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Maternal Separation Exhibits a Sex Dimorphism in Memory Impairments in Adolescent Rats: Acute Methylphenidate Administration as a Treatment

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ABSTRACT

Introduction: Rodents are highly dependent on maternal care after birth. Disturbing mother and pup interactions leads to detrimental alternations for the rat and the mother. Maternal separation (MS) is an accepted model for investigating disruption of mother and pup relationship. In addition to other detrimental effects, MS is a model known to induce permanent changes in learning and memory. Methylphenidate has been effective in memory enhancement in individuals suffering from memory deficits, attention-deficit hyperactive disorder (ADHD), as well as healthy subjects for better performance in exams.

Material and Methods: In this research, a 21-day separation for 3 h was implemented, and the effects of MS on spatial and passive avoidance learning, and memory were evaluated in the mid-adolescence period of rats, in both males and females. Also, a drug intervention of a high therapeutic dose of 5 mg per kg was used in a five-day period in different control and MS groups. Morris water maze was utilized for spatial learning and memory analysis, and a shuttle box paradigm was used for passive avoidance learning and memory.

Results: Through our behavioral tests, we have shown that MS can alter spatial learning and memory in males. On the other hand, females are protected from the detrimental effects of MS on spatial learning and memory. Furthermore, passive avoidance learning was not different among groups, be it male or female. However, in the case of memory evaluation in the passive avoidance test, the male did not exhibit a significant difference in step-through latency. However, maternally separated females had poor performance in the memory phase with shorter step-through latencies.

Conclusion: Methylphenidate compensated for the deleterious effects of MS on learning and spatial memory for the male group and passive avoidance memory in the female group at the behavioral level.

1 | Introduction

In some countries' maternity wards, neonates are deprived of mother-infant contact in the early stages of birth; however, skin contact is crucial for both mother and baby (Vetulani 2013). An animal model looking into early life mother–pup interactions and its effects in later life are maternal separation (MS). MS has been known as a valid model of early life stress. This stress is a result of environmental manipulations as well as lack of mother–pup interactions. The history of this animal model goes back to1950s and mid-1970s (Lehmann and Feldon 2000). Perturbing mother–pup interactions, as a valid model of depression for the

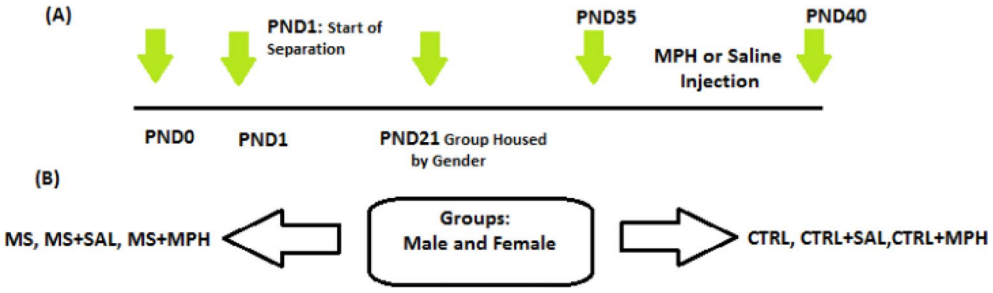

FIGURE 1 | (A) graphical design of the experimental protocol. (B) Graphical design of grouping.

mother (von Poser Toigo et al. 2012) and the offspring (Roque et al. 2014), has been used to shed light on some neurological disorders in rodents. Also MS has been accepted as a valid model to study different childhood experience that may lead to psychiatric illnesses later in life (Sousa et al. 2014).

Hippocampal development happens in postnatal day 1 (PND1) until PND21. The SHRP (stress hyporesponsive period) is PND4–PND14. In this period, normal development depends on the absence of stress. Stress during this period can lead to alternation in development (Aisa et al. 2009). This period is the interval for mossy fiber outgrowth and neurogenesis in the hippocampus, crucial for learning and memory (Huot et al. 2002). Also, PND1–PND21 is the interval when hippocampus granule cells extend their axons (Couto et al. 2012).

Research has revealed memory impairments associated with MS (Huot et al. 2002; Aisa et al. 2007; Cao et al. 2014; Sousa et al. 2014). MS leads to a decline in granule neurons of the dentate gyrus (DG) (Gondré-Lewis et al. 2016). DG in rat's hippocampus is a crucial location for memory-related processing regarding limbic responses. Also, the induced impairments in MS continue to span to adulthood and old age (Sousa et al. 2014). Maternally separated male rats exhibited memory impairments whereas females exhibited more anhedonia in a two-hit model of developmental stress (Hill et al. 2014). Also, MS can make male rats more susceptible to memory impairments when exposed to other stressors later in adulthood (Diehl, Alvares, and Noschang 2011).

Not only is the hippocampus affected by MS but also the whole limbic system can be altered by MS. The limbic system consists of the hypothalamus, amygdala, hippocampus, septal nuclei, and the anterior cingulate gyrus, and it is a component of the reward system. MS alters the HPA axis (Vetulani 2013) dopamine receptor density (Ploj, Roman, and Nylander 2003), and it leads to reductions in dopamine-like neurons in the ventral tegmental area (VTA) and a higher number of neurons in the amygdala. Hence, it was revealed that MS can change neuronal balance (Gondré-Lewis et al. 2016). MS can alter many brain factors in rats, and one needs to investigate the methods to reverse the deleterious effects of MS. For this purpose, some choose drug interventions.

Methylphenidate is a well-known drug used to treat patients with attention-deficit hyperactive disorder (ADHD). The mechanism of methylphenidate has been well shown in research. Briefly, it blocks dopamine, norepinephrine, and serotonin transporters. Hence, it leads to increasing these transmitters in extracellular space in the striatum and prefrontal cortex for dopamine and altering monoamine transmission in nucleus accumbens, temporal cortex including hippocampus, cerebellum, and amygdala (Gray et al. 2007). Some healthy subjects also use methylphenidate for cognitive enhancements and a study has shown that these enhancements are due to feeling of wellbeing in human subjects (Batistela et al. 2016). Acute ip administration of methylphenidate facilitated learning performance which was dependent on D1 type receptor (Tye et al. 2010). Furthermore, methylphenidate administration managed to improve spatial memory (Carmack, Block, et al. 2014). Some doses of methylphenidate managed to enhance long-term memory (Carmack, Howell, et al. 2014). Previous studies have shown that MS can be detrimental to memory and since methylphenidate enhances learning and memory it may help alleviate the harmful effects of MS on memory. We intended to investigate whether MS has detrimental effects on spatial and passive avoidance learning and memory in adolescent rats in both sexes. We tested these memories through Morris water maze (MWM) for evaluating the spatial aspect of learning and memory and shuttle box for passive avoidance learning and memory. We then investigated whether an acute dose of 5 mg per kg in a 5-day period of injection can restore the memory of MS rats.

2 | Materials and Methods

2.1 | Rats and Housing

Virgin female Wistar rats were purchased from animal farm, Kerman University of Medical Sciences, Kerman, Iran. Upon arrival, the rats were acclimatized to the vivarium for 2 weeks. Two females were caged with one male and each female was put in a separate cage after the pregnancy was confirmed by sperm in vaginal lavage. All the cages were provided with fresh wood chip bedding with ad-libitum access to chow and water. The housing condition was set to a 12-h light–dark cycle under controlled temperature of 23°C ± 1°C. The protocols were carried out in strict compliance with the guidelines approved by the ethics committee of the Kerman Neuroscience Research Center (ethics code: 97000028).

2.2 | MS Protocol

The pregnant rats were checked daily for delivery and the day of birth was considered PND0. During the time interval of PND1

until PND21, all the litters were separated from their mothers for 3 h in the morning between 8:00 am and 11:00 am. The mothers were kept in the home cage while the litters were placed in the incubator in separate cages with fresh wood chip bedding. The incubator's temperature was set to 33°C–35°C.

On PND21, all the pups were segregated from the mother, and PND21 was the last weaning day.

All the testing protocols were carried out in mid-adolescence which was PND34–PND46 (Lynn and Brown 2009). The injections started on PND35 and ended on PND39 for 5 days. MWM was performed on PND40, and a passive avoidance test was performed on PND40 for learning and PND41 for memory. A graphical representation is seen in Figure 1 for a general view of the experimental protocol.

2.3 | Study Design

One rat was chosen per mother, and all the other pups were used for other projects. A total number of 84 males and 84 females were used in this study. The number of rats chosen for the test in each group was 7. The number of animals was kept at a minimum and the MWM, and the passive avoidance test had different experimental groups.

The tests were designed with 6 experimental groups. All the animals that experienced standard housing from birth until PND21 and were not disturbed were considered a control group (CTRL). The MS group underwent the MS protocol (MS). The control and MS groups that experienced 5 days of 5 mg per kg of methylphenidate were CTRL + MPH and MS + MPH, respectively. Also, vehicle groups were designed as CTRL + SAL and MS + SAL, which received saline.

2.4 | Drugs

A dose of 5 mg/kg of methylphenidate was administered ip for five consecutive days. The drug was dissolved in 0.5 cc saline for ip injection.

3 | Behavioral Tests

For memory assessment, two memory tests of the shuttle box and MWM were utilized to assess spatial and amygdala-related memory. Behavioral tests were performed between 8 am and 1 pm.

3.1 | Morris Water Maze

MWM is a black circular pool (160 cm in diameter and 80 cm in height filled to a depth of 40 cm), designed to assess spatial learning and memory in rodents. This behavioral test was designed by Richard Morris in 1984 (Morris 1984). The pool consists of four quadrants. A geographical direction is designated for each quadrant, that is, north, south, east, and west. A platform was submerged centrally beneath the water in the northeast quadrant. The distance between the platform surface and the surface of the water was 2 cm. The platform was not visible from underneath the water since the pool and platform were covered with non-toxic black paint. The room temperature was set to 25°C ± 2°C. Also, the room was dimly lit with geometric visual cues attached to the walls surrounding the pool. The water temperature was kept at room temperature.

MWM experimental design consists of two phases. The first phase is the learning phase, and the second phase is the memory phase.

The learning phase includes three blocks separated with a 30-min interval. Each block is made of 4 trials, and each trial lasts 60 s with 60 s resting interval. On each trial, the animal was released from one of the geographical locations each time and it swam to find the hidden platform. If the rat failed to find the platform within 60 s, it was guided toward the platform. The animal was given 20–30 s to stand on the platform, and then, it was given 30 s to rest before the start of the next trial (Rajizadeh et al. 2018). In this phase, the distance moved (cm) and average escape latency (s) in each block were recorded as indicators of learning. The distance moved was calculated as the average of traversed distance in four trials of each block.

The memory phase started 2 h after the last learning block. In this phase, the platform was removed, and all the rats were released once from the same geographical location. The time percentage and the distance percentage in the target quadrant were considered a measure of memory retention (Rajizadeh et al. 2018). All the data were recorded using a tracking system Ethovision (Noldus Ethovision system, version 7, the Netherlands).

3.2 | Passive Avoidance Task (Shuttle Box)

The passive avoidance test is designed to evaluate associative learning and memory in rodents. In this test, the animal learns to suppress the innate tendency for the dark environment and avoid the dark environment associated with an aversive shock stimulus. The apparatus had dimensions of 100 [L] × 25 [W] × 25 [H] (cm) and was composed of two equal compartments. The two compartments were separated with a door and one compartment had a dark environment with wire meshes embedded on the floor, whereas the other had a light environment with similar wiring as the dark compartment.

The apparatus was able to deliver shocks in the dark compartments. The test was composed of three phases known to be habituation, learning, and memory.

In the habituation phase, the animal was placed in the light compartment. Ten seconds later, the door was opened and the rat's latency to enter the dark compartment was measured. If the rat did not enter the dark compartment in a two-minute period, it was excluded from the study.

Two hours after the habituation phase, the learning phase started. In the learning phase, the rat was placed in the light compartment, and after 10 s, the experimenter opened the door.

As soon as the rat entered the dark compartment, the experimenter closed the door and the rat received a shock (0.5 mA, 2 s) through the wires on the surface of the apparatus. The rat remained in the dark compartment for 20 s and then it was removed from the dark compartment. The waiting time between two learning sessions was 2 min for each rat. In the learning session, if the rat remained in the light compartment for a period of 2 min after door opening, the learning criteria were fulfilled.

The memory test was performed 24 h after the learning phase. Like the habituation and learning phases, the rat was put in the light compartment and the door opened after 10 s.

The latency for entering the dark compartment was measured, and it is known as step-through latency (STL). The time to enter the compartment after door opening was measured and the cut-off time was 5 min. The data are reported in seconds.

4 | Data Analysis

All the data were analyzed using GraphPad Prism 8. First, all three blocks of learning were analyzed to check the normality of data. The analyzed parameters were the average traversed distance in each block and average escape latency in each block. After confirmation of normality, two-way ANOVA with repeated measure or mixed model was used to analyze the learning data. $p = 0.05$ was considered statistically significant. For the memory phase of MWM, all the data were checked for normality, and in the case of normality of respective data sets, one-way ANOVA and Tukey's test were utilized. When the data were not normal, Kruskal–Wallis test with Dunn's post hoc test was performed.

For the passive avoidance test, the Kruskal–Wallis test was used to analyze both learning and memory combined with Dunn's post hoc test.

5 | Results

5.1 | Learning Phase of MWM

In the male and female groups during the learning phase, average escape latency in each block and distance moved in each block were analyzed as indicators of learning.

Regarding the escape latency in the male and female groups, in Block 1, no significant differences were observed among groups within their sex. Figure 2A shows female data and Figure 2B shows male data.

In Block 2 of the female group, the CTRL and CTRL + SAL and CTRL + MPH were not significantly different. No significant difference was seen between CTRL, MS, MS + SAL. and MS + MPH. However, our data exhibited a difference between MS + SAL and CTRL + MPH with $p < 0.05$. In Block 3 for the female group, none of the groups exhibited a difference from the control group. Results are summarized in Figure 2A.

While analyzing the escape latency in males, differences were observed among treatment groups.

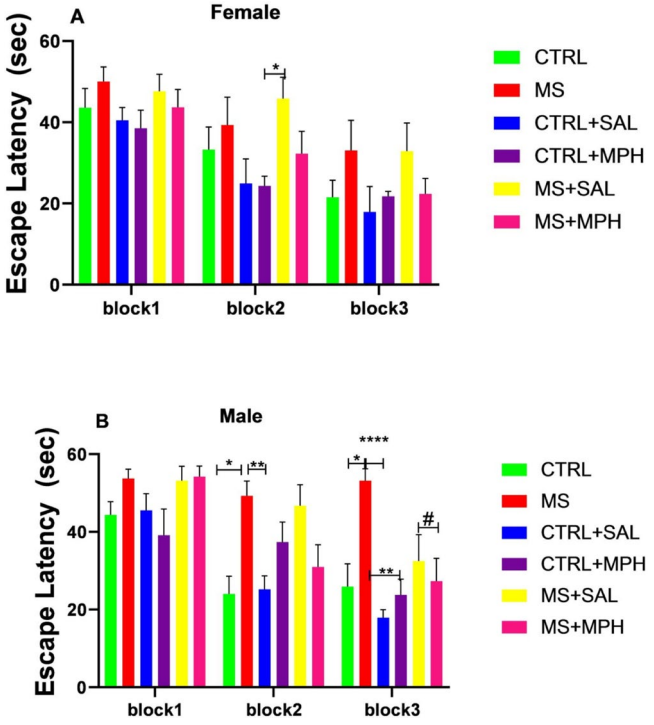

FIGURE 2 | MWM escape latency: (A) average escape latency in the female group in each block, (B) average escape latency in the male group in each block. $p \leq 0.05$ was considered significant. (*) indicates $p < 0.05$ versus control also (**) $p < 0.01$ versus CTRL, (****) $p < 0.0001$ versus CTRL and (#) $p < 0.05$ versus MS. Data depicted as mean ± SEM.

In the male group, a significant difference was seen in the second block between CTRL and MS ($p < 0.05$) as well as CTRL+SAL and MS ($p < 0.01$). In addition, there was no significant difference between CTRL and CTRL + SAL and CTRL + MPH. Also, the CTRL group showed no difference with MS + MPH. Furthermore, our data did not show any significant difference between MS and MS + SAL.

In Block 3 of the learning phase for the males, no significant difference was observed between CTRL and CTRL + SAL and CTRL and CTRL + MPH. Discernable differences were seen for CTRL and MS ($p < 0.05$) and CTRL + SAL and MS ($p < 0.0001$). In addition, a difference was seen between the MS and CTRL + MPH groups ($p < 0.01$). MS and MS + SAL were not significantly different, and MS and MS + MPH were significantly different with $p < 0.05$, indicative of effective treatment.

Results are summarized in Figure 2B.

Regarding the female group, no significant difference was observed in Blocks 1, 2, or 3 when looking at the distance moved. The data are summarized in Figure 3A.

While analyzing the distance moved in each block for the male groups, a significant difference was not observed between groups in Block 1.

In Block 2 of male groups, there was no difference between CTRL and CTRL + SAL, CTRL and CTRL + MPH. A discernable difference was seen between CTRL and MS ($p < 0.05$) as well as CTRL + SAL and MS ($p < 0.05$). Furthermore, no

significant difference was observed in Block 2 between other groups (MS and MS + SAL, MS and MS + MPH and MS + SAL and MS + MPH) when analyzing the distance moved.

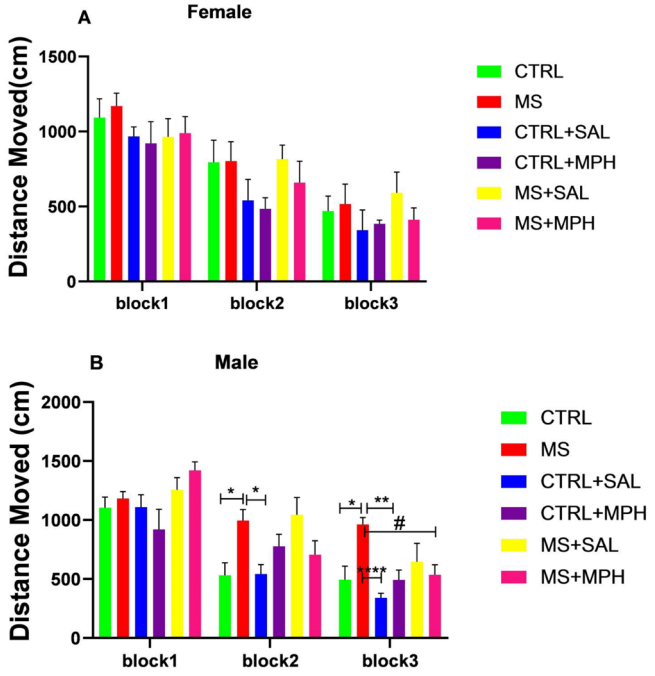

FIGURE 3 | MWM: Distance moved (A) average distance moved in the female group in each block, (B) average distance moved in the male group in each block. $p \leq 0.05$ was considered significant. (*) indicates $p < 0.05$ versus CTRL and () $p < 0.05$ versus MS. Also (**) $p < 0.01$ versus CTRL, (***), $p < 0.001$ versus CTRL, (****) $p < 0.0001$ versus CTRL. Data depicted as mean ± SEM.

In Block 3, CTRL and CTRL + SAL and CTRL + MPH were not significantly different. There was a significant difference between CTRL and MS in the distance moved ($p < 0.05$) as well as MS and CTRL + MPH ($p < 0.01$). MS and CTRL + SAL were also significantly different with $p < 0.001$.

In Block 3 of male groups, MS and MS + MPH showed a significant difference in the distance moved ($p < 0.05$); however, there was no significant difference between MS and MS + SAL. A summary of data can be seen in Figure 3B.

5.2 | Memory Phase of MWM

For the distance percentage in the target quadrant in the female case, the data were not normal, so the Kruskal–Wallis test was performed. No significant difference was observed for the distance percentage in the target quadrant. Data are summarized in Figure 4A.

In the case of distance percentage in the target quadrant for the male group, the data satisfied the normality criteria. Hence, one-way ANOVA was performed. CTRL + SAL and CTRL + MPH were not significantly different from the CTRL group. A significant difference was observed between MS and CTRL with $p < 0.01$. MS and CTRL + SAL showed a discernible difference with the p value 0.05 and CTRL + SAL traveled more distance in the target quadrant. In addition, the MS and CTRL + MPH groups were significantly different ($p < 0.01$). MS and MS + MPH groups were significantly different in the memory phase with $p < 0.05$. No discernible difference was seen between MS and MS + SAL. The data are depicted in Figure 4B.

The time percentage spent in the target quadrant for the female group was proved to follow a normal distribution. One-way

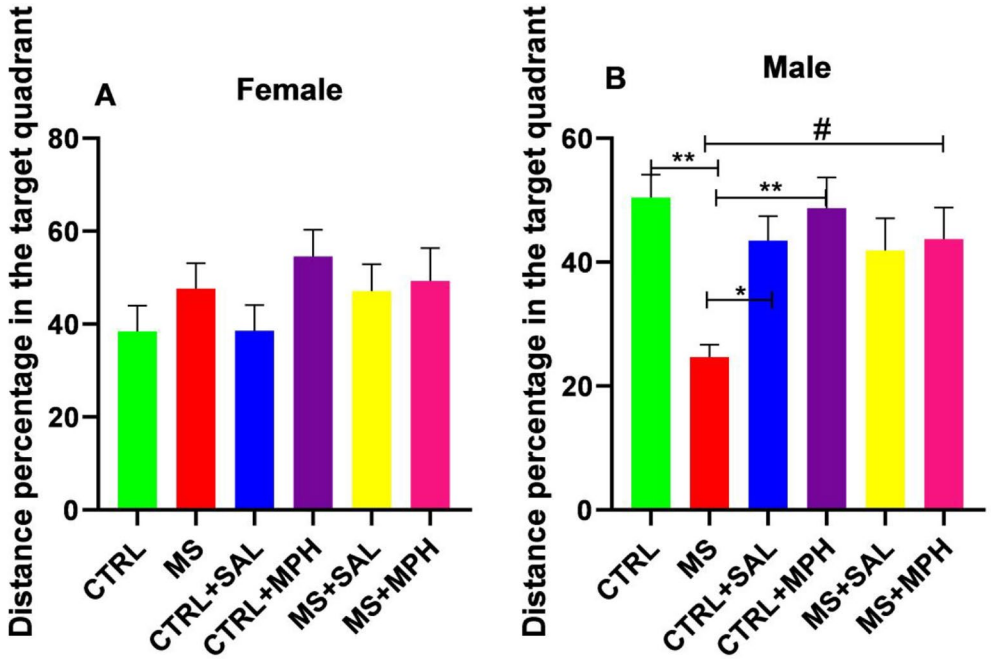

FIGURE 4 | Distance percentage inthe target quadrant for female (A) and male (B). (*) indicates $p < 0.05$ versus CTRL and () $p < 0.05$ versus MS. Also (**) $p < 0.01$ versus CTRL, Data depicted as mean ± SEM.

ANOVA did not show any significant difference among groups. For graphical demonstrations see Figure 5A.

For the time percentage spent in the target quadrant, the male group data were normal and one-way ANOVA with Tukey's post hoc test was performed. The CTRL group was not significantly different from CTRL + SAL and CTRL + MPH.

MS and CTRL groups were significantly different with $p < 0.01$. In addition, MS and MS + SAL were not significantly different. MS and MS + MPH showed a significant difference with $p < 0.05$, indicative of effective treatment. In addition, MS and CTRL + MPH were significantly different ($p < 0.01$). The data are depicted in Figure 5B.

Females were protected from the deleterious effects of MS in MWM and MS males showed impairments restored with methylphenidate in the MWM task.

5.3 | Passive Avoidance Task

For the passive avoidance task, we tested the aversive learning and memory. The Kruskal–Wallis test with Dunn's post hoc tests was used to analyze the data.

When female learning data were analyzed (the number of shocks received until avoiding the dark compartment for more than 2 min), no significant difference was observed among female groups ($p > 0.05$.). Analyzing the male learning phase data (number of shocks) showed no significant difference ($p > 0.05$) among male groups Figure 5A,B.

Also, no significant difference was seen in the memory phase (STL) for the male experimental groups ($p > 0.05$).

However, in the memory phase in female rats, no difference was observed among CTRL and CTRL + SAL as well as CTRL and CTRL + MPH. MS and CTRL were significantly different with $p < 0.05$. Furthermore, MS + SAL and CTRL were significantly different with $p < 0.01$. MS + SAL and MS + MPH were also significantly different ($p < 0.05$). On the other hand, no discernable difference was observed between MS and MS + SAL (see Figure 6A,B). Males did not show impairments among their sex group; however, females showed impairments that can be partially compensated with methylphenidate in the passive avoidance task (Figure 7).

6 | Discussion

Our results have indicated that MS male rats are prone to learning and memory impairments in spatial memory, whereas MS female rats are protected from spatial learning and memory impairments in their sex group. Furthermore, we have observed that the deleterious effects of MS can be partially ameliorated through 5 days of administration of methylphenidate in a high therapeutic dose of 5 mg per kg. The data did not reveal any significant difference in the passive avoidance learning phase in both male and female groups.

In addition, our findings revealed that there is no significant difference between all male groups for the passive avoidance memory task. On the other hand, in our study, MS has made adolescent females more vulnerable to impairments in passive avoidance memory retrieval and methylphenidate has managed

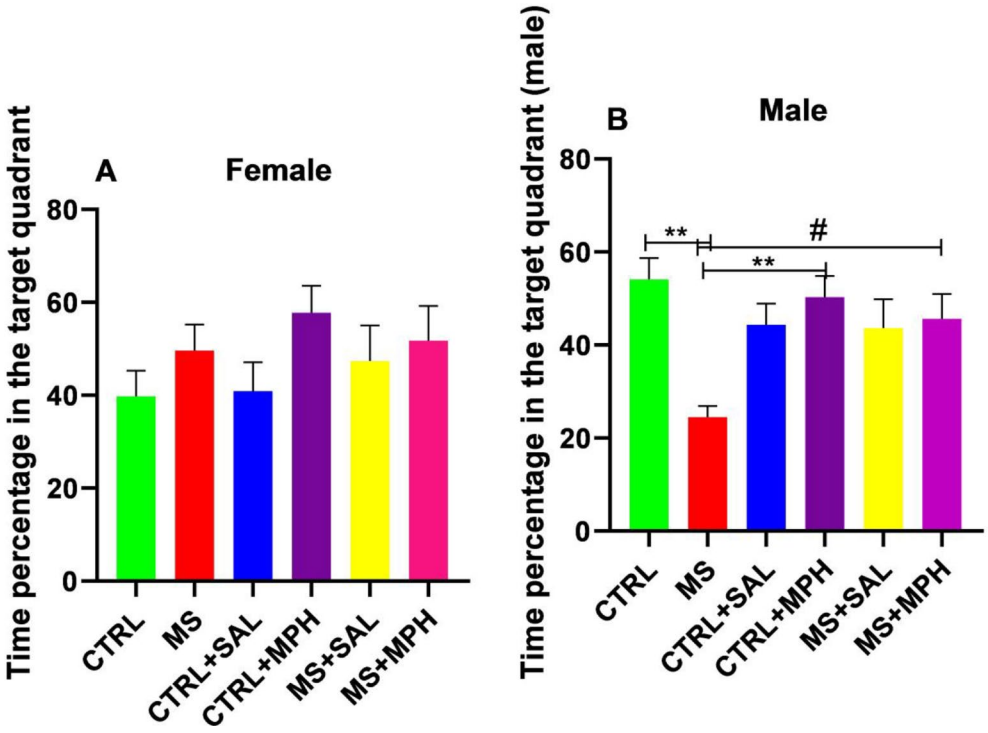

FIGURE 5 | Time percentage in the target quadrant for female (A) and male (B). (**) $p < 0.01$ versus CTRL and (#) $p < 0.05$ versus MS. Data depicted as mean ± SEM.

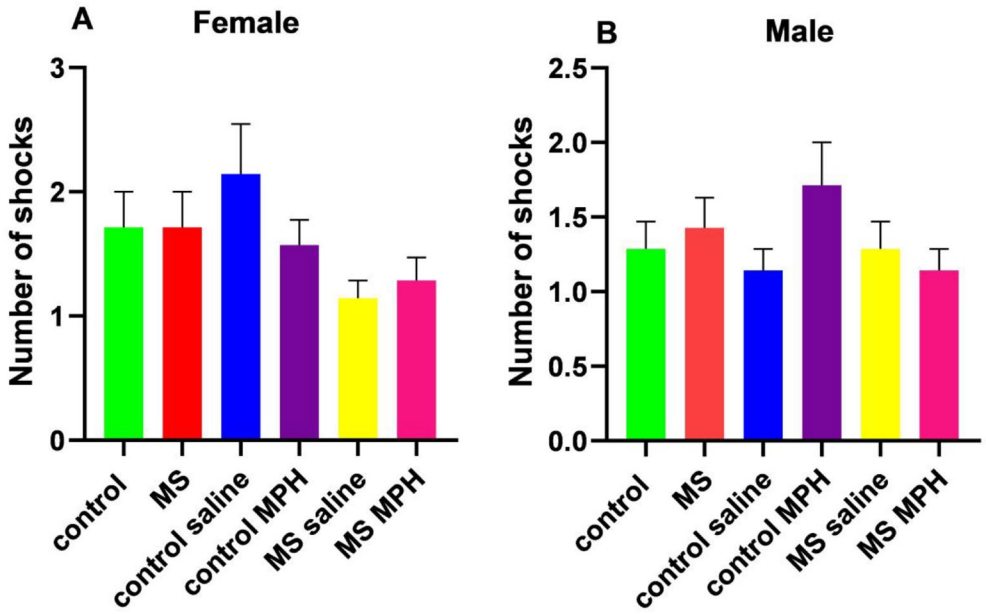

FIGURE 6 | Number of shocks in the learning phase of passive avoidance (A) female, (B) male.

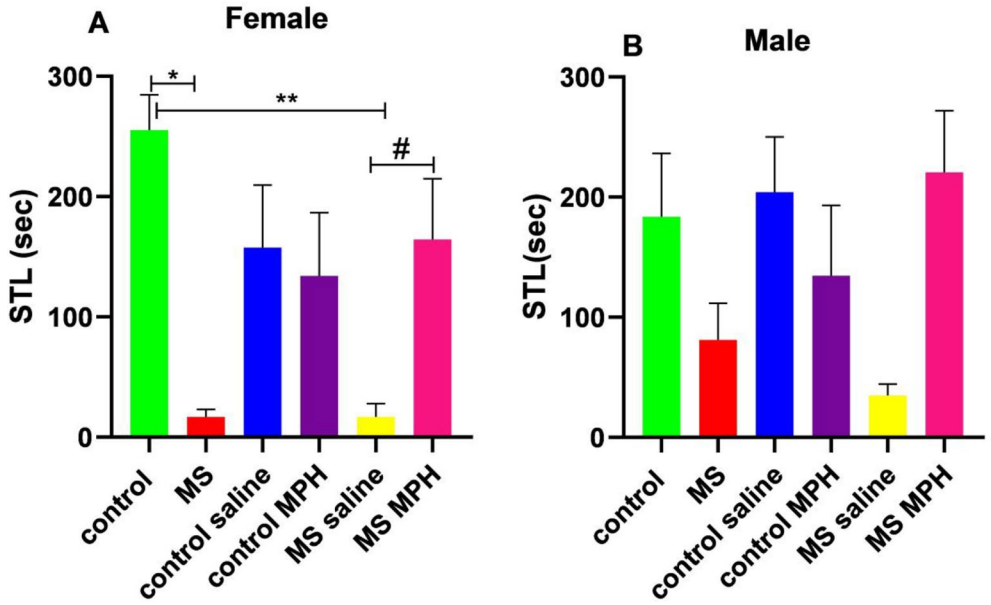

FIGURE 7 | STL for both female (A) and male (B) groups. (*) indicates $p < 0.05$ versus CTRL. Also (**) $p < 0.01$ versus CTRL. (#) $p < 0.05$ versus MS. Data depicted as mean ± SEM.

to partially ameliorate the deleterious effects of MS on passive avoidance memory.

Previous research has revealed that MS can induce spatial memory impairments in males (Huot et al. 2002; Aisa et al. 2007; Hui et al. 2011; Wang et al. 2014; Sousa et al. 2014), and these impairments have been attributed to modifications in hippocampal development due to early life stress and an increase in corticosterone concentrations (Huot et al. 2002). MS can also affect other aspects of cognitive function one known to be recognition memory (Hulshof et al. 2011). Some studies have claimed that memory impairments may be due to elevated secretion of glucocorticoids in the MS model (Aisa et al. 2007). Considering all these impairments, we tried to use a drug known to enhance memory. In line with our findings, research has also shown that a dose of 10 mg per kg methylphenidate successfully improved spatial learning and memory in a 15-day protocol (Carmack, Block, et al. 2014). To our knowledge, this is the first study investigating the impact of acute methylphenidate administration on memory improvement in MS rats with a 5-day protocol, and this protocol managed to ameliorate the deleterious effects of MS on spatial learning and memory at the behavioral level. In addition, a dose of 5 mg per kg showed a better performance compared to other doses for passive avoidance task, and this dose is considered a high therapeutic dose (Gray et al. 2007; Arzi et al. 2014). Research has shown that MS can be detrimental, to declining neurogenesis. Furthermore, 85% of neurogenesis happens in DG in

SHRP and neurogenesis starts in SHRP and extends to adulthood (Cajero et al. 2012). However, one study showed that rats receiving a 5 mg per kg dose of methylphenidate showed better cognition without altering neuronal balance (Ravichandran et al. 2015).

One might need to investigate the mechanisms involved in ameliorating the deleterious effects of MS through methylphenidate administration. This improvement in learning and memory in MWM could be explained by neurogenesis observed after methylphenidate exposure (Oakes et al. 2019). However, some studies did not observe any changes in neuronal population (Gray et al. 2007).

One human study stated that when cognitive processes are below an optimal level, methylphenidate has been effective (Batistela et al. 2016).

It is said that the dorsal hippocampus is crucial for spatial learning through place cells. Dopamine is released from LC and VTA to the dorsal hippocampus (Arzi et al. 2014; McNamara and Dupret 2017). Studies have revealed the importance of neurotransmitter dopamine for stabilizing hippocampus-dependent memory. Blockade of D1/D5 dopaminergic receptors during spatial learning impairs memory (McNamara et al. 2014). In addition, Ploj et al. have shown dopamine receptor binding disturbance in MS protocol (Ploj, Roman, and Nylander 2003). Methylphenidate might act as a modulator and ameliorate these disturbances and improve impaired spatial memory.

DAT has a role in dopamine clearance from the extracellular space. Methylphenidate may act as a modulator agent on DAT transporters by inhibiting DAT reuptake. Hence, further investigations are required to see the effects of methylphenidate on dorsal hippocampal cells with a short-term protocol of 5 days. Also, some studies have mentioned changes in serotonin concentrations in MS rats (Xue et al. 2013) and one needs to delve into the impact of serotonin and dopamine in learning and memory in MS rats. In addition, the role of methylphenidate in the interaction of these two systems needs further investigation.

The data in the passive avoidance memory phase for the male groups are in line with Banqueri et al. In their research MS with a 21-day protocol showed no significant difference in memory retention for control and MS in adolescence for the passive avoidance paradigm in the male (Banqueri, Méndez, and Arias 2017).

On the other hand for the differences observed in females in the passive avoidance memory retention phase, one study has mentioned a sexual dimorphism for passive avoidance behavior in male and female rats, and females have shown more entrance to the dark compartment in the memory phase (van Haaren and van de Poll 1984). In line with our findings, one research has also observed a shorter latency for female group of MS mice with a difference between sexes. In that research, it has indicated that MS females might be more vulnerable when analyzing passive avoidance retention with their relative controls and MS males in adolescence (Gracia-Rubio et al. 2016). In addition, one research has claimed that as age increases, passive avoidance retention increases and in our study the rats were in their adolescence (Ganella and Kim 2014). Our findings show that female MS memory impairments can be partially ameliorated through our proposed protocol.

In addition, the amygdala is the target for regulation of learning, memory as well as fear-related behavior. Neurotensin is a peptide, known to improve passive avoidance memory and its enhancing effects on passive avoidance learning could be due to the modulation of the mesolimbic DA system (László et al. 2012). Since methylphenidate is known to modulate DA, we need to investigate the effects of sex hormones on neurotensin and dopamine interactions. We have observed declined performance for the MS group in a sex and task-dependent manner ameliorated by methylphenidate.

Some studies have mentioned the role of sex hormones in sexual differentiation of neural circuits (Cowan and Richardson 2019). Hence, further investigations are required to investigate the role of specific sexual hormones in mnemonic processes.

The presence of higher levels of testosterone hormone might be a reason for better memory retention in male rats in the passive avoidance memory since testosterone has a protective role for mnemonic processes in avoidance learning in young rats (Schneider-Rivas et al. 2007).

To untangle the enigma behind such dimorphisms and sex-dependent memory disturbances, further investigations are required on the dopamine circuits in the hippocampus, amygdala, and their interactions.

7 | Conclusion

In this research, we have investigated whether MS can negatively affect spatial learning and memory and passive avoidance memory in both male and female in adolescence. Our research has indicated that the male sex is prone to spatial learning and memory impairments and the females were not affected in this type of learning and memory. On the other hand, the females are susceptible to deleterious effects of MS in passive avoidance memory, whereas males were unaffected in this type of memory.

Methylphenidate at a dose of 5 mg per kg ameliorated the negative impact of MS after 5 days of exposure. This effect was observed in both groups with relative memory impairment. Our results have revealed that short-term exposure to a high therapeutic dose of methylphenidate will reduce the negative effects of MS.

Author Contributions

Fatemeh Mohtashami Borzadaran: contributed to sample preparation, performed the experiment, and wrote the manuscript with input from all authors. **Soheila Rezakhani:** contributed to sample preparation and consulted the analytic calculations. **Reyhaneh Kamali:** contributed to sample preparation. **Khadijeh Esmaeilpour:** project supervision, contribution to data analysis, experiment design, model

derivation, and data analysis. All authors made contributions to the final version of the manuscript. All authors provided feedback on every stage of the project and helped shape and develop the research and manuscript. All authors proved that all data was generated in-house, and no paper mill was used in this article.

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Ethics Statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The protocols were carried out in strict compliance with the guidelines approved by the ethics committee of the Kerman Neuroscience Research Center (ethics code: 97000028).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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