Title: The Role of Task Type in Transgenerational Effects of Maternal Spatial Training on Female Offspring Memory

Running Title: Task Type in Transgenerational Effects

Authors: Javad Riyahi[1,*], Parvaneh Shamsipour Dehkordi[1], Behrouz Abdoli[2], Abbas Haghparast[3]

1. *Department of Motor Behavior, Faculty of Sport Sciences, Alzahra University, Tehran, Iran.*
2. *Department of Behavioral and Cognitive Science in Sport, Faculty of Sport Sciences and Health, Shahid Beheshti University, Tehran, Iran.*
3. *Neuroscience Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

***Corresponding Author**: Javad Riyahi, Department of Motor Behavior, Faculty of Sport Sciences, Alzahra University, Tehran, Iran. Email: Javad5r@yahoo.com

To appear in: **Basic and Clinical Neuroscience**

Received date: 2025/02/25

Revised date: 2025/05/11

Accepted date: 2026/06/27

Please cite this article as:

Riyahi, J., Shamsipour Dehkordi, P., Abdoli, B., Haghparast, A. (In Press). The Role of Task Type in Transgenerational Effects of Maternal Spatial Training on Female Offspring Memory. Basic and Clinical Neuroscience. Just Accepted publication Jul. 10, 2026. Doi: http://dx.doi.org/10.32598/bcn.2026.7493.1

DOI: http://dx.doi.org/10.32598/bcn.2026.7493.1

Abstract

This study, building on earlier work on transgenerational influences on cognition, examined whether parental experience in a particular cognitive skill can enhance memory and learning processes in offspring. In particular, we asked whether maternal spatial training not only improves spatial memory in the next generation but also benefits short-term, long-term, and working memory. To address this, we tested groups of female offspring born to spatially trained or untrained mothers using the Morris Water Maze (MWM), Y-maze, and Novel Object Recognition (NOR) paradigms. The Results of implementing the present research protocol indicated that maternal MWM training enhances memory function and the learning process in female offspring when they are tested on the same task, an effect linked to enhanced hippocampal expression of SYT1 and BDNF, as well as increased ERK1/2 phosphorylation. In contrast, maternal spatial training had no significant impact on short- or long-term memory or on working memory more broadly, nor did it modify hippocampal SYT1, BDNF, or ERK1/2 phosphorylation levels in offspring following cognitive testing. Collectively, these results suggest that transgenerational impacts of maternal training in a cognitive task are task-specific and do not generalize to other untrained memory domains.

Keywords: Maternal spatial training; Transgenerational effects; Female offspring; Working memory; Short-term memory; Long-term memory

1. Introduction

Researchers have shown interest in investigating how individuals respond to training for task learning over the past few years. What has attracted researchers' attention more than anything else is the variation in how effective practice and participation in different training sessions are for acquiring a specific skill or creating memory in a particular area among different people. Results from previous studies indicate that individuals differ in the benefits gained from training (Starkes, Deakin, Allard, Hodges, & Hayes, 2014). Individual differences in learning ability can arise from diverse factors, such as variations in mediator genes that influence responses to practice and training (Yarrow, Brown, & Krakauer, 2009). The concept of learning is related to memory. One of the brain's primary functions is the regulation of future behavior through the ability to learn from people's experiences (Mansvelder, Verhoog, & Goriounova, 2019). For an experience to become a part of memory, stable functional and structural changes must be made that form a memory trace of that experience in the brain (Forehand, 2009). Therefore, the formation and storage of memory require many processes at the cellular and molecular level, and the memory engram can be considered as a biological effect of memory (Tonegawa, Pignatelli, Roy, & Ryan, 2015). Today, it is well established that neuroplasticity underlies memory formation and consolidation (Arai & Feig, 2011). Maybe part of the reason for individual differences in learning rates and memory capabilities lies in the neural processes underlying learning and memory.

Long-term potentiation (LTP) is the most important neurophysiological process that underlies neuroplasticity in memory formation and learning. The incidence of LTP requires expression of the gene (Frey, Frey, Schollmeier, & Krug, 1996) and synthesis of the protein (Frey, Krug, Reymann, & Matthies, 1988) in the postsynaptic cell. In fact, among the various processes that control memory formation and consolidation, gene expression regulation is very important (Kim & Kaang, 2017). How epigenetic patterns also regulate gene expression.

Given the significant function of epigenetic factors in neural plasticity underlying memory and learning, scholars are seeking to determine whether parental experiences can influence subsequent generations and, in turn, facilitate memory formation and learning in those generations.

Numerous studies have explored how parental experiences can be inherited by their offspring across generations, demonstrating that parental and ancestral experiences can greatly influence the metabolic, physiological, and cellular roles of living organisms (Arai & Feig, 2011; Azadi, Azizi, & Haghparast, 2019; Dias & Ressler, 2014; Riyahi et al., 2022; Riyahi et al., 2021; Riyahi, Abdoli, Haghparast, & Petrosini, 2019; Zhang et al., 2017), and in certain conditions, these effects can be passed down among generations via epigenetic modifications (Wang, Liu, & Sun, 2017). Epigenetic modifications are caused by individuals' experiences or exposure to various environmental stimuli and may persist for a long time. Key modifications include DNA methylation, where methyl groups are added to DNA to often silence genes; histone modification, which involves adding or removing chemical groups to histone proteins, affecting DNA packaging and gene accessibility; and small non-coding RNAs, which interact with messenger RNAs to regulate their translation into proteins. These epigenetic changes can be dynamically affected by environmental factors, emphasizing the complex link between our environment and gene expression (Wang et al., 2017).

Therefore, the transgenerational transmission of epigenetic patterns provides conditions for organisms to pass acquired traits caused by their ancestors' experience to their offspring (Riyahi, Taslimi, Gelfo, Petrosini, & Haghparast, 2024). One of the most important subjects currently discussed in the studies of epigenetic transmission of acquired traits involves acquired memories and learning capabilities. Despite the considerable body of research on various factors affecting learning and memory, there remains a notable gap in our understanding of how parental instruction methods influence offspring's cognitive

development and memory capabilities.

In the first case, Zhang et al. (2017) found that when parents underwent specialized training to enhance their spatial abilities, their offspring showed significant improvements in spatial cognition. This enhancement in cognitive skills was further linked to a notable increase in synaptic transmission in the offspring's brains, suggesting a biological mechanism that underscores the connection between parental training and their children's cognitive development. This study found that hippocampal SYT1 expression increased in the offspring of trained parents, and that parental training also induced elevated histone acetylation at the SYT1 promoter in both parents and their offspring (Zhang et al., 2017). The findings of this study were very interesting and provided sufficient motivation to research the effects of intergenerational transmission of acquired memory or the facilitation of skill learning through inherited epigenetic factors from previous generations.

Additionally, research by Riyahi et al. (2019) demonstrated that spatial training for parents prior to impregnation enhances their offspring's spatial learning and memory. This transgenerational transfer shows sex-specific patterns: paternal training benefits male offspring, while maternal training improves spatial memory and learning processes in female offspring (Riyahi et al., 2019). In another investigation by our group (Riyahi et al., 2022), we found that paternal training led to enhanced spatial memory and spatial task acquisition in the F1 and F2 generations. Neurobiological evidence corroborated these results, indicating elevated hippocampal BDNF levels and ERK1/2 phosphorylation in both F1 and F2 offspring generations. Additionally, the hippocampal acetylated H3K14 level increased in the offspring of parents who underwent spatial training, supporting the idea that increased histone acetylation may be important for inheriting spatial abilities. Riyahi et al. (2021) found that mothers' participation in spatial training led female offspring to exhibit higher swimming speeds, lower escape latencies, greater duration spent in the target quadrant, shorter total

swimming distance, and enhanced localization memory compared to female offspring of untrained mothers. Furthermore, the study revealed a significant correlation in performance on the MWM between trained mothers and their female offspring. This correlation indicates that when mothers engage in spatial training before becoming pregnant, it positively influences their female offspring's ability to learn and strengthens their spatial memory. These findings suggest that the benefits of maternal training extend beyond the immediate experience, fostering cognitive development in female offspring (Riyahi et al., 2021). Collectively, these findings suggest that parental cognitive experience can be transmitted intergenerationally, thereby enhancing offspring's cognitive abilities, and the results expand the existing body of research in this field. The studies by Riyahi et al. (2019, 2021, 2022) and Zhang et al. (2017) demonstrated that spatial training for parents enhances their offspring's ability to learn and remember spatial information (Riyahi et al., 2022; Riyahi et al., 2021; Riyahi et al., 2019; Zhang et al., 2017). But in the meantime, the field of studies investigating the specificity of the effects of the learned task on parents' cognitive processes in their offspring is extremely underdeveloped. It remains unclear whether the observed improvements in learning and memory are specific to the trained task or if they generally enhance overall cognitive abilities. Therefore, considering the possibility of transferring the created phenotypes to the next generation, as well as the results of recent studies on facilitating learning and the spatial memory formation in offspring of parents who received spatial training before fertilization, the inquiry concerns whether training on a cognitive task can enhance memory and aid the learning of additional cognitive tasks in subsequent generations or not. This research aims to determine whether spatial training of mothers before fecundation enhances spatial memory and improves working-, short-term-, and long-term memory in female offspring, as assessed using the MWM, NOR, and Y-Maze tasks. Additionally, considering the crucial roles of SYT1, BDNF, and ERK1/2 in cognition and memory, and

their possible involvement in mediating the effects of maternal spatial training in female offspring, we assessed hippocampal levels of SYT1, BDNF, and ERK1/2 phosphorylation in the offspring.

2. Materials and methods

2.1. Animals

In this study, we used 16 female Wistar rats, aged 8 weeks (adults). These animals were sourced from the animal development and maintenance laboratory of Neuroscience Research Center at Shahid Beheshti University of Medical Sciences, located in the vibrant city of Tehran, Iran. Selecting these particular rats, known for their genetic uniformity and adaptability in laboratory settings, provided a solid foundation for our research objectives. All rats were housed in a temperature-controlled environment at 22°C ± 2, with a consistent 12-hour light and 12-hour dark cycle (lights on at 7 A.M.), and they had unlimited access to water and food. Every four rats were kept in a standard cage (four animals per cage). All training and behavioral experiments were conducted in the evenings (2:00 PM to 6:00 PM). All procedures conducted in this study adhere to the comprehensive guidelines for the care and use of laboratory animals established by the National Institutes of Health. Additionally, these research procedures have been thoroughly reviewed and officially approved by the Research and Ethics Committee at the School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, with reference number IR.SBMU.PHNS.REC.1399.168. This approval confirms that the study complies with the highest ethical standards in animal research, prioritizing animal welfare and humane treatment while providing meaningful contributions to science.

2.2. Maternal training protocol, mating stages, and how offspring selection

A total of 16 female rats, aged 8 weeks, were randomly assigned to two groups for a spatial learning study. The first group, the trained mothers' group, consisted of eight rats that underwent spatial experience by using the Morris Water Maze (MWM) protocol. In this challenging task, the rats learned to navigate their surroundings to locate a submerged platform just beneath the water's surface. In contrast, the second group, comprised of eight untrained mothers, did not receive any spatial training and therefore remained unfamiliar with the maze's layout and mechanisms. This setup aimed to explore the differences in spatial awareness and cognitive function between the spatially trained and untrained groups.

After completion of a rigorous 5-day MWM (Morris Water Maze) training regimen, the rats were gently returned to their familiar home cages, where they were allowed to acclimatize in a calm, undisturbed environment for an additional 5 days. After this recovery period, each female rat from both experimental groups identified as in the oestrus phase of their reproductive cycle was carefully paired with a standard-reared male of the same species. This intentional pairing was designed to facilitate mating interactions and to observe a range of reproductive behaviors over a subsequent 5-day period (Riyahi et al., 2019). Each breeding pair of rats, consisting of one female and one male, was carefully placed in a separate cage that provided optimal conditions for mating. After successful mating, the females were gently moved to spacious standard home cages, each measuring 40×26×18 cm (length, width, and height). These cages are designed to provide a comfortable, suitable environment for females during gestation.

They remained in these habitats throughout their 22-day pregnancy, the birthing process, and the crucial 21-day period of caring for their young until weaning. During this time, the male rats were removed to prevent any disturbance. Following weaning, the female offspring from both breeding groups were taken away from their mothers. They were then housed

individually in cages, with a setup designed to accommodate up to four animals per cage for social interaction among the females. Meanwhile, the male offspring were also removed from the litters to ensure that they were separately from the female offspring.

When the female rats were born, 4 weeks old, two rats were randomly selected from each of the 16 litters and assigned to two groups.

Sixteen female offspring of mothers spatially trained (Experimental group; n=16), and sixteen female offspring of the mothers not spatially trained (Control group; n=16). Then, assuming that two inbred female rats were not placed in the same group, each group was subdivided into two halves: Control Group 1, and Control Group 2; Experimental Group 1, Experimental Group 2, n=8.

To assess how maternal spatial training influences the cognitive spatial capabilities (learning and memory) of their female offspring, half of the rats in each group (Experimental and Control) were tested for acquisition and retention of spatial memory using the same MWM protocol used to train the mothers. Additionally, to assess the effects of maternal spatial training on both short- and long-term memory in their female offspring, half of the rats in each group (Experimental and Control) were tested with the NOR and Y-Maze tests that different from what the mothers had trained. Ninety minutes following the completion of the NOR (Novel Object Recognition) and Y-maze assessments, or the probe phase of the Morris Water Maze (MWM) test, a total of three rats from each group were euthanized while under deep anesthesia. This procedure was performed to enable thorough analysis of molecular and protein expressions in their brain tissues. Figure 1 provides a summary of the experimental procedures.

2.3. The framework and methods of MWM spatial training

2.3.1. MWM Apparatus

A black circular pool, with a diameter of 150 cm, a depth of 60 cm, and a height of 45 cm, was filled with opaque water maintained at 23ºC. This setting was utilized to evaluate spatial learning capabilities. A black platform with an 11 cm diameter was positioned approximately 2 cm below the water surface. It was situated in the middle of a randomly chosen quadrant—either north-east (NE), north-west (NW), south-east (SE), or south-west (SW). The platform served as the sole escape route from the water. The room was shrouded in a dim ambiance, with heavy, dark curtains that absorbed the light. The walls adorned were a patchwork of diverse photographs that served as spatial cues. These photographs remained in the same position across all training and testing. A CCD camera (Panasonic Inc., Japan) recorded the animal's swimming paths. For automated analysis of animal behavioral data, this setup was linked to a laptop operating EthoVision XT7 (Noldus, Netherlands).

2.3.2 MWM protocol

The MWM protocol was carried out over five straight days: a habituation day, three training days, and a final probe test, consistent with prior research conducted in our lab (Riyahi et al., 2022; Riyahi et al., 2021; Riyahi et al., 2019).

2.3.2.1 Habituation phase

A full day before the commencement of the training phase, the platform was carefully extracted from the pool to ensure appropriate conditions for the upcoming trials. To help the rats adjust to their surroundings and minimize potential stress, they were given a 60-second swim to become more habituated with the environment before training began. During this time, the rats were swimming in the pool without a platform and were immediately removed by the experimenter when the time was up; after drying, they were returned to the cage.

2.3.2.2 Spatial learning phase

The spatial learning reference paradigm involved one set of four trials each day, with a 10-minute interval between trials, conducted over three straight days for a total of 12 trials, based on prior research in our lab (Riyahi et al., 2022; Riyahi et al., 2021; Riyahi et al., 2019).

The platform was strategically positioned in the 4th quadrant (southwest). During each of the four trials, the animals were unpredictably released from one of the four quadrants bordering the pool, creating anticipation as they emerged into the spacious area. Each quadrant offered a unique angle of approach, adding an element of chance to their release and allowing for a diverse range of movements and behaviors as they explored their surroundings. They were given a 60-second time frame to navigate through the water and successfully locate the platform. The challenge required them to rely on their instincts and spatial awareness as they swam to find safety. If they did not find it, the software automatically paused recording, and the experimenter calmly directed the rats to the platform, where they remained for 30 seconds. EthoVision software was used to accurately track the duration each rat took to achieve the escape platform across trials, a metric known as escape latency. After each trial, the animals were returned to their cages to rest before the next session.

2.3.2.3 Spatial memory phase (Probe test)

The day after completing their third training session, the rats were subjected to the probe test (spatial retention), an evaluation designed to assess their learning and memory of the submerged escape platform's location. This test aimed to determine how well the rats could navigate and recall the specific area where they had previously learned to find safety, thereby allowing researchers to gauge the effectiveness of their training. In this test, the platform, which had previously been submerged in a large water-filled arena, was removed to challenge the rats to recall where it had been. The trained rats were then introduced into the pool from the northeastern quadrant, which was the opposite side of the platform's original position,

designated as the target quadrant. Once released, the rats were given 60 seconds to explore the pool and locate the platform that had been removed. The primary focus was to assess their spatial memory by tracking how much time each rat spent in the target quadrant, where the platform had been. This data was meticulously recorded and analyzed using EthoVision software, which facilitated accurate measurements of their behavior and spatial navigation during the test.

2.4. Y-maze

To evaluate the working memory of female offspring from mothers that were spatially trained and those from mothers that were not, the Y-maze test was used. This test measures short-term spatial memory by assessing the rat's ability to recognize its environment. It relies on the natural tendency of rodents to explore novel environments and tests their working spatial recognition memory (Sarnyai et al., 2000). The Y-maze is constructed from Plexiglas and features three arms labeled A, B, and C, arranged in a Y shape. Each arm is 40 cm long, 15 cm wide, and 30 cm high. The Y-maze experiment was conducted in a calm, dim setting to minimize stress on the rats. At the start, each rat, without prior familiarization, was placed in the initial part of arm A for one minute at the beginning of the test (the initial part of arm A, separated from the main section by a guillotine door), with the door shut. After a minute, the door was opened so the animal could enter the maze and explore the environment. Once the rat fully entered, the door was closed at the start, and a 10-minute timer began. The act of entering the arms was noted from the position of the rat's tail when it reached the arm, and the entry sequence was recorded over 10 minutes. The animals were returned to their cages after the testing session. To analyze behavior, the entered arms were categorized in sets of three in the same sequence, and the tally of unique alternations (Actual alternation) was recorded, excluding sequences with repeated arms. The percentage of alternation was determined after calculating the total number of entries using the subsequent formula (Kitanaka et al., 2015;

Sierksma et al., 2014):

Alternation percentage = [(number of actual alternations)/ (total number of arm entries − 2)] × 100

2.5. Novel Object Recognition (NOR)

The NOR test is a commonly used method to examine learning and memory in rats. The test is popular for assessing nonspatial memory and uses rodents' tendency to explore new objects. Researchers measure the time spent exploring each object to estimate memory. The NOR task assesses both short- and long-term memory and does not require motivation, reward, or punishment (Cohen & Stackman Jr, 2015). The testing is in an open-field arena measuring 65 cm on each side and 45 cm high. Small washable objects of different colors and sizes serve as stimuli. Objects are placed 4 cm from the wall, and rats cannot move them. The objects and the area are disinfected with 70% ethanol both before and after each test. The test is conducted in a quiet room with low light and conditions similar to those in the rats' housing. Rats are moved to this room one hour before testing.

The NOR test protocol was carried out over three consecutive days, including a habituation day on the first day, training and short-term memory assessment on the second day, and long-term memory evaluation on the third day. On the initial day, On the first day, we introduced the rats to an empty arena for 5 minutes to help them get used to the experimental conditions and reduce stress. The training session commenced 24 hours after the habituation period. During training, two identical objects were placed in opposite quadrants of an arena, with the rats facing the objects in the center, and the rats were allowed to explore freely for 10 minutes. After the training session, the rats were put back in their cage and stayed in the test room. Sixty minutes after the training session, the rats underwent a short-term recognition assessment. During this stage, one familiar and one new object, different from those used in training, were placed in opposite quadrants of the arena. The animals spent 10 minutes

exploring the space, and the duration they spent near each object was carefully tracked. After 24 hours, the animals participated in a long-term recognition assessment. At this stage, the familiar object from the short-term assessment was kept, while the second object was replaced with a new one. Once again, the rats explored the environment for 10 minutes, and their time around each object was meticulously recorded (Lueptow, 2017).

This assessment uses the discrimination index as a key metric to evaluate rats' responses to various stimuli. It is not affected by total exploration time and ranges from -1 to +1. In this study, the discrimination index is calculated as follows (Lueptow, 2017):

Discrimination index = The difference in the time of exploring a novel object with a familiar object/ Total time exploring the familiar and novel object

2.6. Analysis Using Western Blotting

Ninety minutes post-behavioral evaluation, we measured SYT1, BDNF, and phosphorylated ERK1/2 levels in the hippocampi of female offspring. This analysis focused on two distinct groups of offspring born to mothers who underwent spatial training, referred to as Experimental Groups 1 and 2. In contrast, two additional groups, designated as Control Groups 1 and 2, were formed from offspring of mothers who had not received any spatial training. Using Western blotting techniques, we carefully prepared three rats per group, resulting in a total of 12 subjects. These rats were deeply anesthetized, allowing for the precise extraction of their hippocampi. After extraction, the tissue samples were immediately frozen in liquid nitrogen for 24 hours and subsequently stored at -80ºC to preserve their integrity until homogenization. The hippocampi were homogenized in a lysis buffer containing a protease inhibitor cocktail to prevent protein degradation. To measure protein concentrations, we employed Bradford's method, a well-established technique in protein biochemistry (Bradford, 1976). For each sample, we loaded 75 µg of protein onto 10% SDS-PAGE gels for electrophoresis. Following separation, the proteins were meticulously transferred to

polyvinylidene fluoride (PVDF) membranes, which were then blocked for 75 minutes with a solution of 2% non-fat milk to minimize nonspecific binding. Overnight, the membranes were incubated at 4ºC with primary antibodies specific to the proteins of interest: SYT1 (1:1000 dilution from Abcam), BDNF (1:1000 dilution, also from Santa Cruz Biotechnology), ERK1/2 (1:1000 dilution, sourced from Santa Cruz Biotechnology), and β-Actin (1:1000 dilution, Santa Cruz Biotechnology). Post incubation, we washed the membranes three times for 15 minutes each with TBST to remove any unbound antibodies. After washing, membranes were incubated with a goat anti-rabbit IgG-HRP secondary antibody (1:1000 dilution; Santa Cruz Biotechnology) for 75 minutes at room temperature. Following another set of three washes with Tris-buffered saline, the membranes were developed with ECL advanced reagents, producing a chemiluminescent signal for autoradiographic visualization of the proteins. Finally, we quantified the protein band density using ImageJ (NIH, Bethesda, Maryland, USA), enabling an accurate comparison of protein expression levels across the different experimental groups.

2.7. Statistical analysis

In this research, graphs were generated using GraphPad Prism 6, while STATISTICA 12 (StatSoft) was used to analyze the data. Normality was assessed using the Kolmogorov-Smirnov test, while homoscedasticity was checked with Levene's test. The performance of female offspring in the MWM training from mothers that underwent spatial training was analyzed against a control group using a two-way repeated-measures ANOVA (2×3; Group × Session). The analysis of performance in the MWM probe test, Y-maze, short-term and long-term NOR, as well as the levels of SYT1, BDNF, and ERK1/2 phosphorylation in the hippocampus for female offspring and controls, was conducted employing Student's t-tests. All data are expressed as the mean ± SEM (The standard error of the mean). Statistical

significance is determined using a threshold of $P < 0.05$.

3. Results

3.1. The facilitation of the spatial learning process in offspring

To assess how maternal spatial training affects female offspring's spatial learning across generations, escape latencies were measured over three days in a challenging Morris Water Maze (MWM) test for both the Experimental and Control groups. Results indicated that female offspring of trained mothers exhibited shorter escape latencies than those in the Control group. A two-way repeated measures ANOVA (2×3) analyzing Group and Session showed significant main effects for Group ($F_{(1, 14)} = 6.38$, $p < 0.05$, $\eta 2 = 0.313$) and Session ($F_{(2, 28)} = 81.48$, $p < 0.001$, $\eta 2 = 0.583$). However, the interaction between Group and Session ($F_{(2, 28)} = 0.215$, $p = 0.881$, $\eta 2 = 0.002$) was not significant. The findings indicate that maternal spatial training improves spatial learning abilities in female offspring (Fig. 2a).

3.2. The enhancement of the spatial memory capacity in offspring

Following the third training session, the animals underwent the spatial probe test, a crucial evaluation designed to assess their memory and spatial awareness. During this test, the amounts of time spent in the target quadrant—the area that the animals were trained to associate with rewards—were meticulously recorded. The results of the analysis, as determined by the Student's t-test ($t = 3.93$, $df = 14$, $p < 0.001$), revealed a significant finding: the female offspring of mothers who underwent the spatial training displayed a notably greater time spent in the target quadrant compared to those in the Control group. These compelling results suggest that the spatial training imparted to the mothers not only benefited them but also significantly enhanced the spatial memory capabilities of their female offspring (Fig. 2b).

3.3 The increased levels of SYT1, BDNF, and ERK1/2 phosphorylation in the hippocampus of female offspring during activities related to MWM training

To investigate the transgenerational impact of maternal spatial training, we examined the neurobiological mechanisms underlying enhanced spatial memory in female offspring of trained mothers. We analyzed the expression levels of three crucial proteins in the hippocampus: SYT1, BDNF, and phosphorylated ERK1/2 (p-ERK1/2). This was accomplished using western blotting, in which we compared the results from the Experimental 1 group with those of the Control 1 group. The statistical analysis using Student's t-tests revealed significant increases in several key markers. Specifically, we observed a striking elevation in hippocampal BDNF levels ($t = 4.78$, $df = 16$, $p < 0.001$), as illustrated in Fig 3a. Additionally, p-ERK1/2 levels also showed a significant rise ($t = 2.58$, $df = 16$, $p < 0.05$), depicted in Fig 3b. Interestingly, while SYT1 levels increased, the difference was not as pronounced ($t = 0.64$, $df = 16$, $p < 0.05$), as shown in Fig 3c. These findings highlight an enhanced neurobiological response in female offspring of mothers who engaged in spatial training, compared with those born to untrained mothers.

3.4. No improvement in working memory in female offspring of mothers who had undergone spatial training

To assess the impact of maternal spatial training on the working memory capabilities of their female offspring across generations, the Y-maze test scores of the Experimental group (whose mothers underwent spatial training) were compared with those of the Control group (whose mothers did not). The analysis used a Student's t-test, which indicated there was no significant difference in working memory performance between the two groups of offspring ($t = 0.56$, $df = 14$, $p = 0.956$). Consequently, the findings indicate that the maternal spatial training did not confer any observable benefits to the working memory abilities of their female offspring (Fig.

4a).

3.5. No improvement in short- and long-term memory in female offspring of mothers who had undergone spatial training

To assess how maternal spatial training impacts short- and long-term memory across generations of their female offspring in a task different from the one trained, the discrimination index in the NOR test was analyzed for both the Experimental and Control groups. In the evaluation of short-term memory, the findings from the Student's t-test ($t = 0.395$, $df = 14$, $p = 0.699$) indicated that there was no statistically significant difference between the groups observed (Fig. 4b). Similarly, when assessing long-term memory, the results of the t-test ($t = 2.104$, $df = 14$, $p = 0.054$) also failed to demonstrate a significant difference between the female offspring of mothers who received spatial training and those whose mothers did not receive such training (Fig. 4c). These results suggest that the maternal training in spatial tasks did not lead to any improvements in either short-term or long-term memory abilities in the Novel Object Recognition (NOR) test, a task that was not part of the training regimen for the mothers, in their female offspring.

3.6. No increase in hippocampal expression levels of SYT1, BDNF, or ERK1/2 phosphorylation in female offspring of spatially trained mothers after they performed tasks for which their mothers did not receive training

In this study, we conducted a thorough examination of the hippocampal levels of SYT1, BDNF, and phosphorylated ERK1/2 in two groups: Experimental 2 and Control 2. Utilizing Student's t-test for statistical analysis, we found no significant differences in the levels of BDNF ($t = 1.55$, $df = 16$, $p = 0.145$) (Fig. 5a), our analysis of p-ERK1/2 levels revealed no notable differences ($t = 1.53$, $df = 16$, $p = 0.144$) (Fig. 5b), nor did we observe significant

variations in SYT1 levels ($t = 1.94$, $df = 16$, $p = 0.070$) (Fig. 5c). These findings suggest that the maternal spatial training did not produce any significant changes in the levels of BDNF, p-ERK1/2, or SYT1 within the hippocampus of their female offspring during tasks that their mothers had not specifically trained them for.

4. Discussion

Our latest research on the intergenerational inheritance effects of spatial training of the parents conducted before fertilization reveals compelling evidence that this training significantly enhances offspring's cognitive abilities, particularly learning and memory. This research highlights the profound ways in which early parental experiences can shape the neurological development and cognitive potential of future generations (Riyahi et al., 2022; Riyahi et al., 2021; Riyahi et al., 2019). These findings align with our current study, reinforcing the idea that maternal spatial training before fecundation enhances learning processes and memory consolidation of female offspring. This phenomenon seems to be promoted by alterations in the expression levels of BDNF and ERK1/2 phosphorylation in the hippocampus of the offspring (Riyahi et al., 2022; Riyahi et al., 2019). In fact, this intergenerational effect is mediated by the transmission of epigenetic factors, which serve as the main regulators of neural processes underlying learning and memory.

Evidence indicates that specific epigenetic changes, including histone modifications (Greer, Becker, Latza, Antebi, & Shi, 2016; Katz, Edwards, Reinke, & Kelly, 2009; Öst et al., 2014; Siklenka et al., 2015), are inherited from parents to their offspring, facilitating the transmission of valuable environmental information and experiences accumulated by previous generations (Wang et al., 2017). Histone acetylation is crucial for memory consolidation, as it modifies histones, making DNA more accessible. This chemical modification of histones, which are proteins around which DNA is wrapped, is vital for stabilizing and preserving the

memories we form, allowing them to be stored effectively in the brain (Peixoto & Abel, 2013). Zhang et al. (2017) documented an increase in H3K14 acetylation in the semen of fathers who underwent spatial training, suggesting that this increased histone acetylation can be inherited by their offspring (Zhang et al., 2017). Our recent research revealed that engaging in spatial training as parents significantly increased H3K14 acetylation levels in the hippocampus of their offspring. This modification is essential because it enhances the expression of key proteins, such as SYT1 and BDNF. Moreover, we observed a notable activation of ERK1/2 phosphorylation in the offspring of these trained parents, indicating a robust link between parental training and the molecular mechanisms underlying cognitive development (Riyahi et al., 2019). During the complex process of spermatogenesis in mammals, most histones, the essential proteins that package DNA, are replaced by specialized nucleoproteins. This replacement is important because it helps condense and protect the genetic material during sperm maturation. However, a small subset of nucleosomes and histones is retained, playing a crucial role in regulating early embryonic development. These preserved components are essential for ensuring proper gene expression and overall developmental processes after fertilization (Ihara et al., 2014). In rats, the majority of conserved nucleosomes are associated with a type of histone known as H3.3, which is predominantly located near the promoter regions of GC-rich DNA (Erkek et al., 2013). Additionally, the promoter regions of interest are bordered by activated transcription factor 2 (ATF2). This protein interacts with the P300/CREB complex, a key regulator of gene expression. Notably, ATF2 not only binds to these regulatory elements but also has intrinsic histone acetyltransferase activity, which helps modify histones. This modification is vital for changing chromatin structure and promoting the transcription of target genes (Kawasaki et al., 2000). This potential for chromatin modification can serve as a basis for the intergenerational transmission of phenotypes

resulting from parents' exposure to different environments or stimuli, influencing the behaviors of the next generation.

SYT1, ERK1/2, and BDNF play vital roles in cognitive functions, particularly memory, making them promising factors for understanding the impact of parental spatial training on offspring's spatial learning and memory abilities (Riyahi et al., 2019). The neurotrophic factor BDNF is crucial for regulating processes that influence the survival and specialized development of neurons throughout development (Binder & Scharfman, 2004; Huang & Reichardt, 2003), as well as synaptic plasticity (Bramham & Messaoudi, 2005; Tyler, Alonso, Bramham, & Pozzo-Miller, 2002). Consistent with the understanding that alterations in synaptic strength, driven by activity, are essential for both the processing and retention of memories, a wealth of research highlights the significant role of Brain-Derived Neurotrophic Factor (BDNF) in facilitating spatial learning and memory formation. This evidence points to BDNF as a key player in the intricate mechanisms that underpin how we navigate and remember our environment (Bekinschtein et al., 2007; Tyler et al., 2002). Additionally, the ERK/MAPK signaling pathway plays an essential part in memory formation (Giovannini, 2006; Ji, Gereau IV, Malcangio, & Strichartz, 2009), to the extent that a malfunction in this signaling pathway leads to cognitive impairments, it can significantly impact an individual's thinking, memory, and overall mental functioning (Vithayathil, Pucilowska, Friel, & Landreth, 2017). The ERK pathway, or extracellular signal-regulated kinase pathway, is a significant component of the mitogen-activated protein kinase (MAPK) superfamily. This superfamily comprises a crucial, evolutionarily conserved collection of enzymes that play a key role in cellular signaling. These enzymes are intricately linked to receptors on the cell membrane and various regulatory targets within the cell, facilitating essential communication pathways that influence numerous cellular functions, including growth, differentiation, and responses to external stimuli (Peng, Zhang, Zhang, Wang, & Ren, 2010). Recent evidence

consistently indicates that the ERK signaling pathway is closely linked to learning and memory functions (Feld, Dimant, Delorenzi, Coso, & Romano, 2005; Giovannini, 2006; Ji et al., 2009). SYT1 is a crucial presynaptic protein that plays a vital role in vesicle docking and synaptic vesicle fusion, which is triggered by calcium (Ca2+) (Geppert et al., 1994; Nishiki & Augustine, 2004). This protein is essential for facilitating effective synaptic transmission, which allows communication between neurons. Furthermore, SYT1 is integral to the processes underlying learning and memory, underscoring its role in nervous system function (Zhang et al., 2017).

However, a noteworthy finding in this study, which has not been reported in any other research, is that maternal spatial training did not enhance working, short-term, or long-term memory in the Y-maze and NOR tasks. The cellular and molecular results matched these behavioral observations. Maternal spatial training did not produce any notable differences in the expression levels of BDNF, synaptotagmin1, or phosphorylated ERK1/2 in the hippocampus of female offspring. This was observed during assessments using the Y-maze and novel object recognition (NOR) tests, indicating that the training did not influence the biochemical markers typically associated with learning and memory in these specific tasks. These findings indicate a specificity in the effects of parental training before fertilization on their offspring's learning and memory processes. Previous studies on the impact of an enriched parental environment prior to fertilization suggested that maternal spatial training would enhance female offspring's performance in the Y-maze and NOR tasks (Caporali et al., 2014; Cutuli et al., 2019; Cutuli et al., 2018; Cutuli et al., 2017; Cutuli et al., 2015; Yin et al., 2013). Contrary to this expectation, our study found that maternal spatial training had no effect on offspring performance on these new tasks.

Considering the nature of the Morris Water Maze (MWM) task, which not only poses a cognitive challenge in locating a hidden platform but also increases the physical activity of

the rats, one might see participation in the MWM protocol as a form of environmental enrichment. Previous studies have reported cognitive benefits for offspring from parental enriched cognitive and physical environments, leading us to anticipate improved performance in Y-maze and NOR tasks for the offspring of trained mothers. However, our results showed contradictory findings, as highlighted by several studies (Arai & Feig, 2011; Arai, Li, Hartley, & Feig, 2009; Cutuli et al., 2018; McGreevy et al., 2019; Yin et al., 2013) that generally supports that a stimulating environment, both cognitively and physically, benefits the cognitive development of offspring. Such enriched surroundings are believed to foster improved cognitive abilities in offspring, promoting their intellectual growth and overall mental faculties. But the question is why this effect was not observed in the current study and why maternal training did not have an overall effect on improving offspring's cognitive abilities, nor did it enhance short-term or long-term memory.

The absence of impact from maternal spatial training on the offspring's memory capacity might result from the short duration of MWM training for the mothers. A review of existing research indicates that parental involvement in cognitive and physical environmental enrichment usually lasts 6 to 8 weeks. Therefore, it is clear that only 5 days of MWM training were insufficient to improve the cognitive skills of their female offspring.

Reviewing the first-phase research results shows that maternal spatial training (MWM) enhances offspring's learning and memory specifically in the same task (MWM). However, it does not improve performance in unrelated tasks like the Y-maze and NOR, which the parents were not trained on. This suggests that parental training before fertilization selectively influences the offspring's learning and memory when the task is similar to the parental experience. The unique characteristics are probably due to distinct neural mechanisms and signaling pathways activated during different cognitive processes. Each cognitive function

may tap into specific neural networks and chemical signals in the brain, leading to variations in how we think, learn, and remember.

The core of transgenerational skill transmission is the passing down of epigenetic marks inherited from parental experiences, which can affect development in specific ways. Although neuroplasticity underpins memory formation and learning-related cognitive functions, it's important to recognize that different memory types involve distinct neural processes and regions. Moreover, the neural connections and pathways established in the brain during mastery of each specific task are distinct and tailored to that task.

Creating a memory engram is a biological process that involves the synthesis of various proteins regulated by gene expression (Tonegawa et al., 2015). This regulation, as well as the subsequent protein synthesis, is greatly influenced by epigenetic processes at the chromatin region (Reul, Collins, & Gutièrrez-Mecinas, 2011). t is widely recognized that epigenetic processes primarily regulate gene expression at this level. These intricate biological processes modify the characteristics of the genome or its interactions with essential histone proteins. This modification results in structural transformations within the chromatin, the complex of DNA and proteins found in the nucleus of eukaryotic cells. These changes in chromatin architecture serve an essential function in regulation the transcription of a diverse array of genes that are pivotal for the formation and retention of memories (Kim & Kaang, 2017).

The organization of chromatin is flexible and plays a critical role in regulating cellular functions, such as gene expression (Felsenfeld & Groudine, 2003). Numerous research studies have demonstrated that learning and memory processes induce significant changes in epigenetic marks. These alterations in the chemical modifications of DNA and associated proteins play a crucial role in how we encode, store, and retrieve memories, highlighting the intricate relationship between our experiences and genetic expression (Levenson & Sweatt, 2005; Peixoto & Abel, 2013). Epigenetic mechanisms cause DNA to condense and relax

around histone cores, which, in turn, lead to transcription repression and activation, respectively (Kim & Kaang, 2017). Current research (Miller et al., 2010; Miller & Sweatt, 2007; Zhang et al., 2017) supports the idea that epigenetic changes—such as DNA methylation, histone methylation, and histone acetylation—are essential for the formation of long-term memory engrams.

Therefore, performing a specific task causes unique changes in chromatin, forming a distinct neural memory engram. Consequently, engaging in a particular cognitive activity results in specific epigenetic patterns that can be maintained and passed on to future generations via epigenetic mechanisms.

Nevertheless, this conclusion should be approached cautiously, as it represents the second study on how training in a specific cognitive task influences learning and memory in other tasks across generations. To better understand this issue, we must await future research findings that will provide more clarity.

Acknowledgements

The authors express their heartfelt gratitude to the Neuroscience Research Center at Shahid Beheshti University of Medical Sciences in Tehran, Iran, for their invaluable collaboration throughout this study. Their support has been instrumental in advancing the research. Additionally, they extend their appreciation to the Cognitive Science and Technologies Council (CSTC) for their generous backing. The researchers at the Laboratory of Experimental and Behavioral Neurophysiology at the Santa Lucia Foundation in Rome, Italy, also deserve thanks for their contributions, which have greatly enriched the research efforts presented in this study.

References

Arai, J. A., & Feig, L. A. (2011). Long-lasting and transgenerational effects of an environmental enrichment on memory formation. *Brain research bulletin, 85*(1-2), 30-35.

Arai, J. A., Li, S., Hartley, D. M., & Feig, L. A. (2009). Transgenerational rescue of a genetic defect in long-term potentiation and memory formation by juvenile enrichment. *Journal of neuroscience, 29*(5), 1496-1502.

Azadi, M., Azizi, H., & Haghparast, A. (2019). Paternal exposure to morphine during adolescence induces reward-resistant phenotype to morphine in male offspring. *Brain research bulletin, 147*, 124-132.

Bekinschtein, P., Cammarota, M., Igaz, L. M., Bevilaqua, L. R., Izquierdo, I., & Medina, J. H. (2007). Persistence of long-term memory storage requires a late protein synthesis-and BDNF-dependent phase in the hippocampus. *Neuron, 53*(2), 261-277.

Binder, D. K., & Scharfman, H. E. (2004). Brain-derived neurotrophic factor. *Growth factors (Chur, Switzerland), 22*(3), 123.

Bramham, C. R., & Messaoudi, E. (2005). BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Progress in neurobiology, 76*(2), 99-125.

Caporali, P., Cutuli, D., Gelfo, F., Laricchiuta, D., Foti, F., De Bartolo, P., . . . Petrosini, L. (2014). Pre-reproductive maternal enrichment influences offspring developmental trajectories: motor behavior and neurotrophin expression. *Frontiers in behavioral neuroscience, 8*, 195.

Cohen, S. J., & Stackman Jr, R. W. (2015). Assessing rodent hippocampal involvement in the novel object recognition task. A review. *Behavioural brain research, 285*, 105-117.

Cutuli, D., Berretta, E., Caporali, P., Sampedro-Piquero, P., De Bartolo, P., Laricchiuta, D., . . . Vecchioli, S. F. (2019). Effects of pre-reproductive maternal enrichment on maternal care, offspring's play behavior and oxytocinergic neurons. *Neuropharmacology, 145*, 99-113.

Cutuli, D., Berretta, E., Laricchiuta, D., Caporali, P., Gelfo, F., & Petrosini, L. (2018). Pre-reproductive parental enriching experiences influence progeny's developmental trajectories. *Frontiers in behavioral neuroscience, 12*, 254.

Cutuli, D., Berretta, E., Pasqualini, G., De Bartolo, P., Caporali, P., Laricchiuta, D., . . . Foti, F. (2017). Influence of pre-reproductive maternal enrichment on coping response to stress and expression of c-Fos and glucocorticoid receptors in adolescent offspring. *Frontiers in behavioral neuroscience, 11*, 73.

Cutuli, D., Caporali, P., Gelfo, F., Angelucci, F., Laricchiuta, D., Foti, F., . . . Farioli Vecchioli, S. (2015). Pre-reproductive maternal enrichment influences rat maternal care and offspring developmental trajectories: behavioral performances and neuroplasticity correlates. *Frontiers in behavioral neuroscience, 9*, 66.

Dias, B. G., & Ressler, K. J. (2014). Parental olfactory experience influences behavior and neural structure in subsequent generations. *Nature neuroscience, 17*(1), 89-96.

Erkek, S., Hisano, M., Liang, C.-Y., Gill, M., Murr, R., Dieker, J., . . . Peters, A. H. (2013). Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. *Nature structural & molecular biology, 20*(7), 868-875.

Feld, M., Dimant, B., Delorenzi, A., Coso, O., & Romano, A. (2005). Phosphorylation of

extra-nuclear ERK/MAPK is required for long-term memory consolidation in the crab Chasmagnathus. *Behavioural brain research, 158*(2), 251-261.

Felsenfeld, G., & Groudine, M. (2003). Controlling the double helix. *Nature, 421*(6921), 448-453.

Forehand, C. J. (2009). The action potential, synaptic transmission, and maintenance of nerve function. *Medical Physiology: Principles for Clinical Medicine (Rhoades RA, BD, eds., Lippincott Williams & Wilkins, a Wolters Kluwer business, ed)*, 38-64.

Frey, U., Frey, S., Schollmeier, F., & Krug, M. (1996). Influence of actinomycin D, a RNA synthesis inhibitor, on long-term potentiation in rat hippocampal neurons in vivo and in vitro. *The Journal of physiology, 490*(3), 703-711.

Frey, U., Krug, M., Reymann, K. G., & Matthies, H. (1988). Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. *Brain research, 452*(1-2), 57-65.

Geppert, M., Goda, Y., Hammer, R. E., Li, C., Rosahl, T. W., Stevens, C. F., & Südhof, T. C. (1994). Synaptotagmin I: a major Ca2+ sensor for transmitter release at a central synapse. *Cell, 79*(4), 717-727.

Giovannini, M. G. (2006). The role of the extracellular signal-regulated kinase pathway in memory encoding. *Reviews in the Neurosciences, 17*(6), 619-634.

Greer, E. L., Becker, B., Latza, C., Antebi, A., & Shi, Y. (2016). Mutation of C. elegans demethylase spr-5 extends transgenerational longevity. *Cell Research, 26*(2), 229-238.

Huang, E. J., & Reichardt, L. F. (2003). Trk receptors: roles in neuronal signal transduction. *Annual review of biochemistry, 72*(1), 609-642.

Ihara, M., Meyer-Ficca, M. L., Leu, N. A., Rao, S., Li, F., Gregory, B. D., . . . Meyer, R. G. (2014). Paternal poly (ADP-ribose) metabolism modulates retention of inheritable sperm histones and early embryonic gene expression. *PLoS genetics, 10*(5), e1004317.

Ji, R.-R., Gereau IV, R. W., Malcangio, M., & Strichartz, G. R. (2009). MAP kinase and pain. *Brain research reviews, 60*(1), 135-148.

Katz, D. J., Edwards, T. M., Reinke, V., & Kelly, W. G. (2009). A C. elegans LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. *Cell, 137*(2), 308-320.

Kawasaki, H., Schiltz, L., Chiu, R., Itakura, K., Taira, K., Nakatani, Y., & Yokoyama, K. K. (2000). ATF-2 has intrinsic histone acetyltransferase activity which is modulated by phosphorylation. *Nature, 405*(6783), 195-200.

Kim, S., & Kaang, B.-K. (2017). Epigenetic regulation and chromatin remodeling in learning and memory. *Experimental & molecular medicine, 49*(1), e281-e281.

Kitanaka, J., Kitanaka, N., Hall, F. S., Fujii, M., Goto, A., Kanda, Y., . . . Muranishi, Y. (2015). Memory impairment and reduced exploratory behavior in mice after administration of systemic morphine. *Journal of experimental neuroscience, 9*, JEN. S25057.

Levenson, J. M., & Sweatt, J. D. (2005). Epigenetic mechanisms in memory formation. *Nature Reviews Neuroscience, 6*(2), 108-118.

Lueptow, L. M. (2017). Novel object recognition test for the investigation of learning and memory in mice. *JoVE (Journal of Visualized Experiments)*(126), e55718.

Mansvelder, H. D., Verhoog, M. B., & Goriounova, N. A. (2019). Synaptic plasticity in human cortical circuits: cellular mechanisms of learning and memory in the human brain?

Current opinion in neurobiology, 54, 186-193.

McGreevy, K. R., Tezanos, P., Ferreiro-Villar, I., Pallé, A., Moreno-Serrano, M., Esteve-Codina, A., . . . Martín-Montalvo, A. (2019). Intergenerational transmission of the positive effects of physical exercise on brain and cognition. *Proceedings of the National Academy of Sciences, 116*(20), 10103-10112.

Miller, C. A., Gavin, C. F., White, J. A., Parrish, R. R., Honasoge, A., Yancey, C. R., . . . Sweatt, J. D. (2010). Cortical DNA methylation maintains remote memory. *Nature neuroscience, 13*(6), 664-666.

Miller, C. A., & Sweatt, J. D. (2007). Covalent modification of DNA regulates memory formation. *Neuron, 53*(6), 857-869.

Nishiki, T.-i., & Augustine, G. J. (2004). Synaptotagmin I synchronizes transmitter release in mouse hippocampal neurons. *Journal of neuroscience, 24*(27), 6127-6132.

Öst, A., Lempradl, A., Casas, E., Weigert, M., Tiko, T., Deniz, M., . . . Stoeckius, M. (2014). Paternal diet defines offspring chromatin state and intergenerational obesity. *Cell, 159*(6), 1352-1364.

Peixoto, L., & Abel, T. (2013). The role of histone acetylation in memory formation and cognitive impairments. *Neuropsychopharmacology, 38*(1), 62-76.

Peng, S., Zhang, Y., Zhang, J., Wang, H., & Ren, B. (2010). ERK in learning and memory: a review of recent research. *International journal of molecular sciences, 11*(1), 222-232.

Reul, J. M., Collins, A., & Gutièrrez-Mecinas, M. (2011). Epigenetic mechanisms in memory formation. *Brain, Behavior and Epigenetics*, 287-300.

Riyahi, J., Abdoli, B., Gelfo, F., Petrosini, L., Khatami, L., Meftahi, G. H., & Haghparast, A. (2022). Multigenerational effects of paternal spatial training are lasting in the F1 and F2 male offspring. *Behavioural pharmacology, 33*(5), 342-354.

Riyahi, J., Abdoli, B., Gelfo, F., Petrosini, L., Rezaei, R., & Haghparast, A. (2021). Maternal spatial training before fertilization improves the spatial learning process in female offspring. *Neuroreport, 32*(13), 1106-1112.

Riyahi, J., Abdoli, B., Haghparast, A., & Petrosini, L. (2019). Intergenerational effect of parental spatial training on offspring learning: evidence for sex differences in memory function. *Brain research bulletin, 153*, 314-323.

Riyahi, J., Taslimi, Z., Gelfo, F., Petrosini, L., & Haghparast, A. (2024). Trans-generational Effects of Parental Exposure to Drugs of Abuse on Offspring Memory Functions. *Neuroscience & Biobehavioral Reviews*, 105644.

Sarnyai, Z., Sibille, E. L., Pavlides, C., Fenster, R. J., McEwen, B. S., & Toth, M. (2000). Functional Abnormalities in the Hippocampus and Impaired Hippocampal-dependent Learning in Mice Lacking the 5HT~ 1~ A Receptors. *CNS Drug Reviews, 6*, 40-41.

Sierksma, A., Van Den Hove, D., Pfau, F., Philippens, M., Bruno, O., Fedele, E., . . . Prickaerts, J. (2014). Improvement of spatial memory function in APPswe/PS1dE9 mice after chronic inhibition of phosphodiesterase type 4D. *Neuropharmacology, 77*, 120-130.

Siklenka, K., Erkek, S., Godmann, M., Lambrot, R., McGraw, S., Lafleur, C., . . . Hallett, M. (2015). Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. *Science, 350*(6261), aab2006.

Starkes, J. L., Deakin, J. M., Allard, F., Hodges, N. J., & Hayes, A. (2014). Deliberate practice in sports: What is it anyway? In *The road to excellence* (pp. 81-106): Psychology Press.

Tonegawa, S., Pignatelli, M., Roy, D. S., & Ryan, T. J. (2015). Memory engram storage and retrieval. *Current opinion in neurobiology, 35*, 101-109.

Tyler, W. J., Alonso, M., Bramham, C. R., & Pozzo-Miller, L. D. (2002). From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learning & memory, 9*(5), 224-237.

Vithayathil, J., Pucilowska, J., Friel, D., & Landreth, G. E. (2017). C hronic impairment of ERK signaling in glutamatergic neurons of the forebrain does not affect spatial memory retention and LTP in the same manner as acute blockade of the ERK pathway. *Hippocampus, 27*(12), 1239-1249.

Wang, Y., Liu, H., & Sun, Z. (2017). Lamarck rises from his grave: parental environment-induced epigenetic inheritance in model organisms and humans. *Biological Reviews, 92*(4), 2084-2111.

Yarrow, K., Brown, P., & Krakauer, J. W. (2009). Inside the brain of an elite athlete: the neural processes that support high achievement in sports. *Nature Reviews Neuroscience, 10*(8), 585-596.

Yin, M., Wang, W., Sun, J., Liu, S., Liu, X., Niu, Y., . . . Fu, L. (2013). Paternal treadmill exercise enhances spatial learning and memory related to hippocampus among male offspring. *Behavioural brain research, 253*, 297-304.

Zhang, S., Li, X., Wang, Z., Liu, Y., Gao, Y., Tan, L., . . . Wang, X. (2017). Paternal spatial training enhances offspring's cognitive performance and synaptic plasticity in wild-type but not improve memory deficit in Alzheimer's mice. *Scientific reports, 7*(1), 1521.

Figure legends

Figure 1. Summary of the experimental procedures.

Figure 2. Maternal spatial training improved spatial learning and memory in their female offspring. Female offspring of mothers who received spatial training took significantly less time to find the platform than those of mothers who did not (A). Female offspring of spatially trained mothers spent significantly more time in the target quadrant compared to female offspring of non-trained mothers (B). Data are shown as mean ± SEM. *** $p < 0.001$ and * $p < 0.05$.

Figure 3. Maternal spatial training increased the expression levels of BDNF, SYT1, and p-ERK1/2 in the hippocampus of female offspring during spatial learning and memory. Hippocampal expression levels of BDNF (a), p-ERK1/2 (b), and SYT1 (c) were significantly higher in female offspring of spatially trained mothers compared to those of non-spatially trained mothers. Expression levels of BDNF, p-ERK1/2, and SYT1 in the hippocampus measured by Western blotting (d). Data are shown as mean ± SEM. *** $p < 0.001$ and * $p < 0.05$.

Figure 4. Maternal spatial training did not improve working memory or short- and long-term memory in their female offspring. The maternal spatial training did not enhance the working memory (a), short-term memory (b), or long-term memory (c) of their female offspring. Data are presented as mean ± SEM.

Figure 5. Maternal spatial training did not increase the levels of BDNF (a), p-ERK1/2 (b), or

SYT1 (c) in the hippocampus of female offspring tested on tasks (Y-Maze and NOR) that their mothers did not train in. The expression levels of BDNF, p-ERK1/2, and SYT1 in the hippocampus were measured by western blotting (d). Data are shown as mean ± SEM.

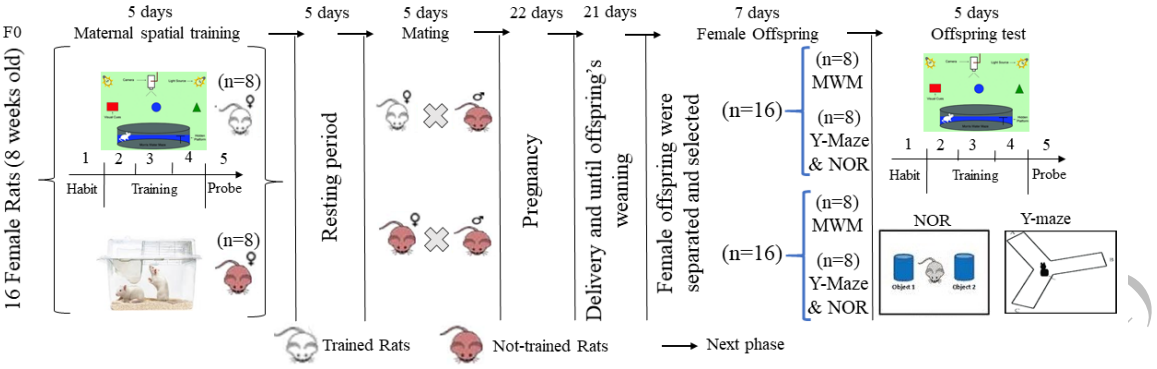

Figure 1

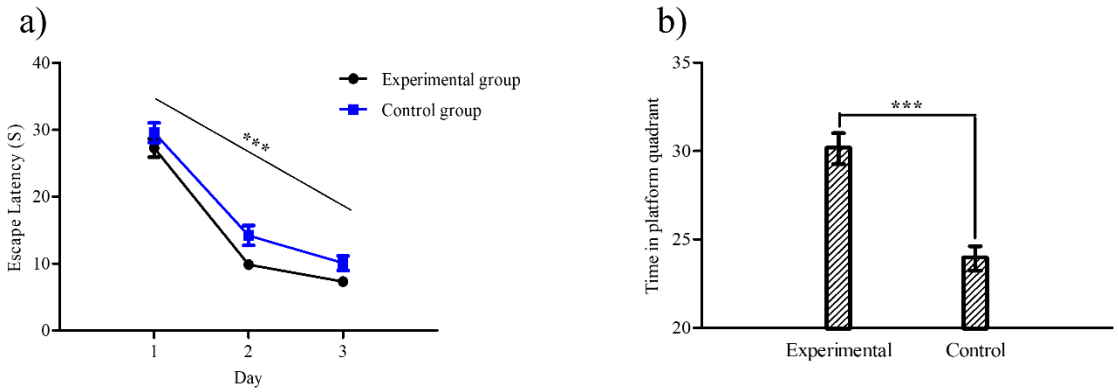

Figure 2

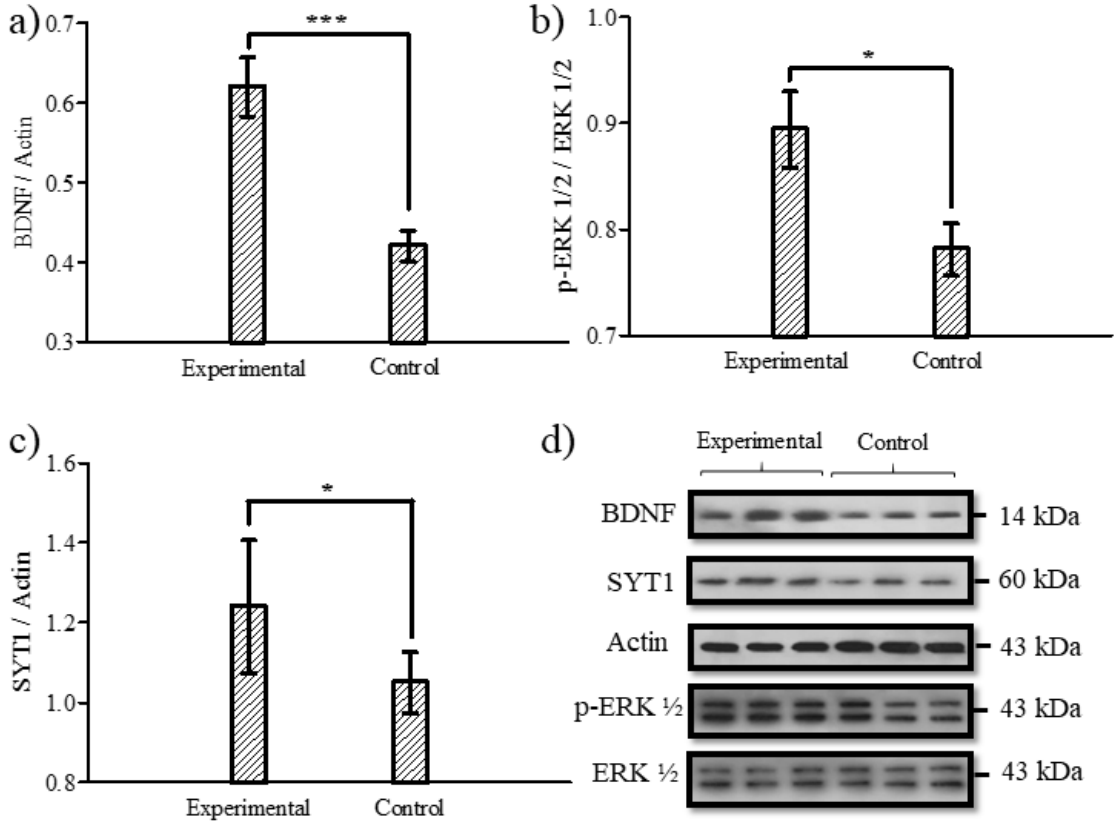

Figure 3

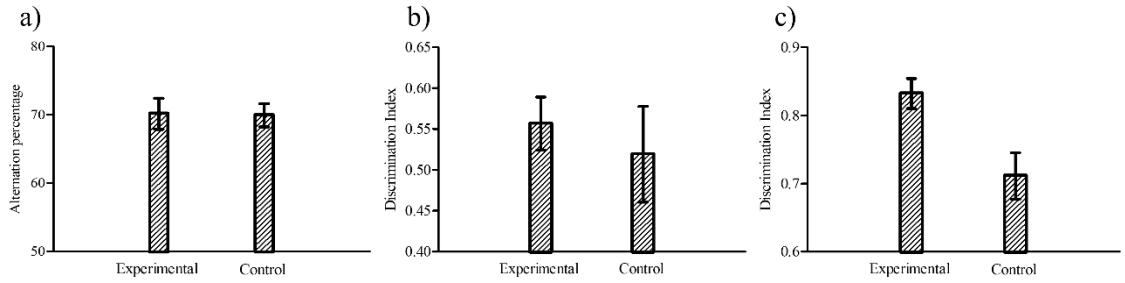

Figure 4

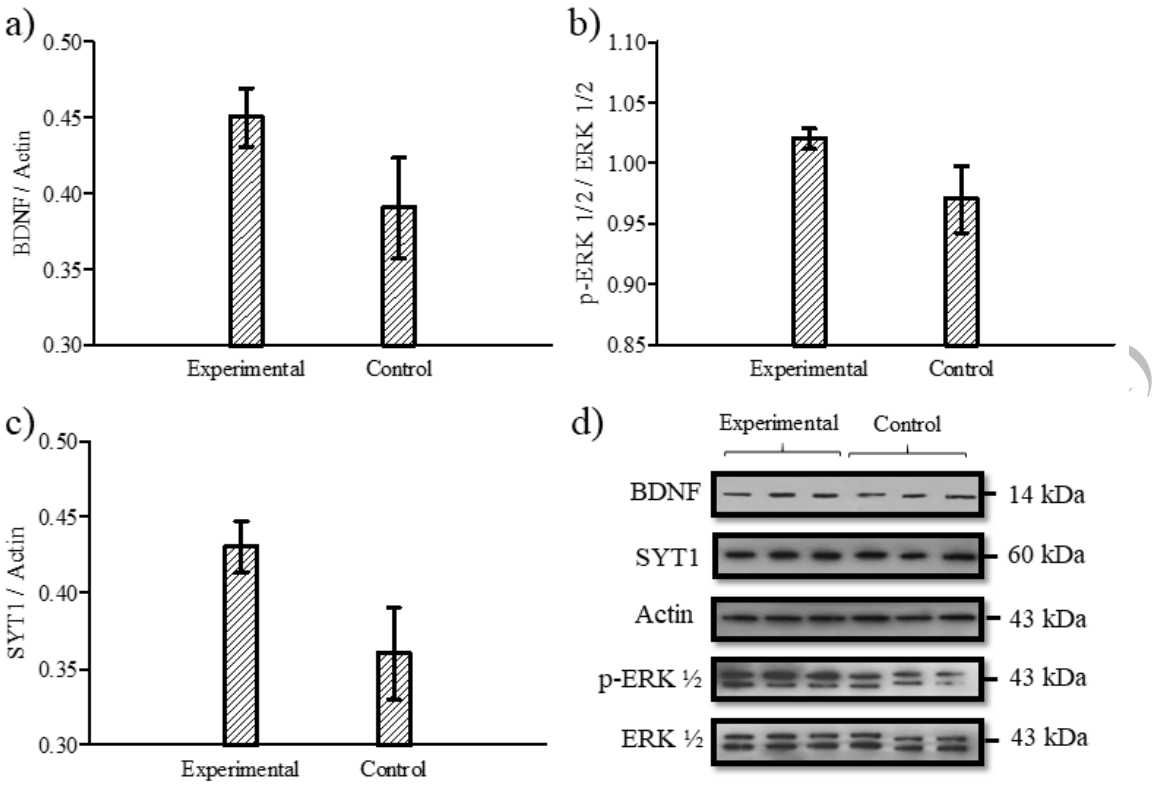

Figure 5