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Research report

Perinatal choline treatment modifies the effects of a visuo–spatial attractive cue upon spatial memory in naive adult rats

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Abstract

The improvement in memory functions by choline supplementation is hypothesized to be due to increased synthesis and release of acetylcholine in the brain. We have found previously that combined pre- and postnatal choline supplementation results in long-lasting facilitation of spatial memory in juvenile rats when training was conducted in presence of a local salient cue. The present work aims to analyze the effects of peri- and postnatal choline supplementation on spatial abilities of naive adult rats. Treated rats were trained in various cued procedures of the Morris navigation task of 5 months of age. The treatment had a specific effect of reducing the escape latency of the rats when the platform was at a fixed location in space and indicated by a suspended cue. This effect was associated with an improved spatial memory when the cue and the platform were removed. In this condition, the control rats showed impaired spatial discrimination following the removal of the target cue, most likely due to an overshadowing of the distant environmental cues. This impairment was not observed in the treated rats. Further training with the suspended cue at unpredictable places in the pool revealed longer escape latencies in the control than in the treated rats suggesting that this procedure induced a selective perturbation of the normal but not of the treated rats. A special probe trial with the cue at an irrelevant location and no escape platform revealed a significant bias of the control rats towards the cue, but in treated rats towards the uncued spatial escape position. This behavioral dissociation suggests that a salient cue associated with the target induces an alternative 'non spatial' guidance strategy in normal rats, with the risk of overshadowing attention towards more distant spatial cues. As a consequence, the improved escape in the presence of the cue in the treated rats is associated with a stronger memory of the spatial position following disappearance of the cue. This and previous observations suggest that a specific spatial attentional process relies on the buffering of highly salient visual cues to facilitate integration of their relative position in the environment.

Theme: Neural basis of behaviour

Topic: Learning and memory: systems and functions

Keywords: Choline supplementation; Morris maze; Cued learning; Attentional processes; Spatial abilities; Rat

1. Introduction

Pre- and postnatal dietary supplementation of choline is assumed to alter brain development and function [18,19]. There are numerous reports of improved memory function in adult animals following dietary supplementation of choline. For example, an improvement in reference memory in a radial-maze task [14], and a lower rate of forgetting in interruption trials with delay in aged rats [11] have been shown. Relative to prenatally deficient and to control animals, choline enrichment during embryonic days 11 to 17 improved performance in temporal processing [12], and reduced proactive interference in a radial-maze task when trials were massed [13]. Changes in ChAT, AchE activity, and muscarinic receptor binding in hippocampus and cortex were correlated with the improvement of spatial performance [6,14]. The morphology and distribution of cholinergic neurons in the basal forebrain were altered by similar perinatal treatment [9,10]. Though the mechanism for this effect of choline upon brain function has not been elucidated, the alterations in brain organization suggest that such supplementation during

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early development is susceptible to modify memory capacity.

Alteration of memory capacity relies on several processes such as attention, selection, organization and encoding of pertinent components of the environment. We have shown that training in the presence of a salient cue indicating a goal position is a task particularly sensitive to alterations of the cholinergic system [3,1,4,5]. A non-specific treatment like perinatal choline enrichment induced an enhancement of spatial memory only following training in the presence of a conspicuous visuo–spatial cue [16]. These results suggested that the selection of an appropriate strategy when an optimal performance requires combining guidance towards a cue with a memory of goal position relative to distant cues might be enhanced by cholinergic treatment.

The aim of the present study is to extend our investigation of the behavioral effects of perinatal choline enrichment upon the encoding of a salient visuo–spatial cue by naive adult rats during a spatial task. To this end, we have developed tests allowing a detailed behavioral analysis in order to determine the changes induced by choline treatment upon the accuracy or the quality of spatial memory. In particular, the following experiments were designed to measure (1) the overshadowing of environmental landmarks by a salient cue signaling the vicinity of the hidden platform; (2) the attraction towards an irrelevantly cued sector; (3) the use of a directional cue to reach a hidden goal.

2. Method

2.1. Treatment

Eight adult female rats of the PVG strain provided by Hofmann SA Basel were exposed to 0 ($n=4$) or 5 ml/l solution of 70% choline chloride ($n=4$), in a saccharine solution (1.2 g/l) as described in Meck et al. [14] and Schenk and Brandner [16]. Briefly, the choline supplementation started at the end of the first week of pregnancy, and was maintained until after weaning. The females were kept separate in standard macrolon cages (38×60×20 cm) provided with a wooden nest box and cellulose as nesting material. The litter size varied between 8 and 12 in both the choline treated and control groups.

During gestation, the treated females consumed a mean of 31 ml/day, and the control females 29 ml/day. This supplementation was maintained until 4 weeks after birth. The total amount of water consumed by the litters was about 80 ml/day at the end of the third week postnatal. From day 29 onward, the young rats were provided daily with choline in diluted condensed milk (0.0 or 0.5 ml 70% choline solution) to provide a supplementary intake of about 40 mg choline. This treatment was progressively discontinued from P.N. day 65 over 10 days. We observed no difference in weight or gross morphology between the choline and the control individuals.

2.2. Subjects

Thirty-two PVG rats (16 females and 16 males) from eight different litters served as subjects. They were 5 month-old and the average weight was 230 g and 435 g for the females and the males respectively at the onset of the experiment. The rats were kept with their respective mothers until the age of 42 days. The rats were then maintained in groups of 4–5 individuals of the same sex in standard macrolon cages (38 cm wide×60 cm long×20 cm high) with a wooden nest box, and ad libitum access to food and water.

2.3. Neurochemistry

At the end of the behavioral experiments sixteen rats (4 male, 4 female choline treated rats and 4 male, 4 female control rats) were sacrificed by decapitation. The entire hippocampi were dissected and weighed. Hippocampi were pooled and homogenized using a sonicator (sonifier, B-30) equipped with a microtip (output no. 3.5×0.5 s at 0.5 s intervals) in 10 vol. of 25 mM sodium phosphate buffer, pH 7.4. Homogenate was centrifuged at 10 000 rpm for 60 min at 4 °C. The resultant supernatant fraction was used to determine ChAT activity level.

ChAT activity was measured following classical published procedures [7] with minor modifications. In particular, sample preparation and incubation conditions were performed according to [8]. In brief, samples were prepared by adding 50 μl of tissue supernatant to 50 μl of incubation mixture that consisted of the following components (final concentrations in parentheses): 2.5 mM choline chloride (1.25 mM), 0.4 mM (^{14}C) actetyl–CoA (Amersham) (0.2 mM; 50 000–60 000 dpm/sample), 0.2 mM eserine sulfate (0.1 mM), 0.3 mM sodium chloride (0.15 mM) in 0.1 mM sodium phosphate buffer, pH 7.4 (0.0625 mM).

3. Experiment 1

The first experiment was designed to assess the effects of peri- and postnatal choline supplementation upon cued spatial learning and memory capacities. To this end, naive five month-old treated and control rats were trained in a Morris navigation task. Rats were first trained in the presence of a conspicuous cue hanging above the hidden platform. The location of the platform and the cue remained fixed throughout this first training phase. Then, whereas the hidden platform was maintained at the same location, the position of the cue was varied. Finally, rats were trained to reach a new platform location in absence of the cue.

3.1. Material and methods

3.1.1. Subjects

Eighteen 5 month-old naive rats (4 males, 5 females control and 4 males, 5 females choline treated) served as subjects. The rats were then kept, 4–5 individuals per group of the same sex, in standard macrolon cages (38×60×20 cm) with a wooden nest box and ad libitum access to food and water.

3.1.2. Data analysis

Each trial was video recorded and the escape latency was measured and pooled in 4-trial blocks. Regarding the latency data, the standard deviation varied with the mean, so a logarithmic transformation was implemented on the original data to stabilize the variance and avoid biases in the ANOVAs [17]. The probe trials were analyzed by an XY video tracker (Kukam S.A.) allowing the measure of time spent in the four sectors (Ø 44 cm) of the pool located in the center of each quadrant.

In experiment 1, the path lengths of four trials (block 11, trials 41–44) of the 'place and variable cue' phase were measured.

3.1.3. Apparatus

A large circular tank of water (diameter 160 cm, wall height 60 cm) painted in white was filled with water (25 °C) and milk (0.5 l) to a depth of 30 cm. This pool was located in a room containing several uncontrolled cues (door, posters, suspended dark cloth) at some distance from the pool wall (min. 100 cm). Four orthogonal starting positions were spaced around the perimeter of the pool, dividing its surface into four quadrants. An invisible escape platform made of transparent Plexiglas (diameter 14 cm) could be placed in the center of each quadrant. A black cylinder (diameter 4.5 cm, length 10 cm) was suspended 30 cm above the platform for cueing procedures. A video camera was placed directly above the center of the pool.

3.1.4. Behavioral procedure

All rats underwent 52 trials and three probe trials over 8 days (4, 8, 8, 4, 4, 8, 8, and 8 trials respectively). A first probe trial was given on day 4 following trial 24, a second probe trial was given on day 5 preceding trial 25, and a third probe trial on day 8 following the last trial, i.e. trial 52.

3.1.5. Phase 1 ('place and cue', trials 1–24)

During phase 1, the rats performed 24 trials over 4 days (i.e. 4, 8, 8 and 4 trials respectively). Control and choline treated rats were trained with a dark cylinder suspended above the hidden platform ('place and cue'). The cue and the platform remained at a fixed position in the pool during the entire phase of the training. A first probe trial during which each rat was left in the pool for 60 s without platform and cue, was given on day 4, following trial 24.

3.1.6. Phase 2 ('place and variable cue', trials 25–44)

During phase 2, the rats received 20 trials over 3 days (4, 8 and 8 trials respectively). The position of the platform remained at the same location, but the position of the dark cylinder was varied pseudo-randomly above the center of each sector. Different cue and starting positions were used on each trial on a pseudo-random schedule such that each starting point was used twice within the eight daily trials. Moreover, the relative location of the start and cue on each trial was arranged so as to avoid more than two identical (right or left) motor responses in sequence. A second probe trial during which the rat was left in the pool for 60 s without platform, but in presence of the cue suspended above one of the three sectors that was never reinforced, was given as the first trial of the day (before trial 25).

3.1.7. Phase 3 ('new place only', trials 45–52)

During phase 3, the rats received 8 trials during 1 day. Training was resumed in the absence of the black cylinder and with the escape platform at a new location. A third probe trial during which the rat was left in the pool for 60 s without platform and cue, was given on day 8, following trial 52.

3.2. Results

In this experiment, we did not detect any significant effect of sex, so the results for males and females were combine for further analyses. The effects of the treatment on the three different phases of the spatial task by naïve adult rats are shown in Fig. 1.

3.2.1. Phase 1: Place and cue training (trials 1–24, blocks 1–6)

A 2-way ANOVA repeated measures (treatment X blocks 1–6) on the escape latencies revealed a significant effect of the treatment ($F[1,16]=7.3$, $P=0.016$). There was also clear evidence of learning in both groups (block effect, $F[5,80]=38$, $P=0.0001$), without a significant interaction between treatment and blocks (Fig. 1a).

3.2.2. Phase 2: 'place and variable cue' (trials 25–44, blocks 7–11)

A 2-way ANOVA repeated measures (treatment X blocks 7–11) on the escape latencies revealed a significant effect of the treatment ($F[1,16]=7.3$, $P=0.011$), as well as a clear evidence of learning in both groups (block effect, $F[4,64]=14.5$, $P=0.0001$). No significant interaction between treatment and blocks was detected (Fig. 1b).

Comparison of Fig. 1a and b shows an increase in escape latency maintained throughout training with the

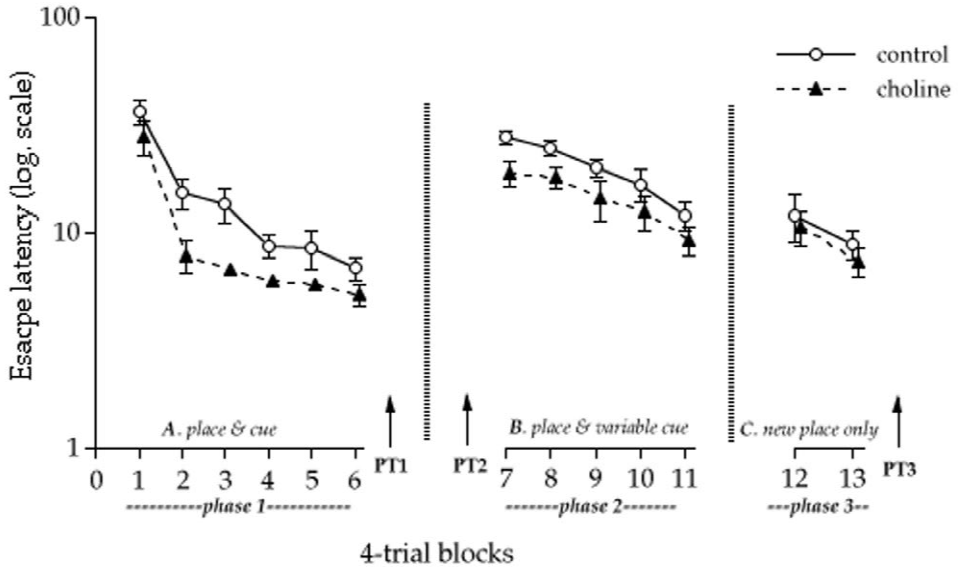

Fig. 1. Mean (±sem) escape latencies (logarithmic scale) during training in the Morris navigation task by control ($N=9$) and choline treated ($N=9$) 5 month-old naive rats. (A) Phase 1,'place and cue', blocks 1–6, the salient cue was hung above the hidden platform. (B) Phase 2, 'place and variable cue', blocks 7–11, the platform location remained fixed in space, but the position of the salient cue was varied pseudo-randomly above the center of each sector. (C) Phase 3, 'new place only', blocks 11–13, the location of the hidden platform was changed, and the salient cue was removed.

variable cue in both groups despite the fact that the hidden platform remained at the same location. This effect was analyzed by a 2-way repeated measures ANOVA [treatment X escape latency of the last block of the phase 1 (block 6) and the last block of the phase 2 (block 11)]. This analysis showed no treatment effect ($F[1,16]=2.3$, ns), but indicated that the escape latency of block 11 remains higher than that of block 6 (block effect: $F[1,16]=18.62$, $P=0.0005$; PLSD block 11>block 6).

Finally, and in order to evaluate the effect of displacement of the cue on the swimming trajectories, path lengths of the trials of block 11 were measured. A one-way ANOVA indicated that mean path length of the choline treated rats was significantly shorter than this of the control ($F[1,16]=4.97$, $P=0.04$; PLSD control>choline).

3.2.3. Phase 3: 'new place only' (trials 45–52, block 12–13)

A 2-way repeated measures ANOVA (treatment X blocks 12–13) revealed no significant effect of treatment or blocks (treatment: $F[1,16]=0.4$, ns; blocks: $F[1,16]=0.10$, ns). As well, no significant effect was shown by a 2-way repeated measures ANOVA treatment X last block (11) of the phase 2 and on the first block (12) of the phase 3 (treatment: $F[1,16]=0.33$, ns; blocks: $F[1,16]=0.73$, ns).

3.2.4. Probe trials

The effects of the treatment upon spatial memory during the probe are shown in Fig. 2.

3.2.4.1. Probe trial 1 (no platform, no local cue). The time spent in the four sectors of the pool in the absence of the platform and the cue is illustrated by Fig. 2a.

A repeated measures ANOVA on the four sectors of the pool showed no treatment effect ($F(1,16)=0.3$, ns). A difference in the amount of time spent in the four sectors ($F(3,48)=62.6$, $P=0.0001$) and a significant interaction between treatment and sectors ($F(3,48)=11.4$, $P=0.0001$) was observed. A one-way ANOVA revealed that the treated group spent a greater amount of time in the training sector as compared to the control group (treatment effect: $F(1,17)=62.6$, $P=0.0015$).

3.2.4.2. Probe trial 2 (no platform, but with the local cue). During the second probe trial, rats were allowed to swim in the pool in the presence of the cue suspended above the opposite quadrant, but without escape platform (Fig. 2b).

A repeated measures ANOVA on the four sectors of the pool showed a significant treatment effect ($F(1,16)=28.9$, $P=0.0001$). A significant difference in the amount of time spent in the four sectors ($F(3,48)=101.3$, $P=0.0001$) as well as a significant interaction between treatment and sectors ($F(3,48)=68.3$, $P=0.0001$) were observed. A one-way ANOVA revealed that the treated rats spent a greater amount of time in the training sector than the cued one ($F(1,17)=66.6$, $P=0.0001$; PLSD treated>control), while control rats spent a greater amount of time in the cued sector than the treated one ($F(1,17)=74$, $P=0.0001$; PLSD control>treated).

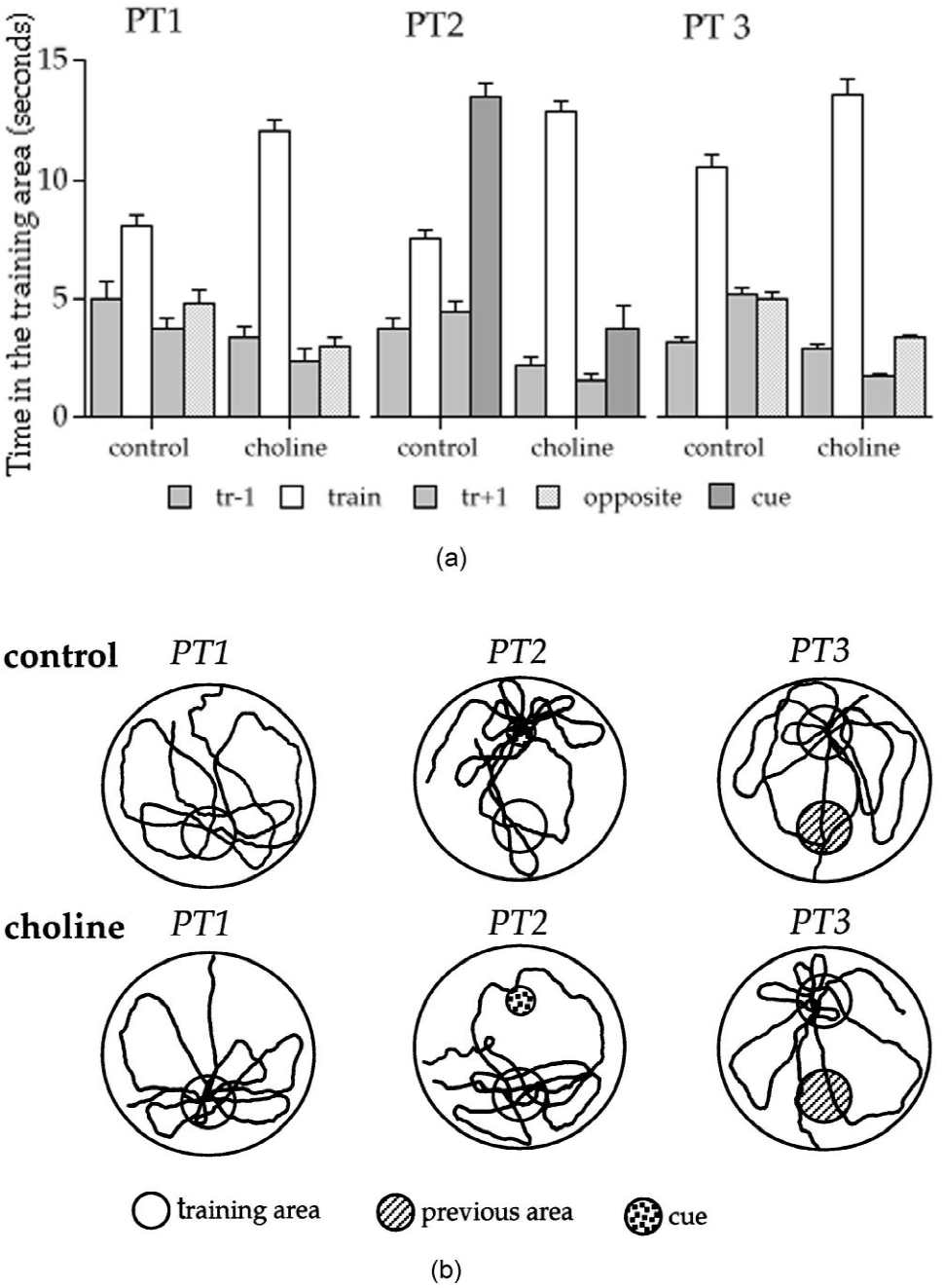

Fig. 2. (A) Mean (±sem) of the time spent in the four sectors (Ø 44 cm) of the pool located in the center of each quadrant during a 60 s probe trials. Probe trial 1 (PT1) was given following trial 24 of phase 1, in the absence of both the platform and the salient cue. Probe trial 2 (PT2) was given as the first trial of the day (before trial 25 of the phase 2) in the absence of the platform but in the presence of the salient cue. Probe trial 3 (PT3) was given following trial 52 in the absence of both the platform and the salient cue. (tr-1=left adjacent training sector; train=training sector; tr+1=right adjacent training sector; opposite=opposite training sector; cue=sector where the cue was hung during the probe trial). (B) Swimming paths taken by representative control and choline treated rats during the probe trials.

3.2.4.3. Probe trial 3 (no platform, no local cue). Fig. 2c shows the time spent in the four sectors of the pool during this third and last probe trial given at the end of the training with the platform at a new location.

A repeated measures ANOVA on the four sectors of the pool showed no significant treatment effect ($F(1,16)=1.6$). However, a difference in the amount of time spent in the four sectors ($F(3,48)=69.2$, $P=0.0001$) and a interaction between treatment and sectors ($F(3,48)=69.2$, $P=0.0001$) was observed.

A one-way ANOVA revealed that the treated group spent a greater amount of time in the training sector as

compared to the control group (treatment effect: $F(1,17)=7.4$, $P=0.0004$).

4. Experiment 2

The first experiment shows that a salient cue placed above the hidden goal can facilitate escape latency in inducing guidance possibly based upon the attractive visuo–spatial features of the cue. Moreover, it shows that control animals are more attracted by the cue than by the spatial location of the goal, while treated rats showed a stronger attraction to the training sector.

This second experiment was designed to assess the effect of the position of the conspicuous cue upon spatial abilities. To this end, the location of the suspended cue was dissociated from the platform position. This procedure was designed in order to evaluate the capacity of the rats to use such a cue as an indicator allowing establishment of their position in relation to the environment.

During the first training phase, the cue was hung above the center of the quadrant adjacent to the hidden platform. Then, the location of the platform and the cue were rotated 180°, but the spatial relation between the cue and the platform was maintained. Finally, training was continued with the platform at the same location, but in absence of the hung cue (see Fig. 3).

4.1. Material and methods

4.1.1. Subjects

Fourteen 5 month-old naive rats (4 males, 3 females as controls, and 4 males, 3 females that were choline treated) served as subjects. The rats were kept in the same conditions as described in Experiment 1.

4.1.2. Apparatus

The same large circular tank as described in Experiment 1 was used.

4.1.3. Behavioral procedure

All the rats underwent 52 trials and three probe trials over 7 days (4, 8, 8, 8, 8, 8 and 8 trials respectively). The first probe trial was given on day 4 following trial 24, the second on day 6 following trial 44, and the third on day 7 following the last trial.

4.1.4. Phase 1 ('place and adjacent cue', trials 1–28)

During phase 1, all the rats received 28 trials over 4 days (i.e. 4, 8, 8 and 8 trials respectively). Control and choline treated rats were trained with a dark cylinder suspended above the center of one of the two quadrants adjacent to the hidden platform (Fig. 3). The cue and the platform remained at a fixed position in the pool during the whole phase of the training. A first probe trial during which the rat was left in the pool for 60 s without platform and cue, was given on day 4, following trial 24.

4.1.5. Phase 2: ('new place and adjacent cue', trials 29–44)

During the phase 2, the rats received 16 trials over 2 days (8, 8 and trials respectively). The position of both the platform and the cue was rotated 180° from the previous location, and training was continued (Fig. 3). A second probe trial during which the rat was left in the pool for 60 s without platform, but in presence of the cue suspended above the opposite quadrant (where is was during the phase 1), was given on day 6, following trial 44.

4.1.6. Phase 3 ('new place only', trials 45–52)

During phase 3, the rats received 8 trials over 1 day. Training was resumed in absence of the black cylinder, but with the escape platform at the same location as in phase 2. A third probe trial during which the rat was left in the pool for 60 s without platform and cue, was given on day 8, following trial 52.

4.2. Results

The effects of choline treatment on the three different phases of the spatial task by naive adult rats are shown in Fig. 4. In this experiment, no significant effect of sex was detected, thus the results of males and females were collapsed for further analyses.

4.2.1. Phase 1: Place and adjacent cue training (trials 1–28, blocks 1–7)

A 2-way repeated measures ANOVA (treatment X

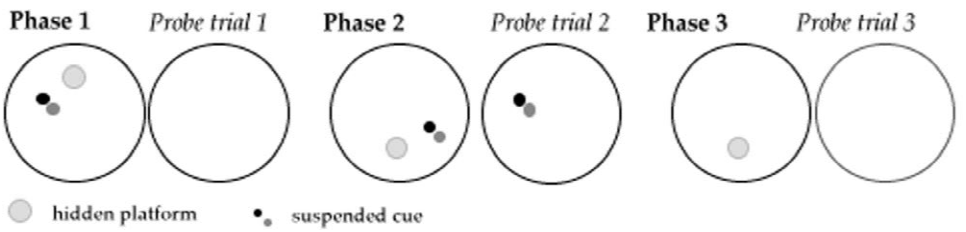

Fig. 3. Schematic representation of the swimming pool with the location of the adjacent cue during training phases 1, 2 and 3 and probe trials of Experiment 2.

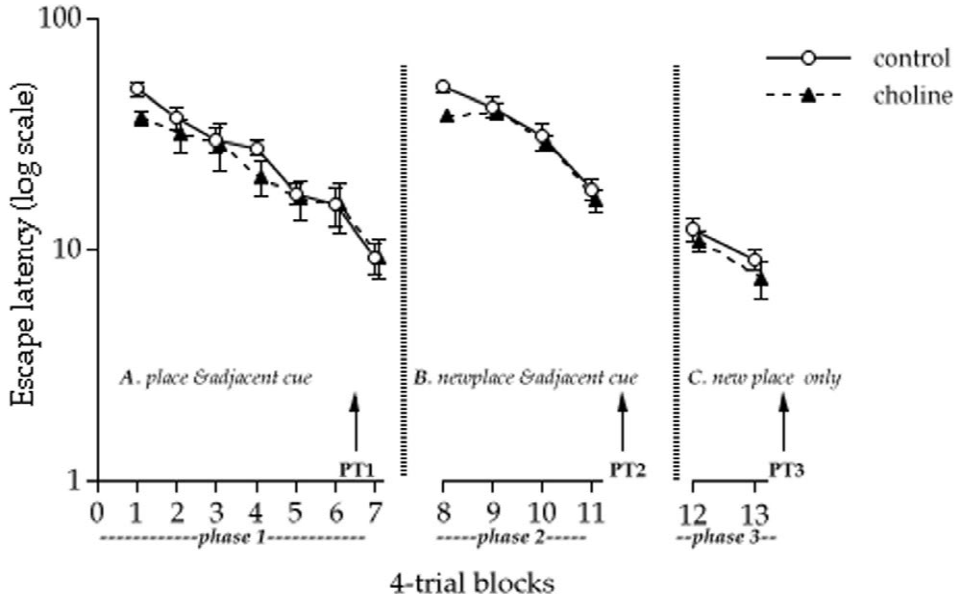

Fig. 4. Mean (±sem) escape latencies (logarithmic scale) during training in the Morris navigation task by control ($N=7$) and choline treated ($N=7$) 5 month-old naive rats. (A) Phase 1,'place and adjacent cue', blocks 1-, the salient cue was hung above the center of one of the two quadrants adjacent to the hidden platform. (B) Phase 2, 'new place and adjacent cue', blocks 8–11, the position of both the platform and the cue was rotated to 180° from the previous location. (C) Phase 3, 'new place only', blocks 12–13, the location of the hidden platform was changed, and the salient cue was removed.

blocks 1–7) of the escape latencies did not shown a significant treatment effect ($F[1,12]=3.2$, ns). Analysis showed a learning effect for both groups (block effect, $F[6,72]=22.5$, $P=0.0001$), without a significant interaction (Fig. 4a).

4.2.2. Phase 2: New place and adjacent cue training (trials 29–44, blocks 8–11)

A two-way repeated measures ANOVA (treatment X blocks 8–11) on the escape latencies did not shown a significant effect of the treatment ($F[1,12]=3.8$, ns), but a significant effect of block ($F[3,36]=42.1$, $P=0.0001$) without a significant interaction (Fig. 4b).

A two-way repeated measures ANOVA on the blocks 7 and 8 (previous location versus new location) revealed a significant effect of treatment ($F[1,12]=11.3$,$P=006$), a significant effect of block ($F[1,12]=217.7$, $P=0.0001$), and a significant interaction between these two factors ($F[1,12]=7.9$, $P=0.016$). Further analysis indicated no significant effect of treatment on the escape latency of the block 7 ($F[1,13]=0.11$, ns), but, as compared to the control group, the escape latency of choline treated rats was significantly shorter during block 8 ($F[1,13]=12.3$, $P=0.004$, PLSD control>choline).

This task appears to be quite difficult since neither choline treated nor control rats were able to reach a performance comparable with that of the last block of phase 1. Indeed, the inversion of cue and platform locations by a 180° rotation induced a two-fold increase in escape latencies in both groups (see Fig. 1).

4.2.3. Phase 3: Place only training (trials 45–52, block 12–13)

The removal of the adjacent cue appears to facilitate escape latency in both groups (Fig. 4c). This effect was confirmed by a 2-way repeated measures ANOVA (treatment X blocks 11 and 12; treatment: $F[1,12]=1.6$, ns; block effect: $F[1,12]=28.7$, $P=0.0002$; PLSD block 11> block 12).

A two-way repeated measures ANOVA on the block 12 and 13 did not show a significant effect of treatment ($F[1,12]=1.5$, ns), but an effect of learning (block effect: $F[1,12]=4.8$, $P=0.05$) in the absence of the suspended cue.

4.2.4. Probe trial 1 (no platform, no local cue)

The time spent in the four sectors of the pool in the absence of the platform and the cue is illustrated by Fig. 5a.

A repeated measures ANOVA (treatment X the four sectors of the pool) showed no treatment effect ($F(1,12)=0.3$, ns). There was a significant difference in the amount of time spent in the four sectors ($F(3,36)=130.6$, $P=0.0001$) and a interaction between treatment and sectors ($F(3,36)=4.3$, $P=0.01$). This interaction was due to the control group which spent a greater amount of time in the sector where the cue was hung during training than did treated rats ($F(1,13)=5$, $P=0.045$; PLSD control>treated).

4.2.5. Probe trial 2 (no platform, but with the local cue)

During the second probe trial (Fig. 5b), rats were allowed to swim in the pool in presence of the cue

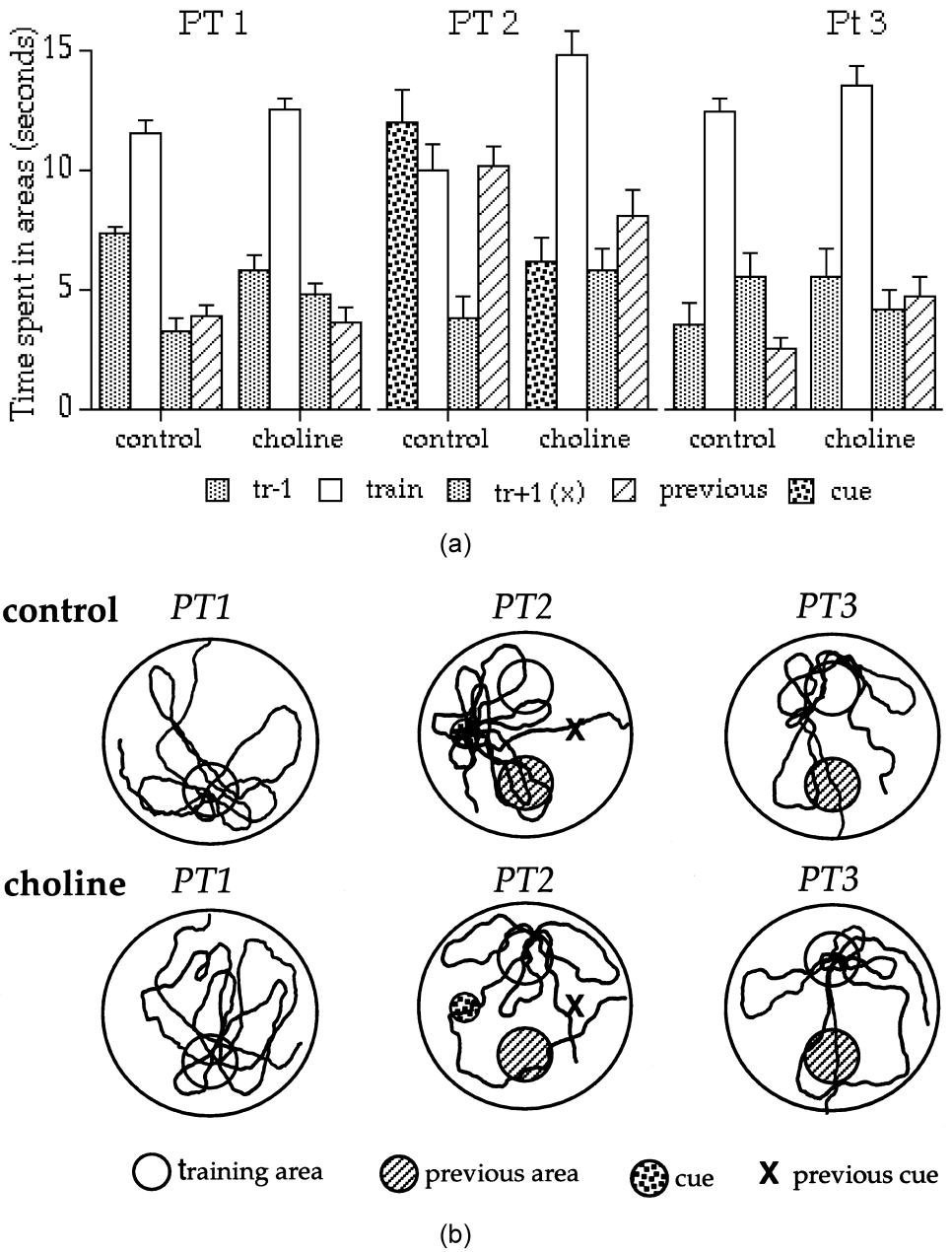

Fig. 5. (A) Mean (±sem) of the time spent in the four sectors (Ø 44 cm) of the pool during a 60 s probe trials. Probe trial 1 (PT1) was given following trial 24 of phase1, in the absence of both the platform and the salient cue. Probe trial 2 (PT2) was given at the end of the phase 2 following trial 44 in the absence of the platform but in the presence of the salient cue. Probe trial 3 (PT3) was given following trial 52 in the absence of both the platform and the salient cue. (tr-1=left adjacent training sector; train=training sector; tr+1=right adjacent training sector; opposite=opposite training sector; cue=sector where the cue was hung during the probe trial). (B) Swimming paths taken by representative control and choline treated rats during the probe trials.

Table 1
Comparison of the mean escape latencies (s) during the last blocks of the phase 1 and 2

Groups	Block 7 (last block of phase 1)	Block 11 (last block of phase 2)	2-way ANOVA
Control	9.17±1.4	18.27±1.8	Treatment: $F[1,12]=0.36$, ns
Choline	9.2±1.7	16.4±1.8	Blocks: $F[1,12]=18.85$, $P=0.001$

Table 2
Comparison of the mean time spent (s) in the four sectors during the second probe trial in presence of the suspended cue

Groups	Training sector	Adjacent right	Opposite sector	Cued sector
Control	7.9±9	3.1±0.7	8.2±0.6	9.6±1.1
Choline	11.9±0.8	5±0.8	4.7±0.7	6.5±0.9

suspended above the opposite quadrant (where it was during the phase 1, see Fig. 3), but without escape platform.

A repeated measures ANOVA on the four sectors of the pool (Table 2) did not show a significant treatment effect ($F(1,12)=0.1$, ns). This analysis revealed, however, a difference in the amount of time spent in the four sectors ($F(3,36)=17.8$, $P=0.0001$) and an interaction between treatment and sectors ($F(3,36)=9.7$, $P=0.0001$).

A two-way repeated measures ANOVA on time spent in the cued and in the training sectors did not show significant effects of treatment or sector (treatment: $F(1,12)=0.26$, ns; sector: $F(1,12)=3.4$, ns). However, a significant interaction between these factors ($F(1,12)=11.9$, $P=0.005$) was shown. Further analysis revealed that the choline treated rats focused on the training sector ($F(1,13)=10.2$, $P=0.008$) when control rats focused on the cued sector (control: 9.6±1.1; choline: 6.5±.91; $F(1,13)=4.8$, $P=0.05$).

Finally, control rats maintained a stronger bias towards the previous training sector (phase 1) than choline treated rats ($F(1,13)=13.8$, $P=0.003$).

4.2.6. Probe trial 3 (no platform, no local cue)

The time spent in the four sectors of the pool during this third and last probe trial given at the end of the training in the absence of the adjacent cue is illustrated in Fig. 5c.

A repeated measures ANOVA (treatment X the four sectors of the pool) showed a significant treatment effect ($F(1,12)=11$, $P=0.006$), a difference in the amount of time spent in the four sectors ($F(3,36)=43.2$, $P=0.0001$) without a significant interaction between treatment and sectors.

An one-way ANOVA on the time spent in the training sector did not show a significant difference between groups (control: 12.4±0.6; choline: 13.57±0.8; $F(1,13)=1.4$, ns).

4.2.7. Neurochemistry

A 2-way ANOVA conducted on ChAT activity expressed as micromoles per hour per 100 milligram of protein in the hippocampi of both the choline treated and the control rats indicated no effect of treatment ($F[1;12]=0.84$; ns) or sex ($F[1;12]=0.38$; ns).

5. General discussion

A modification of spatial navigation measured in a Morris navigation task was observed in both, escape latency and spatial memory in naive 5 months old rats. The improvement of spatial abilities was specific, and related to the presence and the location of a salient visuo–spatial cue signaling the hidden goal during training. These results were not correlated with a change in ChAT activity measured in the hippocampus more than 4 months after the end of the treatment.

5.1. Effects upon spatial learning

Choline treatment reduced escape latencies in rats trained with a salient cue hung above the fixed, hidden platform. This result demonstrates (1) that a salient cue can facilitate escape learning by reducing path lengths, and (2) that this effect is significantly enhanced in treated rats. This result might be explained by an early orientation towards the goal indicated by the cue. However, since the cue does not precisely coincide with the platform, optimal performance requires combining guidance towards the cue with a memory of the platform position relative to distant cues.

In treated rats both escape latency and path length significantly decreased when the cue was randomly suspended above the center of each sector, but with the platform position remaining fixed (see procedure experiment 1).

In this situation, optimal performance requires inhibition of guidance towards the cue in favor of the spatial location of the goal. As treated rats showed shorter path lengths than controls, choline enrichment seems to help rats to prevent guidance when the salient cue does not coincide with the spatial position of the platform.

When the cue was removed, and the rats trained in a new position, the effect of treatment upon escape latency was abolished. This absence of effect seems to indicate that the observed improvement in choline treated rats depended on the presence of the local cue during early training. Moreover, no treatment effect upon escape latency was observed when rats were trained with the cue placed above the sector adjacent to the hidden platform. In that case, the lack of improvement could be based on the fact that the spatial cue cannot be used for guidance towards the goal.

5.2. Effects upon spatial memory

Improved spatial memory following choline treatment was shown during the probe trials after both presence and absence of the salient cue during training. These results demonstrate (1) an enhancement of spatial memory after cued training in treated rats, and (2) an accurate search

behavior despite the presence of the cue placed above a sector that was never reinforced. In this situation, control rats showed a marked preference for the cued sector that induced a neglect of the training sector. In contrast, probe trials following training with the adjacent cue did not reveal an improvement of spatial memory in treated rats. However, the probe trial in the presence of the cue has confirmed a preference for the cued sector that overshadowed place memory in control rats that was not observed in treated subjects.

These results have shown improved spatial abilities in the presence of a salient local cue during training in treated rats. With reference to conditioning theory, a local cue could be considered as a stimulus predicting the direction of the hidden goal. Thus, the importance of distant cues could be reduced or overshadowed because the location of the platform can be predicted with sufficient precision by the local salient cue. It seems that the more the rats rely on the salient cue to find the platform, the more the effect of distant cues will be overshadowed. In other words, it could be hypothesized that the stronger the association between the cue and the platform the larger the decrease of memory based on a spatial representation will be. This overshadowing effect appears to depend on the spatial relation between the cue and the goal. Indeed, when the cue was placed above the sector adjacent to the platform, spatial memory of control rats was not altered but improved. It seems that cued learning could either promote navigation directed by the cue, indicating the location towards which the animal has to move, or allows establishment of the platform position in relation to the environment. Thus, approach directed by the cue reinforces the development of guidance and the acquisition of motor sequences, while navigation oriented by the cue reinforces the initialization of a representation adjusted by the local and contextual cues.

We have observed impaired spatial performance in immature and aged animals trained in the presence of a salient cue placed above the hidden platform. This cued procedure appeared first to facilitate escape latency, but induced a reduction of spatial memory depending on age of the animals. In juvenile (less than 26 days old) Long Evans rats, this procedure prevented discrimination of the training sector during a probe trial in the absence of the cue and the platform [1,16,15]. This effect was also observed in males aged 22 months [1,2]. The most deleterious effect upon spatial memory was observed in medial septum lesioned rats trained in presence of the suspended cue [3]. The opposite effect, a reduction of the overshadowing has been observed in juvenile 22 day-old rats treated with NGF [4,5] and in 80 day-old choline treated rats [16]. The present results have shown that spatial navigation is affected by the selection of visuo–spatial components provided by the environment. A local salient cue that predicts the location of a goal could act only as an attractor, and thus impair spatial learning based on distant cues. Choline treatment appears to improve a specific component of spatial learning and memory in adult rats that allows the development and maintenance of a representation despite the presence of an attractive cue. Taken together, these results seem to indicate that an optimal tuning of the cholinergic system seems require for efficient spatial performance.

In summary, we hypothesize that the improvement observed in spatial capacities is explained by a modification in the hierarchy of relevant components (distant versus local) used for spatial learning. This hierarchy between the relevance of spatial components is likely to be based on their salience. The salience might be ranking with synaptic weight and, in that case, choline treatment could promote fine adjustments of cognitive processes in harmonizing the weight of spatial components by modulations of synaptic plasticity.

These speculations cannot be validated by the data presented above. However, they suggest that the control of environmental cues by a quantification of the spatio–visual salience help to understand how the representation is adapted to environmental requirements.

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