed a significant (p < 0.001) fall in ELT value, when measured after 72 h and 192 h respectively of their last training exposure, as compared to their first exposure ELT. However, these animals did not show any significant decrease in their ELT after 432 h (Figure 1).

Effect of chronic swimming trials on ELT of rats using Hexagonal swimming pool: The animals (group V), which underwent chronic swimming trials (total of 9 trials during first 3 days) exhibited a significant (p < 0.001) decrease in their ELT values as compared to control group, which lasted up to 432 h (Figure1).

Effect of single exposure on time taken to reach reward chamber (TRC) of rats using Hebb-Williams maze: Animals of group VI did not show any significant (p = 0.478) change in their TRC values, when measured after 72 h, 192 h and 432 h, as compared to TRC of their first exposure (Figure 2).

Effect of sub acute training on TRC of rats using Hebb-Williams maze: The animals of group VII, which were subjected to 3 consecutive trials on Hebb-Williams maze, showed a significant decrease in TRC (p < 0.001) when measured after 72 h of their last training trial as compared to TRC of their

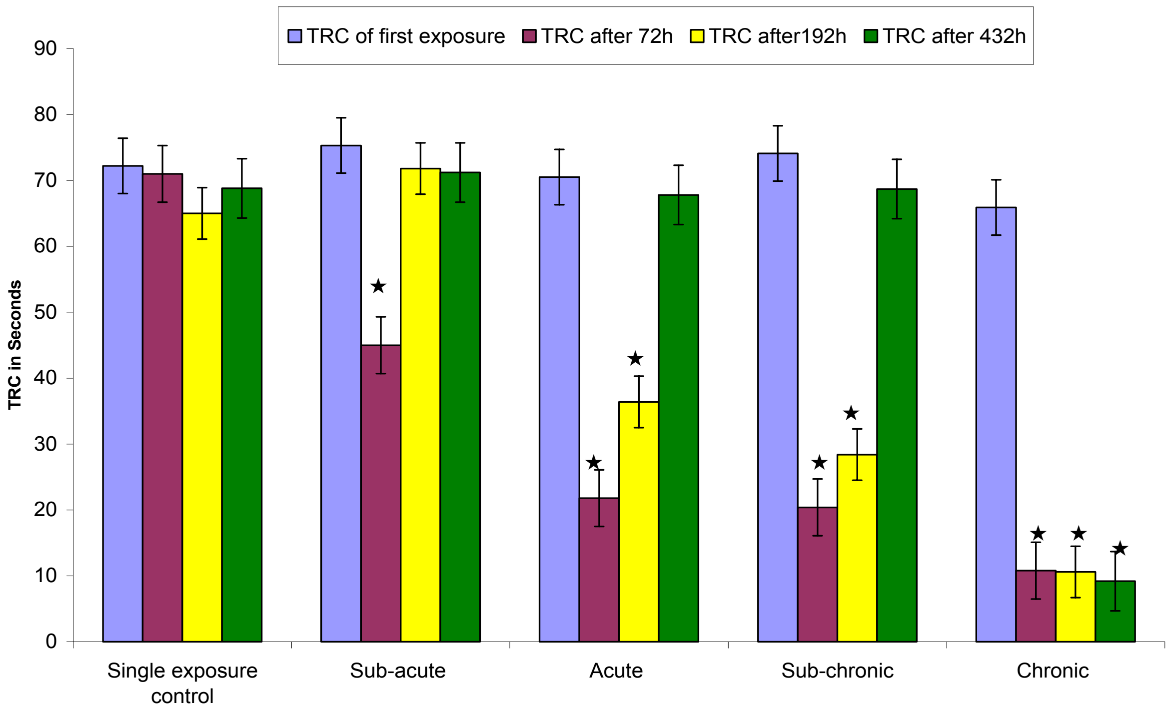

Figure 2. Effect of single, sub-acute, acute, sub-chronic and chronic exposure on TRC of rats using Hebb-Williams maze. Values are means ± S.E.M. ★ denotes p < 0.001 when compared with first exposure group.

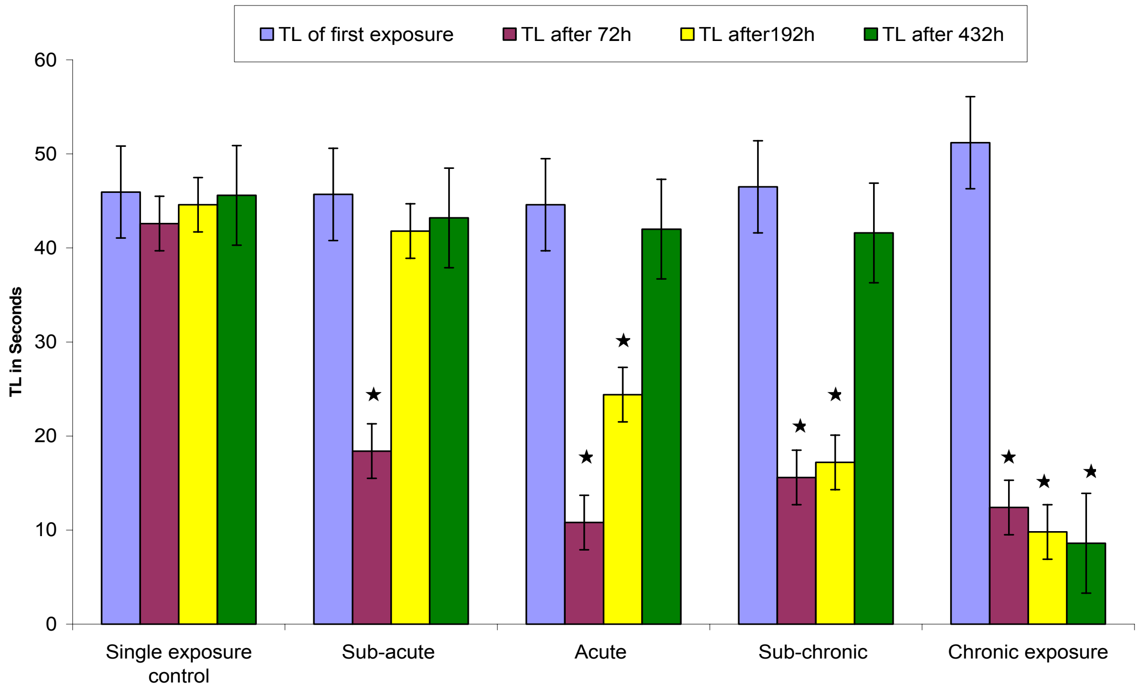

Figure 3. Effect of single, sub-acute, acute, sub-chronic and chronic exposure on TL of rats using Elevated plus-maze. Values are means ± S.E.M. ★ denotes $p < 0.001$ when compared with first exposure group.

first exposure. On the other hand, these animals did not show any significant change in their TRC values, when tested after 192 h and 432 h of their last training (Figure 2).

Effect of acute training on TRC of rats using Hebb-Williams maze: Group VIII animals, which received nine acute trials on Hebb-Williams maze, showed a significant ($p < 0.001$) decrease in their TRC values, when measured after 72 h and 192 h of their last training exposure (Figure 2).

Effect of sub chronic training on TRC of rats using Hebb-Williams maze: Animals of group IX, which received sub chronic training on Hebb-Williams maze (i. e. total of six trials on 1st and 2nd day) showed a significant ($P < 0.001$) fall in TRC value, when measured after 72 h and 192 h respectively of their last training exposure, as compared to their first exposure TRC. However, these animals did not show any significant decrease in their TRC after 432 h (Figure 2).

Effect of chronic training on TRC of rats using Hebb-Williams maze: The animals (group X), which underwent chronic training trials on Hebb-Williams maze (total of 9 trials during first 3 days) exhibited a significant ($P < 0.001$) decrease in their TRC values as compared to control group, which lasted up to 432 h (Figure 2).

Effect of single exposure on transfer latency (TL) of rats using elevated plus maze: Animals of group XI did not show any significant ($p = 0.834$) change in their TL values, when measured after 72 h, 192 h and 432 h, as compared to TL of their first exposure (Figure 3).

Effect of sub acute training on TL of rats using elevated plus-maze: The animals of group XII, which were subjected to 3 consecutive trials on plus-maze, showed a significant decrease in TL ($p < 0.001$), when measured after 72 h of their last training trial as compared to TL of their first exposure. On the other hand, these animals did not show any significant change in their TL values, when tested after 192 h and 432 h of their last training (Figure 3).

Effect of acute training on TL of rats using elevated plus-maze: Group XIII animals, which received nine acute trials on plus-maze, showed a significant ($p < 0.001$) decrease in their TL values, when measured after 72 h and 192 h of their last training exposure (Figure 3).

Effect of sub chronic training on TL of rats using elevated plus-maze: Animals of group XIV, which received sub chronic training on plus-maze (i. e. total of six trials on 1st and 2nd day) showed a significant ($p < 0.001$) fall in TL value, when measured after 72 h and 192 h respectively of their last training exposure, as compared to their first exposure TL. However, these animals did not show any significant decrease in their TL after 432 h (Figure 3).

Effect of chronic training on TL of rats using elevated plus-maze: The animals (group XV), which underwent chronic training on plus-maze (total of 9 trials during first 3 days) exhibited a significant ($p < 0.001$) decrease in their TL values as compared to control group, which lasted up to 432 h (Figure 3).

DISCUSSION

Memory may be looked upon as an ability to remember past events. It is a complex process involving various parts of the brain, several neurotransmitters (GABA, ACh, E, NE, Glutamate etc.) and sensory organs (Parle et al., 2004a; 2004b). Psychologists define memory as a capacity to retain information and later retrieve this information for day to day activities. Memory is comprised of following components: perception (sensation), registration, consolidation, storage, retrieval (recall) and decay. It is observed that the process of decay of information or forgetting is a continuously active process and well learnt information is totally forgotten, if a conscious effort is not made to retain it e.g. we do not remember the poems and theorems, we had well crammed and rehearsed during our school days. Different parts of the brain contribute to different types of sensory (such as visual, olfactory etc) stimuli (Jarrard, 1995) and different kinds of brain damage produce different types of amnesia (memory lose). Hippocampus plays an important role in storing information and hippocampal damage results in serious learning as well as memory deficits (Milner et al., 1968). There are several types of memory such as sensory memory, short term memory, working memory (Anderson et al., 1996), intermediate long term memory and long term memory. Long term memory is further sub classified into implicit (skill or procedural) memory and explicit (declarative) memory (Nyberg and Tulving, 1996). Explicit memory in turn can be further divided into semantic memory, episodic memory and photographic memory (Collins and Quillian, 1969; Dosher and Ma, 1998; Mitchell, 1989; Nesca and Koulack, 1994; Squire and Zola-Morgan, 1998).

Swimming model is based on spatial memory and motor skills (Morris, 1984; Morris et al, 1988). In our earlier study, we showed that swimming strengthens brawn as well as brain (Parle et al., 2005). Therefore, two additional behavioral models unrelated to swimming were also employed in the present study to substantiate the results. Elevated plus-maze is a neutral model, in which the rats show preference towards covered arms (Itoh et al, 1990). Hebb-Williams maze is an incentive based memory model in which food serves as the reward (Parle and Singh, 2004). Since all these different memory models produced consistent results on retention capacity, the built in limitations if any, present in an individual experimental model are taken care of. In the present study, we had provided a hidden platform to the rats for escape. During first exposure, rats swam vigorously and made all attempts to escape from swimming by clinging to the side of the pool. However these rats, preferred to stay on the platform once they succeeded in locating it. The position of the platform in the swimming pool was fixed in such a manner that although, it was not visible from outside, the animal could keep its head and nose comfortably above water surface while standing on the platform with its legs. We observed in the present study that the rats remembered the location of the platform (spatial memory) with respect to the starting point and quickly reached the platform during subsequent trials.

Sensory memory may be taken as perception or registration of new information recorded by sensory system in a raw form, which is stored for an ultra short period of time (Dosher and Ma, 1998). The animals, which were subjected to only single exposure to various behavioral models, did not produce any significant improvement in memory score after 72 h and thereafter. These results suggested that the single exposure group animals failed to retain the newly learned information even for a short period of time. This reflects that sensory memory is capable of holding information for an ultra short interval of time following which there is fast decay of information in the absence of subsequent rehearsals. Sensory memory is converted into short-term memory (STM) upon deliberate effort by an individual in this direction. This short-term memory (STM) is probably expressed through graphical images or perception of words (Baddeley, 1988; Nesca and Koulack, 1994). When the animals were subjected to three consecutive swimming trials on first day in sub-acute group, escape latency time (ELT) was significantly reduced thereby indicating good STM. These animals of sub-acute group retained this information about the location of the platform up to a period of 72 h. However, these animals failed to locate the platform, when tested after 192 h and there after. These findings, suggested that repeated trials are necessary for transferring the learned information (sensory memory) into STM. These observations were found to be consistent in other behavioral models as well, thereby substantiating above results. The animals of the acute group and sub-chronic group performed well on all the three behavioral memory models up to a period of 192 h, as reflected by their respective significant high memory scores (as indicated by markedly reduced ELT, TRC and TL values). In other words, the animals which were exposed to nine trials on first day (acute group) performed almost similar to the animals, which were subjected to six trials divided in two days (sub-chronic) in the present study. These observations suggested that the newly learned information has been successfully transferred from sensory memory to intermediate long term memory via short-term memory, as a consequence of repeated acute or sub-chronic rehearsals. However, it appeared that the impact of rehearsals was not strong enough for formation of long-term memory. Incomplete and improper consolidation may explain the lack of retention after 432 h, since memory retrieval is dependent on strength of memory trace as well as on various input conditions at the time of training (Lamour and Allain, 1996; Morris, 1984). It is essential that the processes of perception, registration, consolidation and storage should be active and long enough so as to form permanent or long-term memory (Izquierdo and Medina, 1992; Nader et al., 2000). We had subjected the animals in chronic group to a regular schedule of three trials on each day for 3 successive days in order to consolidate the learned task. We observed that in all the three behavioral models, the animals of chronic group successfully retained the newly acquired information up to a period of 18 days (432 h). It is note worthy to mention here that ELT, TRC and TL values showed a significant decrease uniformly, in all the three different behavioral models. These results highlight the importance of regular rehearsals in the consolidation of long term memory and probably explain why we forgot our school poems.

CONCLUSIONS

The present study underlines the importance of regular rehearsals in enhancing one's capacity of retaining the learnt information. Single exposure to a new environment is not sufficient enough to form a permanent memory trace in brain. Sub-acute, acute and sub-chronic rehearsals result in storing of information for a limited period of time.

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KEY POINTS

- The present study underlines the importance of regular rehearsals in enhancing one's capacity of retaining the learnt information.
- Sub-acute, acute & sub-chronic rehearsals result in storing of information for a limited period of time.
- Quick decay of information or forgetting is a natural continuously active process designed to wipe out unnecessary and useless information.
- The capacities of grasping, understanding and memory are all crucial for career growth.
- Single exposure to a new environment is not sufficient enough to form a permanent memory trace in brain.

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