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Review

# Learning and metabolic brain differences between juvenile male and female rats in the execution of different training regimes of a spatial memory task

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ABSTRACT

Spatial memory is responsible for encoding spatial information to form a path, storing this mental representation, and evaluating and recovering spatial configurations to find a target location in the environment. It is mainly supported by the hippocampus and its interaction with other structures, such as the prefrontal cortex, and emerges in rodents around postnatal day (PND) 20. Sex differences in spatial tasks have been found in adults, with a supposedly better performance in males. However, few studies have examined sex differences in orientation throughout postnatal development. This study aimed to analyse the performance of juvenile (PND 23) male ($n$ = 18) and female ($n$ = 21) Wistar rats in a spatial reference memory task in the Morris water maze (MWM) with two different training regimes in the acquisition phase, and their subjacent metabolic brain activity. Based on sex, subjects were assigned to two different groups: one that performed four learning trials per day ($n$ = 9 males and $n$ = 8 females) and the other that was submitted to two trials per day ($n$ = 9 males and $n$ = 13 females). After the behavioural protocols, metabolic activity was evaluated using cytochrome c oxidase histochemistry. Results showed no metabolic brain or behavioural differences in the four-trial protocol performance, in which both sexes reached the learning criterion on the fourth day. By contrast, the two-trial protocol revealed an advantage for females, who reached the learning criterion on day four, whereas males needed more training and succeeded on day six. The female group showed lower metabolic activity than the male group in the cingulate and prelimbic cortex. These results suggest a faster consolidation process in the female group than the male group. Further research is needed to understand sex differences in spatial memory at early stages.

## 1. Introduction

Spatial orientation involves essential complex skills that allow us to survive in our surrounding environment [1]. Spatial memory is one of these central functions, responsible for encoding different types of spatial information to form a path, storing this mental representation, and evaluating and recovering that spatial configuration to find a target location in the environment [2,3].

Spatial memory is mainly supported by the hippocampus, a widely studied structure that interacts with other regions, such as the prefrontal cortex, to allow successful spatial navigation [3,4]. Particularly, the dorsal hippocampus, which is closely related to declarative memory in humans, plays an important role in rodents' spatial memory [4–6]. This essential function emerges in rodents around postnatal day (PND) 20 or 21. No research has found evidence of place learning before PND 19, and the complete function emerges much later (PND 45) [7].

The study of spatial memory in rodents and humans has shown sex differences in spatial skills. Extensive literature concurs that males perform better than females regarding different types of memory, such as spatial reference memory (RM) or working memory [8]. Some articles associate this variability with the use of different spatial strategies to navigate through space, and others with the task's difficulty. Regarding the latter, Chen et al. (2020) [9] maintain that these divergences in

*Abbreviations:* BLA, Basolateral Amygdala; CA1, field CA1 of hippocampus; CA3, field CA3 of hippocampus; CCO, Cytochrome c oxidase; CEA, Central Amygdala; CG, Cingulate cortex; DG, Dentate Gyrus; IL, Infralimbic cortex; LAA, Lateral Amygdala; MWM, Morris water maze; PL, Prelimbic cortex; PND, Postnatal day; RM, Reference memory; ZT, Zeitgeber time.

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spatial memory appear when task difficulty increases. However, some research has not found these spatial memory sex differences or has even reported a better performance in females [5]. This controversy could be caused by the scarcity of studies using female samples or by the disparate protocols and features chosen by the researchers.

This problem is aggravated in studies of sex differences during the developmental stage in which the evaluation of spatial memory is almost wholly based on male samples. To contribute to increasing our knowledge of the sex differences in spatial memory and brain activity, the aim of this study was to analyse the execution of juvenile male and female Wistar rats (PND 23 at the start of the experiment) in two spatial RM training regimes using the Morris water maze (MWM) and their subjacent metabolic brain activity. The first protocol entailed four trials in the learning phase, and the second one only included two trials. Several behavioural parameters were analysed, and brain function was evaluated by studying the activity of oxidative metabolism of the areas involved in each task, employing cytochrome c oxidase (CCO) histochemistry.

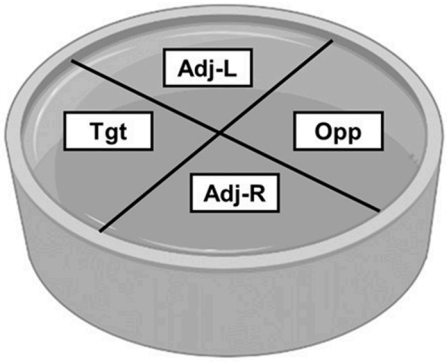


**Fig. 1.** Picture of the Morris water maze where the spatial memory task was conducted. The pool is divided into four quadrants: target quadrant (Tgt), opposite quadrant (Opp), adjacent-right quadrant (Adj-R) and adjacent-left quadrant (Adj-L). Source: Biorender.

## 2. Materials and methods

### 2.1. Subjects

Thirty-nine 23-day-old Wistar rats (18 males and 21 females) that came from five different litters were used. All animals were housed at a constant temperature (22 ± 2 °C) with a relative humidity of 65–70%, a 12-h artificial light-dark cycle (light cycle of 350 luxes from zeitgeber time (ZT) ZT00 to ZT12 and dark cycle from ZT12 to ZT24) and *ad libitum* access to food (pellets compounding of 15.2% crude protein, 3.2% crude fat, 5.1% crude ash, 4.1% crude fibre, Ca, P, Na, Vit A, D3 and E and oligoelements) and tap water. All procedures and manipulation of the animals were carried out following the European Communities Council Directive 2010/63/ EU and the Royal decree N° 53/2013 of the Ministry of the Presidency related to the protection of animals used for experimentation and other scientific purposes. The Ethics Committee of the Principality of Asturias approved the study.

Animals were randomly divided into two groups: a 4-trials RM protocol group, which included 9 males (from two litters) and 8 females (from two litters), and a 2-trials RM protocol group, with 9 males (from three litters) and 13 females (from five litters).

### 2.2. Reference memory (RM) task in the Morris water maze

Animals were trained in the MWM in the light cycle starting the protocols at ZT01. The MWM consisted of a circular pool of 150 cm diameter placed in the centre of a lit room (two lamps of 4000 luxes around the pool). Two sides of the pool were surrounded by two black panels on which different distal spatial cues (yellow circle and blue rectangle with black lines) were placed. The rest of the sides had some cues placed on the room's wall. The water level was 30 cm, and the temperature was 22 ± 2 °C. A black cylinder escape platform (10 cm in diameter) was placed two cm below the surface of the water (not visible to the animals), and the maze was divided into four quadrants: target quadrant (Tgt), opposite quadrant (Opp), adjacent-right quadrant (Adj-R) and adjacent-left quadrant (Adj-L) (Fig. 1). Animals' behaviour was recorded using a video camera (Sony V88E) connected to a computer with the EthoVision Pro-software (Noldus Information Technologies, Wageningen, the Netherlands).

Both learning protocols started on PND 23 with animals' habituation (four trials with a visible platform in the centre of the pool). The 4-trials RM protocol entailed four trials in the learning acquisition phase with a pseudorandomly starting position each trial each day and an escape platform located in the centre of the Tgt. Trials stopped when the animal reached the hidden platform or until 60 s had elapsed. If the animal had not reached the hidden platform after this time, it was placed on the platform for 15 s. During the inter-trial interval, the animals were placed in a black bucket for 30 s. This protocol was performed for 5 days (PND24-PND28), following previous studies carried out in our laboratory and described in Méndez et al. (2008) [10]. After these 4 trials, each day, a 25-second transfer or probe trial was performed. In this case, the platform was removed, and the rat was introduced in the quadrant opposite (Opp) to where the platform had been located to check whether the animal remembered the platform's position.

The 2-trials RM protocol was conducted like the protocol described above but, in this case, we reduced the number of trials in the learning acquisition phase to two trials, and the protocol was conducted for 7 days (PND24-ND30) two days more than the 4-trials protocol, due to the less reinforcement the animals received. Animals were introduced in the pool in a pseudorandomly starting position each trial each day and the escape platform was again located in the centre of the Tgt. The duration of the trials and the inter-trial interval was the same and, after the two trials, a 25-second transfer or probe trial was performed each day.

In both protocols, the time of permanence in the periphery zone, described as an area of 20 cm from the pool edges, and escape latencies during the acquisition trials each day and the time of permanence in each quadrant during the probe test were recorded. A significant difference in the permanence in Tgt compared with the other three quadrants was considered a learning criterion.

### 2.3. CCO histochemistry and optical density quantification

Ninety minutes after the end of each RM protocol, in the light cycle (at ZT03 approximately), subjects were decapitated, brains were removed and frozen in N-methyl butane (Sigma-Aldrich, Madrid, Spain). Coronal sections (30 μm) of the regions of interest were cut in a cryostat (Leica CM1900, Germany). The regions of interest were defined according to the Paxinos and Watson's (2007) [11] stereotactic atlas. The distance in mm of the regions counted from bregma was the following: +3.20 mm for the cingulate (CG), prelimbic (PL) and infralimbic (IL) cortices, and −2.28 mm for the CA1, CA3, and dentate gyrus (DG) subfields of the dorsal hippocampus and amygdala (Basolateral; BLA, Central; CeA, Lateral; LaA).

CCO histochemistry and densitometric quantification were performed as described by Gutiérrez-Menéndez et al. (2021) [12]. CCO enzyme participates in the oxidative phosphorylation process that generates ATP and is considered an indirect marker of the neural activity of some brain regions [13]. In each CCO histochemistry bath, we included sets of tissue homogenate from Wistar rat brains (standards) cut at different thicknesses (10, 30, 50, and 70 μm) that control staining variability. Then, an optical density measure was carried out in each

region for each animal and also in the standard tissue. Regression curves between section thickness and known CCO activity measured spectrophotometrically in each set of standards were calculated for each incubation bath. Finally, average relative optical density values in each region were converted to CCO activity units using the regression curves calculated in each standard (1 unit: μmol of cytochrome c oxidized/min/g tissue wet weight at 23 °C) [14].

### 2.4. Statistical analysis

The Sigma-Stat 12.5 software (Systat Software Inc., Richmond, California) was used to analyse the data obtained. Graphic representation of the results was performed with the SigmaPlot 12.5 software (SPSS Inc. and IBM Company, USA). All data were expressed as mean ± SEM, and the significance level was $p < 0.05$.

Permanence in each quadrant on each day in 4-trials and 2-trials RM protocols were analysed together for each sex, using a two-way repeated-measures ANOVA. Periphery permanency, escape latencies and permanence in Tgt in both protocols were analysed, also using a two-way repeated-measures ANOVA (*Sex* x *Day)*. Post hoc comparisons using the Holm-Sidak method were carried out when significant differences were found. An additional graph was designed to show the progression of the acquisition of the learning criterion (longer permanence in Tgt). Brain metabolic activity was tested using a one-way-repeated-measures ANOVA, and the analysis of interregional correlations was carried out calculating Pearson product-moment correlations using the "jackknife" procedure.

## 3. Results

### 3.1. RM protocols

A two-way repeated measures ANOVA was conducted to analyse the escape latency differences of the acquisition phase on the 4-trials and 2-trials RM tests taking into account factors *Sex (Male, Female)* and *Day* (4-trials: *Day 1* to *Day 5*; 2-trials: *Day 1* to *Day 7*). On the 4-trials RM protocol (PND24-PND28), the interaction *Sex x Day* ($F_{4,60} = 0.125$, $p = 0.973$) and the factor *Sex* ($F_{1,60} = 1.991$, $p = 0.179$) were not significant. However, the factor *Day* was significant ($F_{4,60} = 12.021$, $p < 0.001$). Multiple comparisons using the Holm-Sidak method showed latency differences between *Day 1* and *Day 4* ($t = 4.890$, $p < 0.001$) and *5* ($t = 6.190$, $p < 0.001$), between *Day 2* and *Day 4* ($t = 2.999$, $p = 0.023$) and *5* ($t = 4.299$, $p < 0.001$), and between *Day 3* and *Day 5* ($t = 3.560$, $p = 0.005$), showing shorter escape latencies in the last days (Fig. 2A).

On the 2-trials RM test (PND24-PND30), results showed, as in the 4-trials protocol, that the interaction *Sex x Day* ($F_{6,120} = 2.161$, $p$=.051) and the factor *Sex* ($F_{1,120} = 3.036$, $p = 0.097$) were not significant but there were differences in the variable *Day* ($F_{6,120} = 11.656$, $p < 0.001$). Multiple comparisons using the Holm-Sidak method showed differences between *Day 1* and *Day 3* ($t = 3.772$, $p = 0.004$), *4* ($t = 4.792$, $p < 0.001$), *5* ($t = 4.792$, $p < 0.001$), *6* ($t = 6.271$, $p < 0.001$) and *7* ($t = 6.764$, $p < 0.001$), between *Day 2* and *Day 4* ($t = 2.999$, $p = 0.045$), *5* ($t = 2.999$, $p = 0.042$), *6* ($t = 4.478$, $p < 0.001$) and *7* ($t = 4.971$, $p < 0.001$), and between *Day 3* and *Day 7* ($t = 2.99$, $p = 0.040$) (Fig. 2B).

Then, we carried out a two-way repeated measures ANOVA taking into account the variables *Quadrants* and *Day* for each *Sex* group (*Male, Female)* in each training protocol (4-trials RM and 2-trials RM). Concerning the time spent in quadrants in the probe phase of the 4-trials RM protocol (PND24-PND28), results showed that in the *Male* group, the interaction *Quadrant x Day* ($F_{12,96} = 3554$, $p < 0.001$) and the factor *Quadrant* ($F_{3,96} = 39{,}642$, $p < .001$) were significant. There were no differences in the *Day* factor ($F_{4,96} = 1080$, $p = 0.383$). Post-hoc comparisons showed differences on Day 2 between Tgt and Adj-R ($t = 2940$) and Adj-L ($t = 3537$); on Day 3 between Adj-R and Adj-L ($t = 3057$), Opp ($t = 2747$) and Tgt ($t = 4095$); on Day 4 between Adj-R and Adj-L ($t = 2601$), and between Tgt and Adj-R ($t = 5929$), Adj-L ($t = 3328$) and Opp ($t = 3926$). On the last day, Day 5, differences were observed between Tgt and the rest of the quadrants: Adj-R ($t = 7625$), Adj- L ($t = 7231$) and Opp ($t = 6716$) (all $p < 0.05$). These analyses revealed that the *Male* group reached the learning criterion (more permanence in the Tgt than in the rest of the quadrants) on the fourth day ($p < 0.05$) (Fig. 3A). Conversely, in the *Female* 4-trials RM group, we also found that the interaction *Quadrant x Day* ($F_{18,126} = 4495$, $p < 0.001$) and the factor *Quadrant* ($F_{3,126} = 22{,}016$, $p < 0.001$) were significant. The factor *Day* was not significant in this group ($F_{6,126} = 0{,}389$, $p = 0.882$). Post-hoc comparisons showed permanence differences on Day 2 between Tgt and Adj-L ($t = 2886$); on Day 3 between Tgt and Adj-L ($t = 3389$); on Day 4 between Tgt and Adj-R ($t = 4140$), Adj-L ($t = 3753$) and Opp ($t = 2639$) and finally, on Day 5 between Tgt and Adj-R ($t = 6013$), Adj-L ($t = 3485$) and Opp ($t = 2774$), and between Adj-R and Adj-L ($t = 2528$) and Opp ($t = 3239$) (all $p < 0.05$). Like the Male group, the Female group also reached the learning criterion on the fourth day ($p < 0.05$) (Fig.3B).

Regarding the permanence in quadrants in the probe test of the 2-trials RM protocol (PND24 and PND30), results showed that in the juvenile *Male* group, the interaction *Quadrant x Day* ($F_{18,144} = 2261$, $p = 0.004$) and the factor *Quadrant* were significant ($F_{3,144} = 28{,}263$, $p < 0.001$). There were no differences in the *Day* factor ($F_{6,144} = 1679$, $p = 0.147$). The post-hoc analysis showed differences on Day 2 between Opp and Adj-R ($t = 4136$), Adj-L ($t = 3242$) and Tgt ($t = 2751$); on Day 3 between

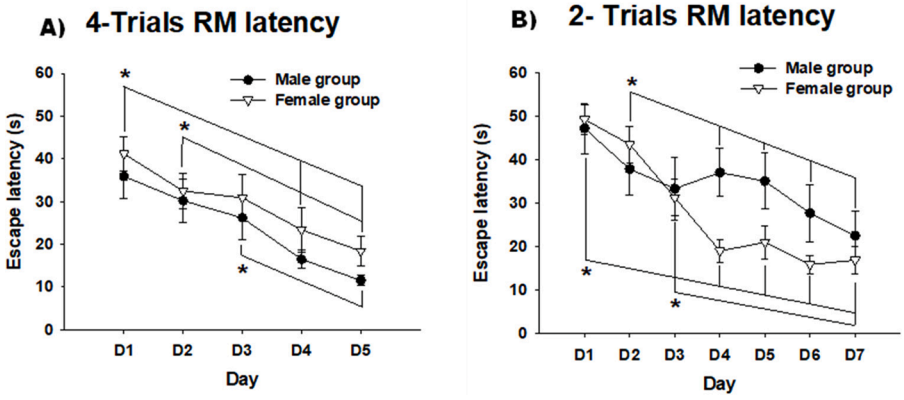


**Fig. 2.** Escape latency (s) results in the acquisition phase of juvenile *Male* and *Female* groups during the 4-trials RM protocol (PND24-PND28) (**A)** and the 2-trials RM protocol (PND24-PND30) (**B**) (Mean ± SEM). **A.** There was a decrease in escape latency on the fourth and fifth days (*$p < 0.05$) but there were no differences between *Sex* ($p > 0.05$). **B.** There was a decrease in escape latency along the days (*$p < 0.05$) but there were no differences between *Sex* ($p > 0.05$). The *x*-axis shows the days. PND, postnatal day; RM, reference memory.

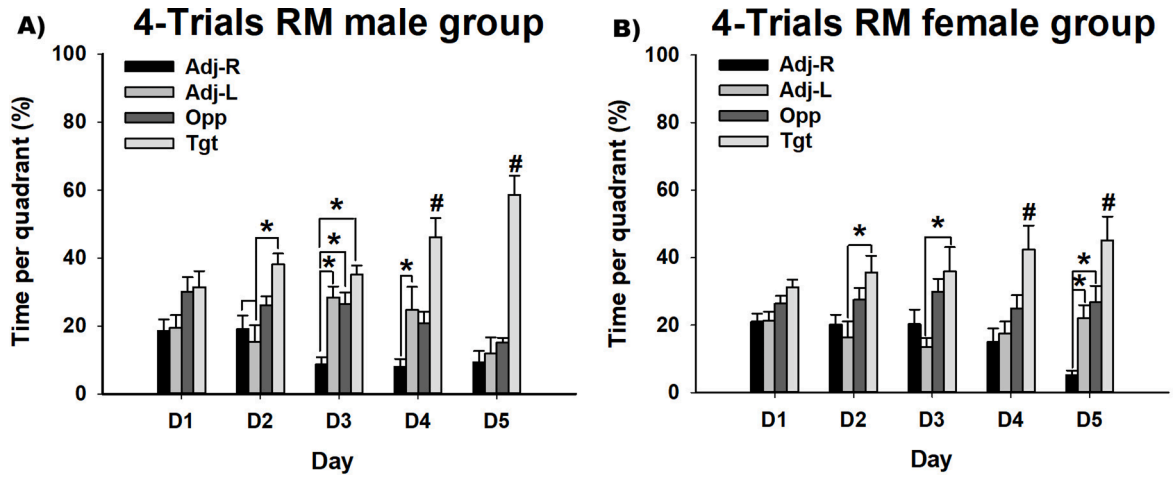


**Fig. 3.** Permanence in the probe phase of juvenile *Male* and *Female* groups in each quadrant (Tgt, Opp, Adj-R and Adjt-L) during the 4-trials RM protocol (PND24-PND28) (Mean ± SEM). A. Permanence of the juvenile *Male* group. They reached the learning criterion on the fourth day (#$p < 0.05$). B. Permanence of the juvenile *Female* group. This group also learned the protocol on the fourth day (#$p < 0.05$). The *x*-axis represents the days. * symbol represents differences between individual quadrants; # symbol represents differences between a quadrant and all other quadrants. Adj-*L* = adjacent-left quadrant; Adj-*R* = adjacent-right quadrant; Opp = opposite quadrant; PND = postnatal day; RM = reference memory; Tgt = target quadrant.

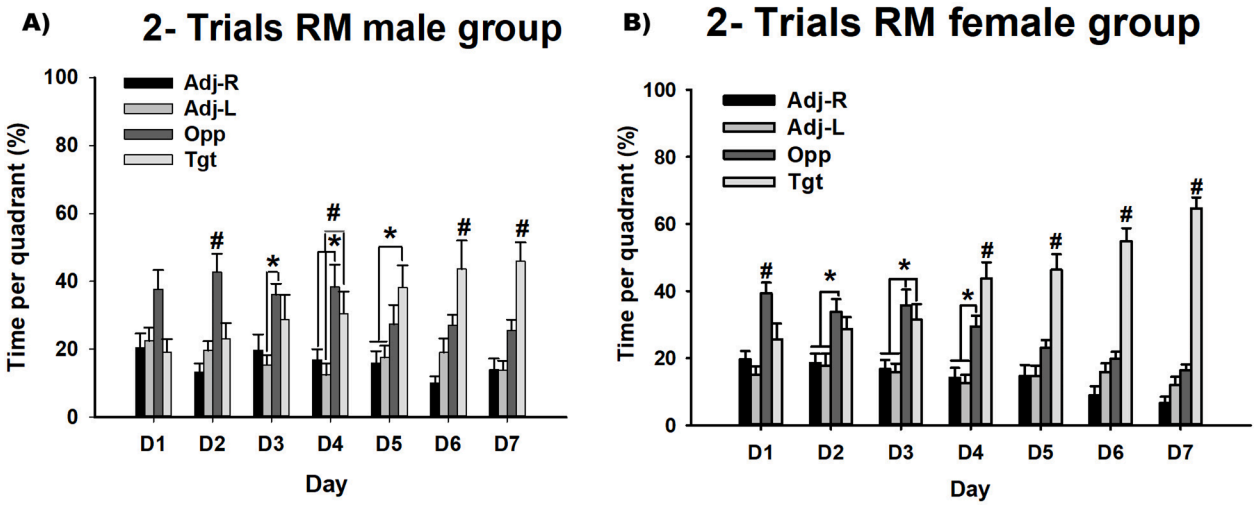


**Fig. 4.** Permanence in the probe test of juvenile *Male* and *Female* groups in each quadrant (Tgt, Opp, Adj-R and Adj-L) during the 2-trials RM protocol (PND24-PND30) (Mean ± SEM). **A.** Permanence of the juvenile *Male* group. They reached the learning criterion on *Day 6* (#$p < 0.05$). **B.** Permanence of the juvenile *Female* group. This group learned the protocol on the fourth day (#$p < 0.05$). * symbol represents differences between individual quadrants; # symbol represents differences between a quadrant and all other quadrants. The *x*-axis represents the days. Adj-*L* = adjacent-left quadrant; Adj-*R* = adjacent-right quadrant; Opp = opposite quadrant; PND = postnatal day; RM = reference memory; Tgt = target quadrant.

Opp and Adj-L ($t = 2895$); on Day 4 between Opp and Adj-R ($t = 3012$) and Adj-L ($t = 3633$), and between Tgt and Adj-L ($t = 2530$); on Day 5 between Tgt and Adj-R ($t = 3132$) and Adj-L ($t = 2902$) and finally, on Day 6 and 7 between Tgt and the rest of the quadrants (Day 6: between Tgt and Adj-R [$t = 4104$], Adj-L [$t = 3734$] and Opp [$t = 2726$] and Day 7: between Tgt and Adj-R [$t = 4484$], Adj-L [$t = 4507$] and Opp [$t = 2859$]) (all $p < 0.05$). The Male group reached the learning criterion on Day 6 ($p < 0.05$) (more permanence in the Tgt than in the rest of the quadrants) (Fig. 4A). In the juvenile *Female* group, we also found that the interaction *Quadrant x Day* ($F_{18,198} = 6733$, $p < 0.001$) and the factor *Quadrant* ($F_{3,198} = 77{,}542$, $p < 0.001$) were significant but the factor *Day* was not significant ($F_{6,198} = 0{,}763$, $p = 0.602$). The Holm-Sidak method revealed differences on *Day 1* between Opp and Adj-R ($t = 3823$), Adj-L ($t = 4560$) and Tgt ($t = 2832$); on *Day 2* between Opp and Adj-R ($t = 2725$) and Adj-L ($t = 2881$); on *Day 3* between Opp and Adj-R ($t = 3542$) and Adj-L ($t = 3879$), and between Tgt and Adj-R ($t = 2702$) and Adj-L ($t = 3039$); on *Day 4* between Opp and Adj-R ($t = 2841$) and Adj-L ($t = 3134$), and between Tgt and Adj-R ($t = 5151$), Adj-L ($t = 5444$) and Opp ($t = 2310$) (all $p < 0.05$). Finally, we found differences on *Days 5, 6* and *7* between Tgt and the rest of the quadrants (*Day 5:* between Tgt and Adj-R [$t = 5633$], Adj-L [$t = 5695$] and Opp [$t = 3943$]; *Day 6*: between Tgt and Adj-R [$t = 8432$], Adj-L [$t = 6827$] and Opp [$t = 6185$]; *Day 7:* between Tgt and Adj-R [$t = 10{,}366$], Adj-L [$t = 9176$] and Opp [$t = 8431$]) (all $p < 0.05$). The *Female* group reached the learning criterion on the fourth day ($p < 0.05$) (Fig. 4B).

In an attempt to find potential *Sex* differences, we carried out several in-depth analyses. A two-way repeated measures ANOVA was conducted to examine permanence differences in the acquisition phase between the *Male* and *Female* groups in the periphery area (20 cm from the pool edges) and Tgt in the probe phase, considering the variables *Sex* and *Day*. Results of the 4-trials RM protocol showed that the interaction *Sex* x *Day* was nonsignificant, both in the periphery ($F_{4,60} = 0.16$, $p = 0.954$) and Tgt ($F_{4,60} = 0.798$, $p = .531$). This shows that there were no differences between the male and female groups over the days. Also, the

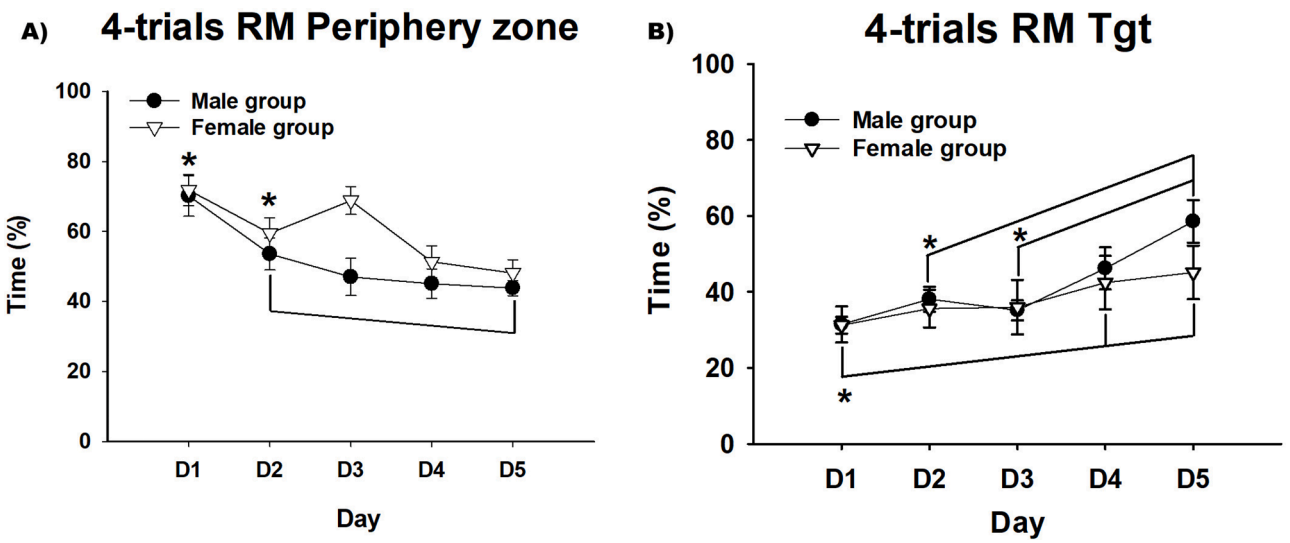


**Fig. 5.** Permanence of the *Male* and *Female* groups during the 4-trials RM protocol (PND24-PND28) in two areas of interest (Mean ± SEM). **A.** Permanence of the *Male* and *Female* groups in the periphery (area of 20 cm from the pool edges) in the acquisition trials. We did not find any group differences over the days ($p > 0.05$) but there were differences between days, observing less permanence over the days (*$p < 0.05$). **B.** Permanence of the *Male* and *Female* groups in Tgt during the probe test. Both groups displayed similar permanence in Tgt over the days ($p > 0.05$). There were differences in the factor *Day,* with longer permanence in the last days (*$p < 0.05$). The *x*-axis represents the days. PND = postnatal day; RM = reference memory, Tgt = target quadrant.

factor *Sex* was nonsignificant in both areas (Periphery: $F_{1,60} = 1.055$, $p = 0.321$ and Tgt: $F_{1,60} = 0.642$, $p = 0.435$). However, *Day* was a significant factor in the periphery ($F_{4,60} = 16{,}105$, $p < 0.001$) (differences between *Day 1* and *Days* 2 [$t = 4.077$], *3* [$t = 5.814$], *4* [$t = 6.420$] and *5* [$t = 7.053$] and between *Day 2* and *Day 5* [$t = 3.220$]) (Fig. 5A) and Tgt area ($F_{4,60} = 6543$), finding differences between *Day 1* and *Days 4* ($t = 2.892$) and *5* ($t = 4.567$), and between *Days 2* and *3* and the last day, *Day 5* (*Day 2-Day 5:* $t = 3.344$; *Day 3- Day 5:* $t = 3.628$) (all $p < 0.05$) (Fig. 5B).

In the 2-trials RM protocol, results showed that the interaction *Sex* x *Day* was not significant in the periphery ($F_{6,120} = 1.974$, $p = 0.075$) or the Tgt ($F_{6,119} = 0.616$, $p = 0.717$), showing no differences in permanence in the two areas between the *Male* and *Female* groups over the days. However, *Day* was a significant factor in the periphery ($F_{6,120} = 21{,}017$, $p < 0.001$) with differences between *Day 1* and *Days 2* ($t = 2882$), *3* ($t = 4122$), *4* ($t = 6288$), *5* ($t = 7012$), *6* ($t = 7599$) and *7* ($t = 9608$); between *Day 2* and *Days 4* ($t = 3405$), *5* ($t = 4130$), *6* ($t = 4717$) and *7* ($t = 6725$); between *Day 3* and *Days 5* ($t = 2891$), *6* ($t = 3478$) and *7* ($t = 5486$); and finally, between *Day 4* and *Day 7* ($t = 3320$) (Fig. 6A) and even in Tgt ($F_{6,119} = 12{,}604$) between *Day 1* and *Days 4* ($t = 3033$), *5* ($t = 4108$), *6* ($t = 5528$) and *7* ($t = 6800$); between *Day 2* and *Days 5* ($t = 3332$), *6* ($t = 4738$) and *7* ($t = 5997$); between *Day 3* and *Days 6* ($t = 3933$) and *7* ($t = 5205$); and finally, between *Day 4* and *Day 7* ($t = 3767$) (all $p < 0.05$) (Fig. 6B). The factor *Sex* was also significant in the Tgt

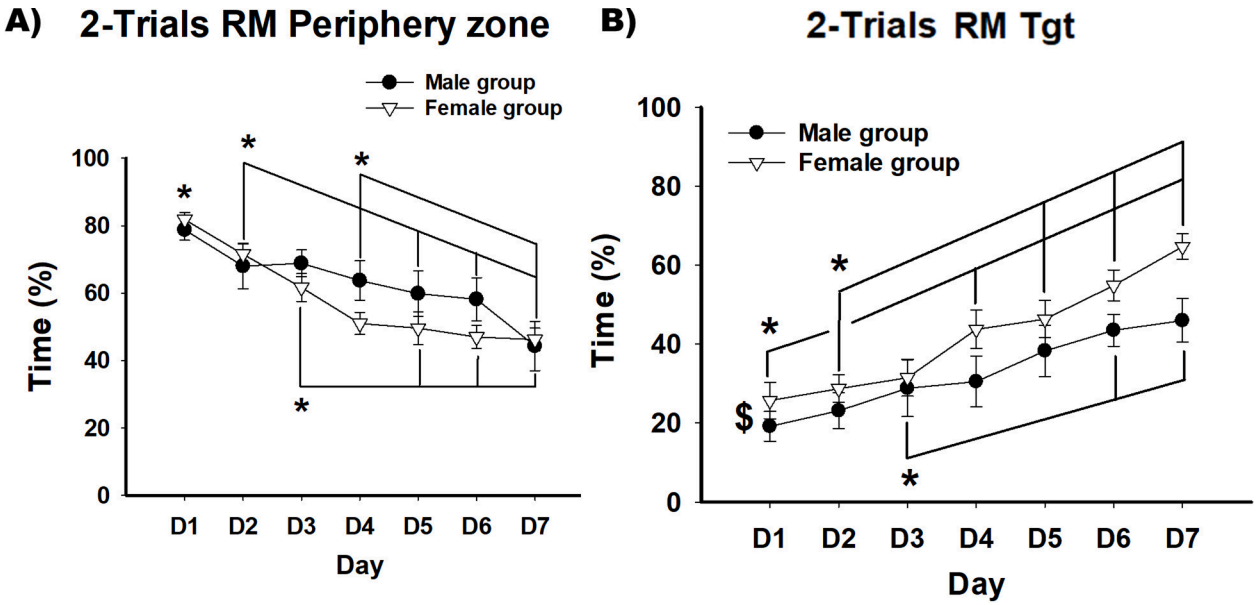


**Fig. 6.** Permanence of the *Male* and *Female* groups during the 2-trials RM protocol (PND24-PND30) in the two areas of interest (Mean ± SEM). A. Permanence of the *Male* and *Female* groups in the periphery (area of 20 cm from the pool edges) during the acquisition phase. We found no group differences over the days ($p > 0.05$), but permanence decreased in the last days (*$p < 0.05$). C. Permanence of the *Male* and *Female* groups in Tgt during the probe test. The *Female* group displayed longer permanence than the *Male* group in Tgt ($p < 0.05$), and there was an increase in permanence in this quadrant over the days in both groups (*$p < 0.05$). The *x*-axis represents the days. PND = postnatal day; RM = reference memory, Tgt = target quadrant.

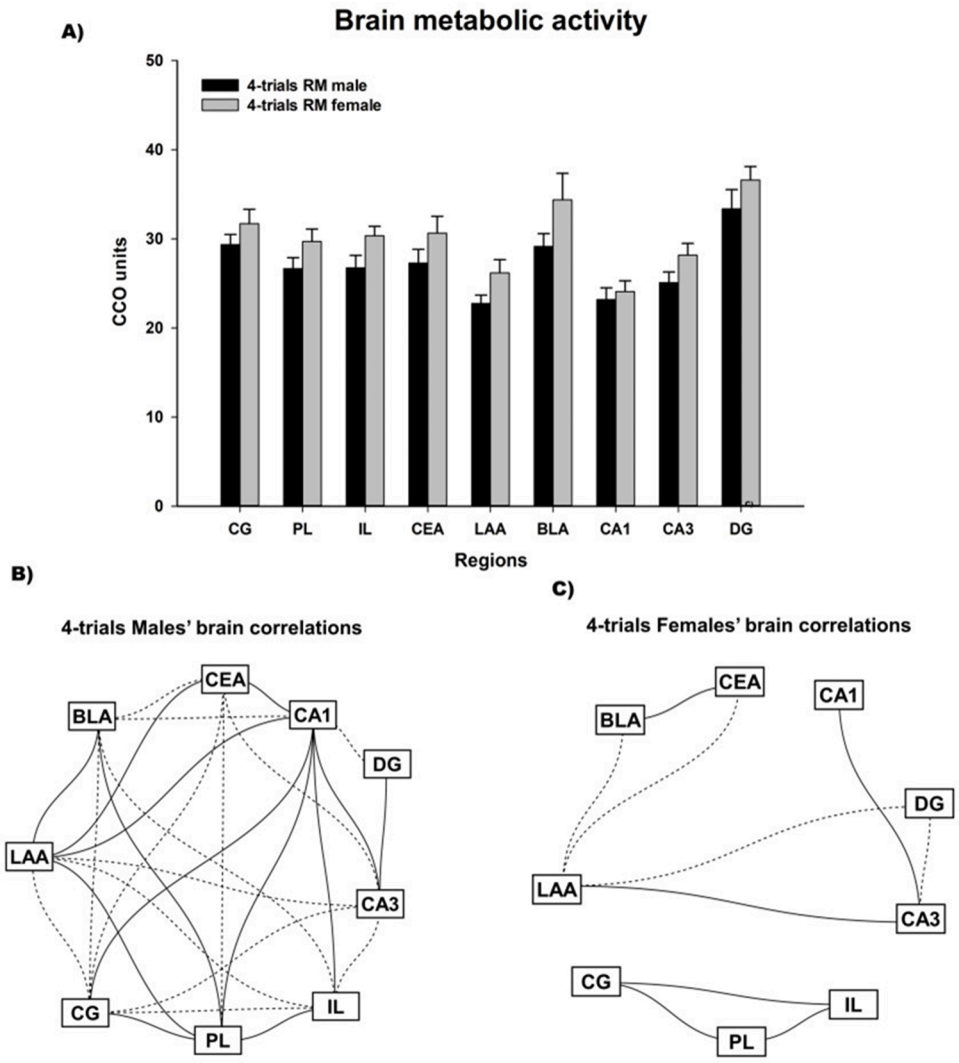


**Fig. 7.** CCO results (mean ± SEM) and brain correlations of the *Male* and *Female* groups in the 4-trials MR protocol. **A.** CCO values of the *Male* and *Female* groups. There were no significant group differences in any of the regions of interest ($p > 0.05$). **B.** Pearson correlations between brain areas in the *Male* group for all the structures studied. **C.** Pearson correlations between brain areas in the *Female* group for all the structures studied. Discontinuous lines: $p < 0.05$; solid lines: $p < 0.01$. Areas: CG = Cingulate cortex, PL = Prelimbic cortex, IL = Infralimbic cortex, CEA = Central Amygdala, LAA = Lateral Amygdala, BLA = Basolateral Amygdala, CA1 = field CA1 of hippocampus, CA· = field CA3 of hippocampus, DG = Dentate Gyrus.

($F_{1,119} = 12{,}150$, $p = 0.002$) with less *Male* permanence in Tgt, but it was not a significant factor in the periphery ($F_{1,120} = 1125$, $p = 0.301$) (Fig. 6).

### 3.2. Brain metabolic activity

Analysis of the brain metabolic differences between the *Male* and *Female* groups in the 4-trials RM protocol releveled no differences in any of the areas of interest (CG: $F_{1,14} = 1.393$, $p = 0.258$; PL: $F_{1,14} = 2.653$, $p = 0.126$; IL: $F_{1,14} = 4.208$, $p = 0.059$; CEA: $F_{1,12} = 1.682$, $p = 0.219$; LAA: $F_{1,12} = 3.557$, $p = 0.084$; BLA: $F_{1,12} = 2.389$, $p = 0.148$; CA1: $F_{1,14} = 0.245$, $p = 0.629$; CA3: $F_{1,14} = 2.995$, $p = 0.106$; DG: $F_{1,14} = 1.508$, $p = 0.240$) (Fig. 7A). However, we observed differences between *the Male* (Fig. 7B) and *Female* (Fig. 7C) groups in the correlations between the regions involved in the RM task. In the *Male* group strong positive correlations were found between CG, PL ($r = 0.897$) and CA1 ($r = 0.890$); between PL, IL ($r = 0.952$), LAA ($r = 0.938$), BLA ($r = 0.962$) and CA1 ($r = 0.898$); between IL and CA1 ($r = 0.919$); between CEA, LAA ($r = 0.888$) and CA1($r = 0.917$); between LAA, BLA ($r = 0.965$) and CA1 ($r = 0.920$), between CA1 and CA3 ($r = 0.937$) and finally, between CA3 and DG ($r = 0.892$) (all $p < 0.01$) (Table 1, Fig. 7B). A pattern of interactivity was also observed between CG, IL ($r = 0.828$), CEA ($r = 0.815$), LAA ($r = 0.846$) and BLA ($r = 0.863$), between PL and CEA ($r = 0.795$); between IL, LAA ($r = 0.848$), BLA ($r = 0.874$) and CA3 ($r = 0.803$); between CEA, BLA ($r = 0.820$) and CA3 ($r = 0.818$); between BLA and CA1 ($r = 0.842$) and finally, between CA1 and DG ($r = 0.795$) (all $p < 0.05$). However, in the *Female* group showed high positive correlations between CG, PL ($r = 0.875$) and IL ($r = 0.917$); between PL and IL ($r = 0.954$); between CEA and BLA ($r = 0.959$); between LAA and CA3 ($r = 0.968$) and finally, between CA1 and CA3 ($r = 0.908$) (all $p < 0.01$). Other positive correlations were found between CEA and LAA ($r = 0.946$); between LAA, BLA ($r = 0.864$) and DG ($r = 0.875$) and finally, between CA3 and DG ($r = 0.856$) (all $p < 0.05$) (Table 1, Fig. 7C).

In the 2-trials RM protocol, we found differences only in CG ($F_{1,13} = 5.877$, $p = 0.031$) and PL ($F_{1,13} = 4.825$, $p = 0.047$), where the *Female* group displayed less brain activity than the *Male* group (Fig. 8A). We found no differences in the rest of the regions (IL: $F_{1,13} = 3.698$, $p = 0.077$; CEA: $F_{1,13} = 0.428$, $p = 0.525$; LAA: $F_{1,13} = 0.441$, $p = 0.518$; BLA: $F_{1,13} = 1.121$, $p = 0.309$; CA1: $F_{1,14} = 0.768$, $p = 0.396$; CA3: $F_{1,14} = 0.728$, $p = 0.408$; DG: $F_{1,14} = 0.166$, $p = 0.690$). Also, brain correlations showed different patterns in each *Sex* (Fig. 8B and C): the *Male* group showed that CEA was strong positive correlated with BLA ($r = 0.951$, $p < 0.01$) and CA3 was positively correlated with DG ($r = 0.818$, $p = 0.05$) (Table 2, Fig. 8B) while the *Female* group displayed high positive interactivity between PL and IL ($r = 0.818$) and CEA and BLA ($r = 0.935$) (all $p < 0.01$) and positive correlations between CG, PL ($r = 0.873$) and IL ($r = 0.861$); PL and CA3 ($r = 0.779$); IL and CA3 ($r = 0.778$); CEA and LAA ($r = 0.828$); LAA and BLA ($r = 0.800$) and finally, between CA1 and CA3 ($r = 0.809$) (all $p < 0.05$) (Table 2, Fig. 8C).

**Table 1**
Pearson correlations between brain areas in 4-Trials male group and in 4-Trials female group for all the region studied.

| | PL | IL | CEA | LAA | BLA | CA1 | CA3 | DG |
|---|---|---|---|---|---|---|---|---|
| ***4-Trials Male group*** | | | | | | | | |
| CG | **0.897** | **0.828** | **0.815** | **0.846** | **0.863** | **0.890** | 0.744 | 0.699 |
| | **0.00614** | **0.0214** | **0.0255** | **0.0163** | **0.0125** | **0.00727** | 0.0552 | 0.0804 |
| PL | | **0.952** | **0.795** | **0.938** | **0.962** | **0.898** | 0.708 | 0.550 |
| | | **0.000932** | **0.0327** | **0.00177** | **0.000522** | **0.00603** | 0.0753 | 0.201 |
| IL | | | 0.740 | **0.848** | **0.874** | **0.919** | **0.803** | 0.570 |
| | | | 0.0574 | **0.0160** | **0.0100** | **0.00344** | **0.0296** | 0.181 |
| CEA | | | | **0.888** | **0.820** | **0.917** | **0.818** | 0.630 |
| | | | | **0.00754** | **0.0240** | **0.00367** | **0.0246** | 0.129 |
| LAA | | | | | **0.965** | **0.920** | 0.753 | 0.536 |
| | | | | | **0.000440** | **0.00330** | 0.0505 | 0.215 |
| BLA | | | | | | **0.842** | 0.605 | 0.425 |
| | | | | | | **0.0174** | 0.150 | 0.342 |
| CA1 | | | | | | | **0.937** | **0.795** |
| | | | | | | | **0.00187** | **0.0324** |
| CA3 | | | | | | | | **0.892** |
| | | | | | | | | **0.00687** |
| ***4-Trials Female group*** | | | | | | | | |
| CG | **0.875** | **0.917** | 0.00113 | −0.0471 | −0.0757 | −0.703 | −0.601 | −0.259 |
| | **0.00982** | **0.00367** | 0.999 | 0.940 | 0.904 | 0.0783 | 0.153 | 0.576 |
| PL | | **0.954** | 0.753 | 0.798 | 0.539 | −0.478 | −0.332 | −0.0209 |
| | | **0.000862** | 0.142 | 0.105 | 0.348 | 0.279 | 0.467 | 0.965 |
| IL | | | 0.847 | 0.798 | 0.785 | −0.644 | −0.456 | −0.0730 |
| | | | 0.0702 | 0.106 | 0.116 | 0.119 | 0.304 | 0.876 |
| CEA | | | | **0.946** | **0.959** | 0.582 | 0.839 | 0.685 |
| | | | | **0.0147** | **0.00992** | 0.303 | 0.0759 | 0.202 |
| LAA | | | | | **0.864** | 0.770 | **0.968** | **0.875** |
| | | | | | **0.0264** | 0.128 | **0.00675** | **0.0225** |
| BLA | | | | | | 0.362 | 0.733 | 0.603 |
| | | | | | | 0.550 | 0.159 | 0.281 |
| CA1 | | | | | | | **0.908** | 0.679 |
| | | | | | | | **0.00473** | 0.0937 |
| CA3 | | | | | | | | **0.856** |
| | | | | | | | | **0.0139** |

Significant correlations are in bold. Each table cell shows the calculated Pearson's correlation *r*-value and the *p*-level for the calculated correlation coefficient. Areas: CG = Cingulate cortex, PL = Prelimbic cortex, IL = Infralimbic cortex, CEA = Central Amygdala, LAA = Lateral Amygdala, BLA = Basolateral Amygdala, CA1 = field CA1 of hippocampus, CA· = field CA3 of hippocampus, DG = Dentate Gyrus.

## 4. Discussion

This may be the first study to examine sexual brain and behaviour dimorphism in the spatial memory of juvenile Wistar rats, adding a complexity variable by reducing daily reinforcement. We analysed spatial RM sex differences in two protocols with different training regimes in the acquisition phase: a protocol of 4 trials with the platform allowing the rats to escape from the water and a protocol of 2-trials with the escape platform in the same location. We found no general sex differences in the permanence in the quadrants in the 4-trials protocol. However, we found a remarkable dimorphism in the 2-trials protocol in which the *Female* group showed less metabolic activity in CG and PL and learned the task two days earlier than the *Male* group. That is, the *Female* group showed faster acquisition of the learning criterion over the days and longer permanence in Tgt than the *Male* group.

Spatial memory is a vital cognitive function that allows us to learn and explore our surrounding context [9]. Regarding sexual dimorphism in spatial memory, most studies have shown a general male adult advantage over females in different types of spatial memory [8]. These results are supported by the use of different spatial information and specific spatial search strategies in each sex: allocentric or egocentric strategies [15–17]. The former is based on the contextual configuration and the spatial relationships between landmarks and objects that allow creating a cognitive map of the environment [18,19]. By contrast, egocentric navigation uses an internal reference system based on proprioceptive and vestibular cues [20]. In this regard, less research is found focusing on the differences between males and females during the developmental stage. Studies that evaluated RM in juvenile rats have found better performance in male subjects than in females [21–23]. In this research, we first evaluated *Male* and *Female* Wistar rats using a 4-trials RM protocol that starts at PND 24, placing distal cues around the pool to prompt the animals' use of allocentric strategies to guide their path. Both groups learned the task on the fourth day (PND27) and displayed no differences in the periphery and Tgt. These outcomes are in accordance with the results of Akers and Hamilton (2007) [24], who found similar performance between sexes in a distal cue task. In our second protocol. when only 2 acquisition trials were administered per day and less reinforcement was available per session, the *Female* group showed a clear advantage over *Male* group in the acquisition of the learning criterion reaching the criterion on Day 4 (PND27), two days before the *Male* group (Day 6, PND 29), and showing significant permanence in Tgt. Despite the higher complexity of the task, the *Female* group maintained the performance that we found in the 4-trials RM protocol. It can be noted that in this protocol the *Male* group showed more variability in their permanence in quadrants than the *Female* group. This could be related to the sample size, since the *Female* group is composed of four more animals and this could reduce the final variability of the group.

Spatial memory is dependant on the entorhinal cortex, hippocampus and other surrounding structures. The development of rats' allocentric spatial memory coincides with the functional maturation of the hippocampus place cells [2]. The interactions between the hippocampus and prefrontal cortex also seem relevant to code prospective goals and location by the place cells during navigation [3]. In our research, we study the brain activity of oxidative metabolism using CCO histochemistry. CCO enzyme participates in the oxidative phosphorylation process that generates ATP and is considered an indirect marker of the neural activity of some brain regions [13]. According to the behavioural analysis in the 4-trails RM protocol, we found no brain metabolic activity differences between the *Male* and *Female* groups in the prefrontal

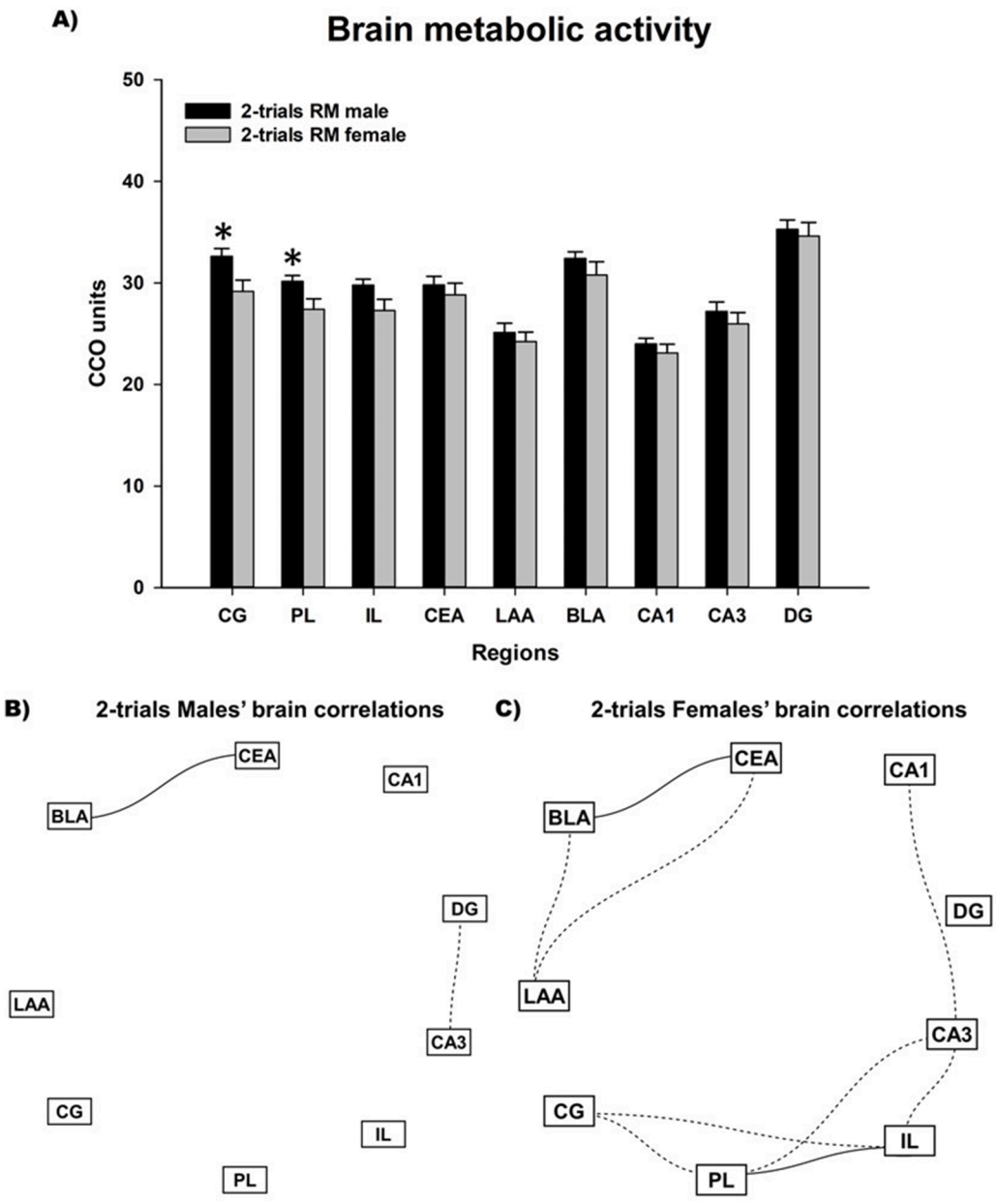


**Fig. 8.** CCO results (mean ± SEM) and brain correlations of the *Male* and *Female* groups in the 2-trials MR protocol. **A.** CCO values of the *Male* and *Female* groups. The *Female* group displayed less brain metabolic activity in CG and PL than the *Male* group (*$p < 0.05$). **B.** Pearson correlations between brain areas in the *Male* group for all the structures studied. **C.** Pearson correlations between brain areas in the *Female* group for all the structures studied. Discontinuous lines: $p < 0.05$; solid lines: $p < 0.01$. Areas: CG = Cingulate cortex, PL = Prelimbic cortex, IL = Infralimbic cortex, CEA = Central Amygdala, LAA = Lateral Amygdala, BLA = Basolateral Amygdala, CA1 = field CA1 of hippocampus, CA· = field CA3 of hippocampus, DG = Dentate Gyrus.

cortex or hippocampus. However, we identified a different pattern of correlations between the regions involved in the 4-trials RM task in the *Male* and *Female* groups, suggesting the potentially varying contribution of these brain structures in the RM task during the postnatal period. The *Male* group showed a great number of interactions between regions. The pattern that this group showed was the interconnection between most of the areas, being the PL and the CA1 regions of the brain that displayed more interactions. On the other hand, the *Female* group showed independent interactions between brain regions. This group did not present an extensive network, as it was found in the *Male* group. *Female* group showed correlations between the subregions of the prefrontal cortex, the nuclei of the amygdala, some subregions of the hippocampus and interactions between LAA and some regions of the hippocampal formation. Despite these differences between groups, both displayed positive interactions between areas.

Regarding brain activity during the execution of the 2-trials RM task, we found that the *Female* group showed less metabolic activity than the *Male* group in CG and PL, subregions of the prefrontal cortex. Moreover, we also discovered a different pattern of correlations between brain areas involved in the 2-trials task. In this task, the *Male* group showed a reduced number of interactions displaying only a correlation between BLA and CEA and between DG and CA3 while the *Female* group exhibited a pattern of individual interactivity between the nuclei of the amygdala and the subregions of the prefrontal cortex and a connection between the prefrontal cortex, PL and IL, and the CA3 subregion of the hippocampus. In both groups, the interactions between areas were positive. These findings suggest that different networks mediate the acquisition of this complex RM task in a sex-dependant way: in the *Female* group, the hippocampus and the prefrontal cortex were indicated as central structures, also linked to emotion-related structures, whereas in the *Male* group, other structures such as the hippocampus and the amygdala were implicated. Previous literature linked these sex-brain differences to the use of different learning strategies to locate hidden spatial goals [5]. In our research, the *Male* group may use place-learning strategies related to the hippocampus [5]. The amygdala, which is involved in the association of cues with their affective significance, was also activated. The BLA is part of an integrated system that can modulate spatial navigation by integrating a reward with spatial information [25]. In the *Female* group,

**Table 2**
Pearson correlations between brain areas in 2-trials male group and in 2-trials female group for all the region studied.

| | PL | IL | CEA | LAA | BLA | CA1 | CA3 | DG |
|---|---|---|---|---|---|---|---|---|
| ***2-Trials Male group*** | | | | | | | | |
| CG | 0.295 | 0.501 | −0.184 | −0.501 | −0.0218 | −0.462 | 0.0193 | −0.210 |
| | 0.520 | 0.253 | 0.727 | 0.311 | 0.967 | 0.297 | 0.967 | 0.651 |
| PL | | 0.724 | −0.0320 | −0.503 | −0.0452 | 0.0833 | −0.283 | −0.408 |
| | | 0.0658 | 0.952 | 0.309 | 0.932 | 0.859 | 0.538 | 0.363 |
| IL | | | 0.0980 | −0.210 | 0.146 | −0.00229 | 0.0209 | −0.0580 |
| | | | 0.853 | 0.690 | 0.783 | 0.996 | 0.965 | 0.902 |
| CEA | | | | 0.602 | **0.951** | 0.195 | 0.524 | 0.439 |
| | | | | 0.153 | **0.00102** | 0.674 | 0.228 | 0.325 |
| LAA | | | | | 0.523 | 0.495 | 0.553 | 0.347 |
| | | | | | 0.229 | 0.259 | 0.198 | 0.445 |
| BLA | | | | | | 0.247 | 0.655 | 0.515 |
| | | | | | | 0.594 | 0.110 | 0.237 |
| CA1 | | | | | | | 0.558 | 0.201 |
| | | | | | | | 0.150 | 0.633 |
| CA3 | | | | | | | | **0.818** |
| | | | | | | | | **0.0131** |
| ***2-Trials Female group*** | | | | | | | | |
| CG | **0.873** | **0.861** | 0.396 | 0.138 | 0.111 | 0.481 | 0.749 | 0.0749 |
| | **0.0103** | **0.0129** | 0.380 | 0.768 | 0.812 | 0.275 | 0.0529 | 0.873 |
| PL | | **0.926** | 0.482 | 0.212 | 0.306 | 0.332 | **0.779** | 0.229 |
| | | **0.00273** | 0.273 | 0.649 | 0.505 | 0.466 | **0.0390** | 0.622 |
| IL | | | 0.282 | −0.0418 | 0.0793 | 0.315 | **0.778** | 0.213 |
| | | | 0.541 | 0.929 | 0.866 | 0.492 | **0.0394** | 0.647 |
| CEA | | | | **0.828** | **0.935** | 0.270 | 0.427 | 0.0148 |
| | | | | **0.0213** | **0.00198** | 0.559 | 0.340 | 0.975 |
| LAA | | | | | **0.800** | 0.320 | 0.298 | 0.376 |
| | | | | | **0.0309** | 0.484 | 0.516 | 0.406 |
| BLA | | | | | | 0.199 | 0.302 | 0.0114 |
| | | | | | | 0.669 | 0.510 | 0.981 |
| CA1 | | | | | | | **0.809** | 0.434 |
| | | | | | | | **0.0276** | 0.331 |
| CA3 | | | | | | | | 0.510 |
| | | | | | | | | 0.243 |

Significant correlations are in bold. Each table cell shows the calculated Pearson's correlation *r*-value and the *p*-level for the calculated correlation coefficient. Areas: CG = Cingulate cortex, PL = Prelimbic cortex, IL = Infralimbic cortex, CEA = Central Amygdala, LAA = Lateral Amygdala, BLA = Basolateral Amygdala, CA1 = field CA1 of hippocampus, CA· = field CA3 of hippocampus, DG = Dentate Gyrus.

not only the hippocampus and the amygdala were involved, but also the prefrontal cortex. The prefrontal cortex participates in spatial memory, displaying a significant portion of neurons that fire in a specific location, and it is related to egocentric strategies [26]. It also plays a role in reward-guided learning, predicting reward location based on spatial contingencies in the environment [27,28].

Several studies also attributed sex differences in spatial performance to the gonadal hormones' effects on early development and adult life [5]. It is known that oestrogens affect neuroplasticity in several brain regions. Concretely, they modulate spine and synapsis formation and neurogenesis in the hippocampal formation [29]. Our better female performance may be unrelated to oestrogens circulating because their concentrations are low and remain equivalent in both sexes until puberty (PND30-PND42 in females and PND42- PND55 in males). Besides, oestrous cycle regulation and sperm maturation occur in puberty [30]. The rat strain also seems to be an important factor. Sprague–Dawley rats, for example, showed substantially higher male advantages than any other strain [31]. We used Wistar rats, which exhibited lower male advantages in comparison with other strains [31].

Another important factor could be related to the use of a pretraining protocol that allows animals to familiarize themselves with the task and reduce swim performance stress [32]. The use of these habituation procedures tends to reduce sex differences, and a lower thigmotaxis response, a wall-hugging stress behaviour, is shown [5,32]. Despite the tendency of the female rats to display more thigmotaxis behaviour [32], we found no differences in this parameter. This could explain the similar performance found in the 4-trials RM protocol. In both cases, we performed a habituation day, and these pretraining trials may be sufficient to reduce the animals' stress and achieve a correct task performance, as found in previous articles [5]. However, the lack of stress cannot explain the better *Female* performance in the 2-trials RM task.

An important factor to consider in the female advantage in the 2-trials RM task is the process of memory consolidation, which refers to a gradual process of reorganization of brain areas that support a specific memory trace in a time-dependant way [33,34]. Studies in animal models suggest that memory encoding starts in the hippocampus and becomes increasingly dependant on cortical areas, emphasizing the role of the prefrontal cortex in consolidating memories, with the anterior CG being a central region [28,33]. However, as shown Tse et al. (2007) [35], sometimes the neocortex can consolidate very rapidly. The investigation of temporal factors in the consolidation process, showed that learning emerged more rapidly when trials were spaced in time compared with a presentation of an amount of training massed in one day [36]. Previous authors such as Redolar-Ripoll et al. (2002) [36], assessing associative learning in an active avoidance task, suggested that more intensive training in that paradigm could facilitate memory consolidation and, then, improve subsequent remembering. In line with these results, Uzakov, Frey and Korz (2005) [37] suggested that animals that received higher number of trials were better habituated to the procedure than animals that submitted to only a few trials. These differences could result in different stress responses that, in turn, could have different modulatory effects on learning and memory. Despite abundant literature can be found about the consolidation process and spaced/massive training, there are no studies focused on the learning and memory process using different training regimes. In the 2-trials training regimen of our research, the *Female* group displayed faster learning criterion acquisition compared to the *Male* group and showed less metabolic activity in CG and PL than the *Males*. Less CCO activity after learning may be associated with a reduction in metabolic costs, increasing the efficiency of the process, which involves the prefrontal

cortex [16]. This metabolic activity reduction could reflect a faster consolidation process in the *Female* group than in the *Male* group, and may explain the behavioural advantage of this group despite receiving less reinforcement than the 4-trials RM protocol. At the beginning of the training, as the animals did not learn the task, they cannot retrieve any information. But when the animals were exposed to successive learning trials, they can retrieve the information that they learnt and reach the correct quadrant. Memories may have been stored initially in the hippocampus network [38,39] but, as days passed, there may have been a strong synchronization of neural activity between the hippocampus and the prefrontal cortex that may reflect the slow memory consolidation in the neocortex for permanent storage [39,40]. As the protocol lasted seven days, after memory consolidation in the prefrontal cortex, at the end of training, two subregions of this cortex (CG and PL) would have reduced its metabolic costs as an efficiency mechanism, prompting a faster learning criterion acquisition. However, as a limitation in our study, we only assessed the metabolic brain activity by CCO histochemistry that was analysed ninety minutes after training completion. We selected the sampling time of ninety minutes after the last trial because previous literature assessing CCO histochemistry in spatial learning use this period for metabolic changes detection after training completion [12,41–44], enabling comparison of results between different studies. Also, this period of ninety minutes after the last trial was demonstrated to be an optimal time point for signal detection of neural changes after training [45,46]. At a first attend, with this analysis, we only can conclude about brain activity networks after training completion and more research is needed to understand brain involvement at learning progression. At the same time, other neural markers of learning and memory consolidation, such as immediate early gene activation and dendritic spine formation, could be used to assess in more detail other brain changes apart from metabolic activity modifications [45]. Thus, more analysis are necessary to evaluate in depth further potential brain changes and also to clarify how the number of reinforced trials and the number of sessions delivered could affect the male and female acquisition of spatial learning at early ages.

## 5. Conclusions

In this article, we carried out two spatial RM protocols in the MWM with different training regimes (4-trials or 2-trials in the acquisition phase) to analyse the potential sexual brain and behaviour dimorphism of developing rats (PND24). We found that in the first protocol with four acquisition trials per day for five days, both sexes reached the learning criterion on Day 4 (PND27), with no variations in brain metabolic activity between sexes. However, in the protocol of two acquisition trials per day for seven days, the female group showed a better performance than the males, reaching the learning criterion two days earlier (PND27) and showing longer permanence in Tgt. Also, the female group showed less metabolic activity in CG and PL, subregions of the prefrontal cortex. These results suggest a faster consolidation process in the female group than in the male group and may explain the behavioural advantage. However, more studies are necessary to evaluate in depth further potential brain changes and to clarify how the number of reinforced trials and the number of sessions delivered could affect males' and females' acquisition of spatial learning at early ages.

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## Declaration of Competing Interest

The authors declare that they have no conflict of interest to disclose.

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## References

[1] E. Coluccia, G. Louse, Gender differences in spatial orientation: a review, J. Environ. Psychol. 24 (2004) 329–340, https://doi.org/10.1016/j.jenvp.2004.08.006.
[2] T.Z. Baram, F. Donato, G.L. Holmes, Construction and disruption of spatial memory networks during development, Learn. Mem. 26 (2019) 206–218, https://doi.org/10.1101/lm.049239.118.
[3] F. Glöckner, N.W. Schuck, S.C. Li, Differential prioritization of intramaze cue and boundary information during spatial navigation across the human lifespan, Sci. Rep. 11 (2021) 1–16, https://doi.org/10.1038/s41598-021-94530-9.
[4] H.M. Tan, T.J. Wills, F. Cacucci, The development of spatial and memory circuits in the rat, Wiley Interdiscip. Rev. Cogn. Sci. 8 (2017), https://doi.org/10.1002/wcs.1424.
[5] M. Méndez-López, M. Méndez, L. López, J.L. Arias, Sexually dimorphic c-Fos expression following spatial working memory in young and adult rats, Physiol. Behav. 98 (2009) 307–317, https://doi.org/10.1016/j.physbeh.2009.06.006.
[6] J.R. Manns, H. Eichenbaum, Evolution of declarative memory, Hippocampus 1031 (2006) 1026–1031, https://doi.org/10.1002/hipo.
[7] T.J. Wills, F. Cacucci, The development of the hippocampal neural representation of space, Curr. Opin. Neurobiol. 24 (2014) 111–119, https://doi.org/10.1016/j.conb.2013.09.006.
[8] S. Yagi, D. Drewczynski, S.R. Wainwright, C.K. Barha, O. Hershorn, L.A.M. Galea, Sex and estrous cycle differences in immediate early gene activation in the hippocampus and the dorsal striatum after the cue competition task, Horm. Behav. 87 (2017) 69–79, https://doi.org/10.1016/j.yhbeh.2016.10.019.
[9] W. Chen, B. Liu, X. Li, P. Wang, B. Wang, Sex differences in spatial memory, Neuroscience 443 (2020) 140–147, https://doi.org/10.1016/j.neuroscience.2020.06.016.
[10] M. Méndez, M. Méndez-López, L. López, M.Á. Aller, J. Arias, J.M. Cimadevilla, J. L. Árias, Spatial memory alterations in three models of hepatic encephalopathy, Behav. Brain Res. 188 (2008) 32–40, https://doi.org/10.1016/j.bbr.2007.10.019.
[11] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, 6th Edit, Elsevier, 2007.
[12] A. Gutiérrez-Menéndez, S. Cid-Duarte, M. Banqueri, J.A. Martínez, M. Méndez, J. L. Arias, Photobiomodulation effects on active brain networks during a spatial memory task, Physiol. Behav. (2021) 230, https://doi.org/10.1016/j.physbeh.2020.113291.
[13] M. Méndez-López, M. Méndez, L. López, J.L. Arias, Spatial working memory in Wistar rats: brain sex differences in metabolic activity, Brain Res. Bull. 79 (2009) 187–192, https://doi.org/10.1016/j.brainresbull.2009.02.007.
[14] P. Sampedro-Piquero, C. Zancada-Menendez, A. Begega, M. Mendez, J.L. Arias, Effects of forced exercise on spatial memory and cytochrome c oxidase activity in aged rats, Brain Res. 1502 (2013) 20–29, https://doi.org/10.1016/j.brainres.2012.12.036.
[15] C. Wang, X. Chen, J.J. Knierim, Egocentric and allocentric representations of space in the rodent brain, Curr. Opin. Neurobiol. 60 (2020) 12–20, https://doi.org/10.1016/j.conb.2019.11.005.
[16] M. Méndez-López, M. Méndez, L. López, J.L. Arias, Spatial working memory learning in young male and female rats: involvement of different limbic system regions revealed by cytochrome oxidase activity, Neurosci. Res. 65 (2009) 28–34, https://doi.org/10.1016/j.neures.2009.05.001.
[17] S. Wang, K. Li, S. Zhao, X. Zhang, Z. Yang, J. Zhang, T. Zhang, Early-stage dysfunction of hippocampal theta and gamma oscillations and its modulation of neural network in a transgenic 5xFAD mouse model, Neurobiol. Aging 94 (2020) 121–129, https://doi.org/10.1016/j.neurobiolaging.2020.05.002.
[18] A. Begega, M. Cuesta, S. Rubio, M. Méndez, L.J. Santín, J.L. Arias, Functional networks involved in spatial learning strategies in middle-aged rats, Neurobiol. Learn. Mem. 97 (2012) 346–353, https://doi.org/10.1016/j.nlm.2012.02.007.
[19] W.G. Brake, J.M. Lacasse, Sex differences in spatial navigation: the role of gonadal hormones, Curr. Opin. Behav. Sci. 23 (2018) 176–182, https://doi.org/10.1016/j.cobeha.2018.08.002.
[20] S. Rubio, A. Begega, M. Méndez, M. Méndez-López, J.L. Arias, Similarities and differences between the brain networks underlying allocentric and egocentric spatial learning in rat revealed by cytochrome oxidase histochemistry, Neuroscience 223 (2012) 174–182, https://doi.org/10.1016/j.neuroscience.2012.07.066.
[21] N.M. Conejo, H. González-Pardo, G. Vallejo, J.L. Arias, Involvement of the mammillary bodies in spatial working memory revealed by cytochrome oxidase activity, Brain Res. 1011 (2004) 107–114, https://doi.org/10.1016/j.brainres.2004.03.025.
[22] C. Chow, J.R. Epp, S.E. Lieblich, C.K. Barha, L.A.M. Galea, Sex differences in neurogenesis and activation of new neurons in response to spatial learning and

memory, Psychoneuroendocrinology 38 (2013) 1236–1250, https://doi.org/10.1016/j.psyneuen.2012.11.007.
[23] J.M. Cimadevilla, H. González-Pardo, L. López, F. Díaz, E.G. Cueto, L.M. García-Moreno, J.L. Arias, Sex-related differences in spatial learning during the early postnatal development of the rat, Behav. Processes. 46 (1999) 159–171, https://doi.org/10.1016/S0376-6357(99)00034-0.
[24] K.G. Akers, D.A. Hamilton, Comparison of developmental trajectories for place and cued navigation in the Morris water task, Dev. Psychobiol. 49 (2007) 553–564, https://doi.org/10.1002/dev.20227.
[25] W.E. Pratt, S.J.Y. Mizumori, Characteristics of basolateral amygdala neuronal firing on a spatial memory task involving differential reward, Behav. Neurosci. 112 (1998) 554–570, https://doi.org/10.1037//0735-7044.112.3.554.
[26] K. Ethier, N.Le Marec, P.P. Rompré, R. Godbout, Spatial strategy elaboration in egocentric and allocentric tasks following medial prefrontal cortex lesions in the rat, Brain Cogn. 46 (2001) 134–135, https://doi.org/10.1016/S0278-2626(01)80050-6.
[27] W.E. Pratt, S.J.Y. Mizumori, Neurons in rat medial prefrontal cortex show anticipatory rate changes to predictable differential rewards in a spatial memory task, Behav. Brain Res. 123 (2001) 165–183, https://doi.org/10.1016/S0166-4328(01)00204-2.
[28] D.R. Euston, A.J. Gruber, B.L. McNaughton, The role of medial prefrontal cortex in memory and decision making, Neuron 76 (2012) 1057–1070, https://doi.org/10.1016/j.neuron.2012.12.002.
[29] P.A.S. Sheppard, E. Choleris, L.A.M. Galea, Structural plasticity of the hippocampus in response to estrogens in female rodents, Mol. Brain. 12 (2019) 28–30, https://doi.org/10.1186/s13041-019-0442-7.
[30] M.R. Bell, Comparing postnatal development of gonadal hormones and associated social behaviors in rats, mice, and humans, Endocrinology 159 (2018) 2596–2613, https://doi.org/10.1210/en.2018-00220.
[31] Z. Jonasson, Meta-analysis of sex differences in rodent models of learning and memory: a review of behavioral and biological data, Neurosci. Biobehav. Rev. 28 (2005) 811–825, https://doi.org/10.1016/j.neubiorev.2004.10.006.
[32] J. Simpson, J.P. Kelly, An investigation of whether there are sex differences in certain behavioural and neurochemical parameters in the rat, Behav. Brain Res. 229 (2012) 289–300, https://doi.org/10.1016/j.bbr.2011.12.036.
[33] B.C. Wartman, M.R. Holahan, Neurobiology of learning and memory the use of sequential hippocampal-dependent and -non-dependent tasks to study the activation profile of the anterior cingulate cortex during recent and remote memory tests, Neurobiol. Learn. Mem. 106 (2013) 334–342, https://doi.org/10.1016/j.nlm.2013.08.011.
[34] C. Zorzo, J.L. Arias, M. Méndez, Functional neuroanatomy of allocentric remote spatial memory in rodents, Neurosci. Biobehav. Rev. 136 (2022), https://doi.org/10.1016/j.neubiorev.2022.104609.
[35] D. Tse, R.F. Langston, M. Kakeyama, I. Bethus, P.A. Spooner, E.R. Wood, M. P. Witter, R.G.M. Morris, Schemas and memory consolidation, Science 316 (2007) 76–82, https://doi.org/10.1126/science.1135935, 80-.
[36] D. Redolar-Ripoll, L. Aldavert-Vera, C. Soriano-Mas, P. Segura-Torres, I. Morgado-Bernal, Intracranial self-stimulation facilitates memory consolidation, but not retrieval: its effects are more effective than increased training, Behav. Brain Res. 129 (2002) 65–75, https://doi.org/10.1016/S0166-4328(01)00325-4.
[37] S. Uzakov, J.U. Frey, V. Korz, Reinforcement of rat hippocampal LTP by holeboard training, Learn. Mem. 12 (2005) 165–171, https://doi.org/10.1101/lm.89305.
[38] N.M. Conejo, H. González-Pardo, G. Vallejo, J.L. Arias, Changes in brain oxidative metabolism induced by water maze training, Neuroscience 145 (2007) 403–412, https://doi.org/10.1016/j.neuroscience.2006.11.057.
[39] T. Kitamura, S.K. Ogawa, D.S. Roy, T. Okuyama, Engrams and Circuits Crucial For Systems Consolidation of a Memory (Matt Jones).pdf, 78, 2017, pp. 73–78. http://proxy.library.upenn.edu:2341/content/sci/356/6333/73.full.pdf.
[40] A.R. Preston, H. Eichenbaum, Interplay of hippocampus and prefrontal cortex in memory, Curr. Biol. 23 (2013) R764–R773, https://doi.org/10.1016/j.cub.2013.05.041.
[41] M. Méndez-Couz, H. González-Pardo, G. Vallejo, J.L. Arias, N.M. Conejo, Spatial memory extinction differentially affects dorsal and ventral hippocampal metabolic activity and associated functional brain networks, Hippocampus 26 (2016) 1265–1275, https://doi.org/10.1002/hipo.22602.
[42] M. Banqueri, A. Gutiérrez-Menéndez, M. Méndez, N.M. Conejo, J.L. Arias, Early life stress due to repeated maternal separation alters the working memory acquisition brain functional network, Stress 24 (2021) 87–95, https://doi.org/10.1080/10253890.2020.1777974.
[43] S. Tronel, V. Lemaire, V. Charrier, M.F. Montaron, D.N. Abrous, Influence of ontogenetic age on the role of dentate granule neurons, Brain Struct. Funct. 220 (2015) 645–661, https://doi.org/10.1007/s00429-014-0715-y.
[44] L.A. Teather, M.G. Packard, D.E. Smith, R.G. Ellis-Behnke, N.G. Bazan, Differential induction of c-Jun and Fos-like proteins in rat hippocampus and dorsal striatum after training in two water maze tasks, Neurobiol. Learn. Mem. 84 (2005) 75–84, https://doi.org/10.1016/j.nlm.2005.03.006.
[45] D. Tse, T. Takeuchi, M. Kakeyama, Y. Kajii, H. Okuno, C. Tohyama, H. Bito, R.G. M. Morris, Schema-dependent gene activation and memory encoding in neocortex, Science 333 (2011) 891–895, https://doi.org/10.1126/science.1205274, 80-.
[46] Y. Dudai, A. Karni, J. Born, The consolidation and transformation of memory, Neuron 88 (2015) 20–32, https://doi.org/10.1016/j.neuron.2015.09.004.