ORIGINAL ARTICLES

Choline Supplementation Facilitates Short-term Memory Consolidation into Intermediate Long-term Memory of Young Sprague-Dawley Rats

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ABSTRACT

Choline is important for the synthesis of acetylcholine, an integral neurotransmitter involved in memory formation. In order to investigate the effect of choline supplementation on memory consolidation, the study utilized a T-maze to facilitate passive avoidance learning and memory in young female Sprague-Dawley rats. Rats were placed in two groups; choline-supplemented that received choline chloride daily for two weeks, and control that received vehicle daily for two weeks. Rats were evaluated to determine their ability to avoid an aversive electric foot-shock (0.1mA at 60V) when they characteristically entered the preferred dark area (DA) of the T-maze. Both groups of rats showed preference, without significant difference, for entry into DA of the T-maze. However, fifteen minutes after passive avoidance both choline supplemented and control rats avoided entry into DA. This display of DA avoidance 15 minutes after training, suggests that both groups of rats had acquired short-term memory of the aversive stimulus. However, when the test was repeated 24 hours after training, the control group did not avoid entry into DA, whereas the choline-supplemented group either avoided entry or entered after a significantly longer latency period ($p < 0.01$). These results suggest that supplementation with choline facilitated the consolidation of short-term memory of the avoidance learning into intermediate long-term memory in young rats.

La Suplementación con Colina Facilita la Consolidación de la Memoria a Corto Plazo y su Transformación en Memoria Intermedia a Largo Plazo, en las Ratas Sprague-Dawley Jóvenes

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RESUMEN

La colina es importante para la síntesis de la acetilcolina – un neurotransmisor integral que participa en la formación de la memoria. Para investigar el efecto de la suplementación con colina en la consolidación de la memoria, el estudio utilizó un laberinto T para facilitar la memoria y el aprendizaje de evitación pasiva en ratas hembras jóvenes Sprague-Dawley. Las ratas fueron colo-cadas en dos grupos: uno que recibió cloruro de colina diariamente por espacio de dos semanas, y uno de control que recibió vehículo diariamente por dos semanas. Las ratas fueron evaluadas a fin de determinar su habilidad para evitar un choque eléctrico aversivo (0.1mA a 60V) cuando entraban característica-mente a la preferida área oscura (AO) del laberinto en T. Ambos grupos de ratas mostraron preferencia – sin diferencia significativa – por entrar en el área oscura del laberinto en T. Sin embargo, quince minutos después de la evitación pasiva, tanto las ratas que recibieron la suplementación con colina como las ratas de control, evitaban entrar al área oscura. El hecho de que se observe la evitación del área oscura15 minutos después del entrenamiento, sugiere que ambos grupos de ratas habían adquirido una memoria a corto plazo del estímulo aversivo. Sin embargo, cuando la prueba se repitió 24 horas después del entrenamiento, el grupo de control no evitó el entrar al AO, mientras que el grupo

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que recibió el complemento de colina, o evitó entrar o entró luego de un período de latencia significativamente más largo ($P < 0.01$). Por lo tanto, estos resultados sugieren por consiguiente que la suplementación con colina facilitó la consolidación de la memoria a corto plazo del aprendizaje de la evitación, y su transformación en memoria a largo plazo en las ratas jóvenes.

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INTRODUCTION

Cholinergic neurons are abundant in the hippocampus of the brain and it is well established that cholinergic innervation of the hippocampus plays an integral role in both the formation of short-term and long-term memories (1–3). For example, treatment of rats with cholinergic receptor antagonists such as atropine disrupted both forms of memories (4); while intraseptal administration of the muscarinic agonist, oxetremorine, disrupted only memory consolidation in rats in a delayed-non-match-to-sample radial maze task (5). Studies in which rats were placed in a radial arm maze task modified by environmental enrichment (*ie* toy presentation) also showed significant involvement of cholinergic neurons of the hippocampus in memory consolidation (6). Cholinergic neurons require choline for synthesis of the neurotransmitter, acetylcholine, and therefore choline supplementation may improve the establishment of long-term memories. Therefore the aim of the study was to determine whether choline supplementation could facilitate the consolidation of long-term memory in young rats.

MATERIALS AND METHODS

Animal groups used and treatment before training

Eight to ten week old female Sprague-Dawley rats weighing 240–310 g were divided into two groups and housed under standard laboratory conditions. All rats were allowed access to water and standardize rat food (LabDiet 5001 purchased from PMI Nutrition International that contains 2250 part per million choline chloride) *ad libitum*. The experimental group (n = 8) received daily, over a period of 14 days, intraperitoneal choline chloride (Sigma Chemicals) supplementation at a dose of 100 mg/ml solution at 0.2 ml/100g body weight; while the control group (n = 8) received distilled water (vehicle) at a volume of 0.2 ml/100g body weight, intraperitoneally for the same period.

Description of the Training Apparatus

Verification of the effect of choline supplementation was tested in an animal model that utilized passive avoidance learning during exploratory behaviour in a T-maze. The T-maze was a wooden T-shaped runway with two arms. The neutral (grey) arm (NA) with dimensions 51 cm (length) x 26 cm (width) x 30 cm (height), acted as the starting runway from which rats could begin to explore the maze. The NA was illuminated by a 40-watt bulb placed directly above it. The longer arm with dimensions 128 cm (length) x 26 cm (width) x 30 cm (height) was divided into two chambers, a white area (WA) and a dark area (DA). The sides of WA were lined with white cartridge paper and illuminated by a 40-watt bulb positioned directly above it. The DA was enclosed by a lid of black cartridge paper (the roof) and the sides were also lined with black cartridge paper. The WA was separated from DA by a removable partition. The floor of DA was fitted with a plate that could deliver electric foot shocks of 0.1mA at 60 V intensity.

Training and Testing in the T-maze

Following the 14-day choline supplementation, rats were placed singly in NA on day 15 and allowed to explore the T-maze for 300 seconds. Entry into DA or WA was recorded when all four paws of the rat were present in the respective chamber. Rats were given three trials, at ten-minute intervals to determine chamber preference. Only those rats that moved out of NA within 300 seconds and showed a preference for DA were considered for the next stage of the experiment. For each rat, the result of the first trial was used to calculate the median latency for the pre-acquisition period.

Passive avoidance training was carried out 10 minutes after the pre-acquisition period. During this period, rats were placed singly in NA once again and allowed 300 seconds to explore the maze. Entry into DA resulted in the application of an electric foot-shock and the animal was allowed to escape, leading to avoidance of DA.

Short-term memory of the aversive event was evaluated by determining whether the avoidance behaviour was retained 15 minutes after foot-shock training (*ie* 15-minute retention period). Recall of foot-shock association with DA would result in rat entering WA or remaining in NA for the duration of the observational period. Those rats that re-entered DA at 15 minutes, and therefore did not recall the foot-shock association with DA, received a second foot-shock. Intermediate long-term memory was evaluated by determining whether learnt avoidance behaviour was retained 24 hours after foot-shock training (*ie* on Day 16 or 24 hour-retention period).

Data Analysis: Pre-acquisition and retention scores

A standardized scoring system combining the chamber selection and latency was designed to characterize the exploratory behaviour of each rat at the pre-acquisition and retention (15-minute and 24-hour) periods. The scores for chamber selection were as follows: score = 0 if the rat remains in NA; score = 1 if the rat entered WA and score = 2 if the rat entered DA.

These scores were distributed as indicated above, because it is the normal propensity of rats to explore and

show a preference for entry into dark areas. Therefore, entry into DA was given the maximum score for this normal behaviour, whereas the lowest score was given to a rat that did not explore (*ie* remained in NA) and the intermediate score was given to a rat that explored, but did not show preference for the dark chamber (*ie* entered WA).

The scores for latency period were as follows: score = 1, if the latency was less than or equal to the median latency of the group at pre-acquisition and score = 0, if the latency was greater than the median latency for the group at pre-acquisition. The latency scores were distributed as indicated above, because it is the natural propensity for rats to explore; therefore a shorter latency of movement would indicate a greater propensity to explore and hence the greater score. The final score given to each rat for a specific period (pre-acquisition and retention) was a summation of the scores for chamber selection and latency.

Statistical analysis

Chi-square analysis was used to examine preference of rats for selective entry into DA for both groups before training, as well as differences in preferred arm between the the groups. The two sample median test was used to determine significant differences in the median latency between groups before training. The Wilcoxon paired-sample test was used to evaluate significant changes in behaviour before (pre-acquisition period) and after training (retention period) for each group. These statistical tests were performed using SPSS for windows version 12.

RESULTS

When the exploratory behaviour of rats was examined in the T-maze before passive avoidance training (designated the pre-acquisition period), all 16 rats showed a preference for DA ($p < 0.01$; Table 1) and moved out of NA within 300 seconds (Figure 1). Additionally, the control group and the choline-supplemented group showed no significant difference in preference for DA (Table 1) or in the median latency (Fig. 1) for preferential entry into DA. Therefore, the exploratory behaviour of both groups of rats during the pre-acquisition period was unaffected by 14 days of choline supplementation or vehicle treatment. The exploratory behaviour was standardized in terms of chamber selection and latency of entry, which when computed gave a maximum achievable pre-acquisition score of 3 for each rat. During the pre-acquisition period, rats from both groups obtained pre-acquisition scores of 2 or 3 (Fig. 2). All rats advanced to the training stage for the experiment.

Table 1: Probability of arm selection made by rats during three trials in T-maze. Rats were placed in NA (neutral arm) and choosed to remain in NA or enter either DA (dark area) or WA (white area) of the box. All rats exited NA and both groups showed similar preference for entry into DA

Group of rats	**Probability of entry (%) into T-maze arms (Calculated from 3 trials per rat)**		
	DA (Dark area)	WA (White area)	NA (Neutral arm)
Control group (n = 8)	75**	25	0
Choline-supplemented group (n = 8)	78**	22	0

** represents significant preference for entry into DA ($p < 0.01$)

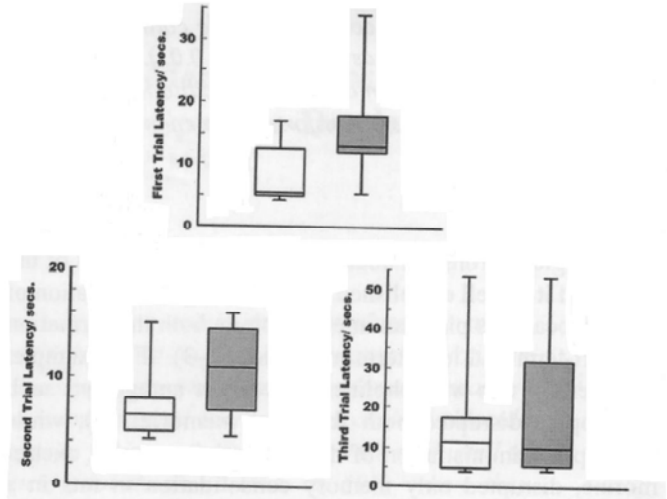
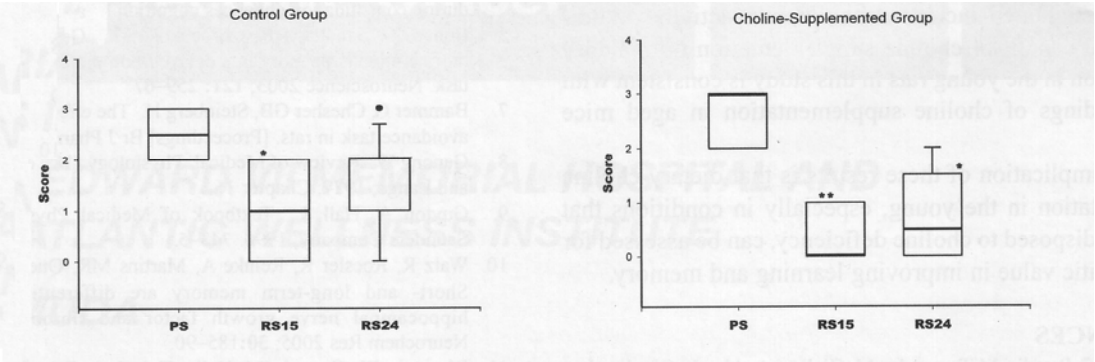


Fig. 1: Graphs showing the latency of entry into DA for control (white box) and choline-supplemented groups (gray box) during the pre-acquisition period. There was no significant difference in the latencies between the groups for the three trials.

When the rats were tested in the T-maze during passive avoidance training, involving brief application of an electric foot-shock on entering DA, both the control group (n = 8) and the choline-supplemented group of rats (n = 8) exited DA immediately after receiving the foot-shock. Subsequent to this training, the rats were examined in the T-maze to determine whether exploratory behaviour reflected memory retention of the avoidance procedure. The exploratory behaviour was again standardized in terms of chamber selection and latency of entry, which when computed gave a maximum achievable retention score of 3 for each rat. Neither the control nor the choline-supplemented group of rats achieved the maximum retention score for exploratory behaviour when they were examined 15 minutes after the avoidance learning procedure. In fact, the retention score of the control group and the choline-supplemented group had significantly decreased (Fig. 2). These lower scores (0 to 2, $p < 0.05$ for control group and 0 to1, $p = 0.01$ for choline-supplemented group) indicated that both groups of rats retained the memory of the foot-shock and avoided entry into DA and consequently their exploratory behaviour was retarded 15 minutes after the training period.

However, when the rats were examined 24 hours after training, the control group of rats did not retain memory of



Signficant differences from the score at PS indicated by * and ** for $p < 0.05$ and $p < 0.01$ respectively.

Fig. 2: Box plot representing distribution of scores for exploratory behaviour of each rat during pre-acquisition (PS) and retention periods 15 minutes (RS15) and 24 hours (RS24) of avoidance procedure. Bold line for each box represents the median. The control group and the choline-supplemented group had median values at PS that were not significanly different. Both control rats (vehicle) and choline-supplemented rats remembered the aversive stimulus 15 minutes after training, as reflected by a significant decrease from the scores obtained at pre-acquisition ($p < 0.05$ for control rats and $p < 0.01$ for choline-supplemented rats), but only choline-supplemented rats remembered the aversive stimulus at 24 hours retention period, producing a decrease in the scores of rats that was significantly different from the score attained at pre-acquisitions ($p < 0.05$).

the avoidance training and therefore produced retention scores that were not significantly different from the pre-acquisition period (Fig. 2). By contrast, the choline-supplemented group of rats retained memory of the avoidance training and consequently their exploratory behaviour was retarded giving retention scores that remained significantly lower (0 to 2; $p < 0.05$) than that of the pre-acquisition period (Fig. 2).

DISCUSSION

The exploratory behaviour of young rats, including preference for dark areas, was used in this study to investigate the effects of choline on the retention of memory of an avoidance procedure in a T-maze. However, before the rats were trained in the avoidance procedure, they were allowed to explore the T-maze in order to confirm their preference for dark areas as previously reported by Banner (7). This preference was demonstrated in this study when both the control group and the choline-supplemented group of rats selected entry into the dark chamber, which was one of three options for exploration in the T-maze.

Advantage was taken of this demonstrated propensity of rats to select DA during exploration to develop a passive avoidance procedure through delivery of an aversive electric foot-shock upon entry into the dark chamber of the T-maze. In a single training session, rats received an electric foot-shock when they demonstrated their natural preference for entry into the dark chamber and they all exited the chamber immediately. Memory of this aversive stimulus would reduce exploratory behaviour, observed as rats avoiding entry, either by not entering the dark area or entering with a longer latency.

Memory retention of this aversive stimulus was present 15 minutes after avoidance training in the control and the choline-supplemented rats, as both groups avoided entry into the dark area of the T-maze. Recall of activity 15 minutes from occurrence is usually taken as an indication of the presence of short-term memory of the event (8–10). Therefore, from the results of the study, the rats in both the control and the choline-supplemented group displayed short-term memory of aversive stimulus.

However, when memory retention of the avoidance procedure was assessed 24 hours after the training session, only the choline-supplemented group of rats avoided entry, indicating a significantly reduced exploratory behaviour compared to the pre-acquisition period. By contrast, the control rats did not avoid entry into the dark chamber of the T-maze when they were assessed 24 hours after training. Intermediate long-term memory is characterized by the 24-hour retention time of the event (8–10). Based on this designation of intermediate long-term memory, only the choline-supplemented group of rats showed evidence of intermediate long-term memory of the avoidance procedure. Therefore, it is reasonable to conclude that only the choline-supplemented rats were able to consolidate short-term memory of the avoidance procedure into intermediate long-term memory.

The consolidation of short-term memory into long-term memory in the choline-supplemented rats is in agreement with the reported involvement of brain cholinergic nerves in the formation and retention of memory (4–6). In previous studies, choline supplementation was shown to facilitate increased cholinergic nerve activity in the brain including the hippocampus, by serving as a precursor for the synthesis of the neurotransmitter acetylcholine (11, 12).

Therefore, it is likely that in this study choline supplementation facilitated increased cholinergic activity. Additionally, the benefit of choline supplementation on memory consolidation in the young rats in this study is consistent with similar findings of choline supplementation in aged mice (13).

The implication of these results is that dietary choline supplementation in the young, especially in conditions that may be predisposed to choline deficiency, can be assessed for its therapeutic value in improving learning and memory.

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