Maternal Separation Impairs Mother's Cognition One Month Beyond The Separation

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Abstract

Background: Separation of pups from their dam during the infancy not only has detrimental effects on the pups' brain but also affects dams' behavior. Postpartum stress may affect mothers' cognitive functions. In the present study, by using a 180 min/day maternal separation (MS) paradigm, we assessed anxiety-like behavior (in the elevated plus maze) and locomotor activity (in the open field) of rat dams which experienced separation from postnatal day (PND) 1 until weaning. Since no study has ever investigated MS effects on spatial learning and memory of dams in the Morris water maze, we tried for the first time to clarify whether such separation impairs dam's performance in the Morris water maze.

Methods: We assessed dams' behavior at three time points; 24 hours, 1 week and 1 month after weaning. Therefore, the six groups consisted of control (CTRL); 24h, 1week and 1month and MS; 24h, 1week and 1month was allocated in this study.

Results: Our results revealed that although MS-24h and MS-1week groups had intact locomotor activity, MS-1month group showed less locomotor activity in the open field. Moreover, MS induced anxiety-like behavior was more pronounced in MS-1week and MS-1month dams. Spatial learning and memory was also impaired only in MS-1month dams.

Conclusions: We can conclude that MS induces cognitive impairments in dam that may appear not immediately after the separation, but a few weeks after such stressful event.

Background

Maternal separation (MS) is an extensively used model to study the effects of early-life adversity in laboratory animals. In MS studies, pups are separated from the dam during the first postnatal days of life. MS is associated with disruptions in normal development of hypothalamic pituitary adrenal (HPA) axis (Doreste-Mendez, Ríos-Ruiz, Rivera-López, Gutierrez, & Torres-Reveron, 2019). The literature on the field suggests that MS in critical periods of brain development affects some aspects of neuroplasticity and may lead to behavioral and cognitive malfunctioning such as spatial (Bárbara Aisa, Tordera, Lasheras, Del Río, & Ramírez, 2007; Akillioglu, Yilmaz, Boga, Binokay, & Kocaturk-Sel, 2015; Batalha et al., 2013; Cao et al., 2014; Frankola et al., 2010; Huot, Plotsky, Lenox, & McNamara, 2002; Yang, Li, Han, & Zhu, 2017) and non-spatial (Bárbara Aisa et al., 2007; Banqueri, Mendez, & Arias, 2017; Frankola et al., 2010; Hill et al., 2014; Hulshof et al., 2011; Wearick-Silva et al., 2017) learning and memory as well as depressive- or anxiety-like behaviors (Wang, Shao, & Wang, 2015). Hippocampus, an essential structure for spatial learning and memory, is especially vulnerable to early life stress (Cao et al., 2014). Accordingly, stress affects hippocampal LTP, facilitates long-term depression and exposure to glucocorticoids reduces hippocampal LTP (Sousa et al., 2014). However, the data mostly focus on the consequences of MS on the offspring. During early life, mother and infant establish a very close relationship (Reis et al., 2014). Since the mother-pup relationship is dynamic and reciprocal, it is expected that MS would affect both members of the dyad (Alves, Portugal, Summavielle, Barbosa, & Magalhães, 2019). Recently, it has been shown that

MS affects dam's maternal behavior (J. Aguggia, Paola, Magdalena, & Rivarola, 2019; Alves et al., 2019; Burenkova, Averkina, Aleksandrova, & Zarayskaya, 2020) and induces anxiety- and depressive-like behaviors in the dams. However, in the case of anxiety, there is contradictory with the results of the previous studies for the impact of MS on mothers' anxiety (Alves et al., 2019). While some studies have shown that dams were more anxious (J. P. Aguggia, Suárez, & Rivarola, 2013; Bousalham, B, Hessni, Ali, & Mesfioui, 2013; Maghami et al., 2018; Orso et al., 2018), some showed a reduction in the anxiety levels (Kalinichev, Easterling, & Holtzman, 2000).

Mothers submitted to MS protocols showed short- and long-term memory impairments (J. P. Aguggia et al., 2013; Sung et al., 2010). There are only two studies evaluating memory alterations in the mothers after MS, both using the Step-Down Inhibitory Avoidance Task, reporting memory impairments after MS (J. P. Aguggia et al., 2013; Sung et al., 2010).

Most of the previous studies have investigated dam's behavior immediately after weaning. In the present study, we investigated dam's anxiety, locomotor activity and spatial learning and memory in three time points after weaning to see whether MS effects on the dams' behavior would be long-lasting. Moreover, for the first time we studied spatial learning and memory of dams in the Morris water maze since to the best of our knowledge, no study had ever done such assessment.

Materials And Methods

2.1 Animals

Male and female Wistar rats were used in this experiment. Animals were kept under a 12-h light–dark cycle, temperature at 23 ± 2 °C, and had *ad libitum* access to food and water. All experimental procedures were conducted in accordance with guidelines relevant to the care of experimental animals, as approved by the Institutional Animal Research Ethics Committee of Kerman University of Medical Sciences (Ethic code: KNRC-96-63).

2.2 Maternal separation protocol

Pregnant females were kept separately at least three days before parturition. From postnatal day (PND) 1 to PND21, MS litters were separated from dams for 180 min/day, starting at 08:30 AM and ending at 11:30 AM. MS dams were first removed from the home cage, after which pups were moved to a cage and placed in an incubator, which kept the temperature at 30 ± 0.5 °C, and $52 \pm 4\%$ humidity. (Sara Joushi, Esmaeilpour, Masoumi-Ardakani, Esmaeili-Mahani, & Sheibani, 2021) to avoid hypothermia. The dam was kept in a separate room for the duration of the separation to inhibit olfactory or ultrasound vocalization exchanges between dams and their pups. After 3 hours, pups were returned to the home cage followed by dam reunion.

2.3 Experimental Groups

At PND 22, all pups were weaned. The dams were classified in six groups. Control (CTRL) and MS dams were tested at three time points; 24 h, 1 week and 1 month after weaning. Hence, groups consisted of CTRL-24h, CTRL-1week and CTRL-1month, MS-24h, MS-1week and MS-1month. The sample size was $n=7$ per condition. Since in the Morris Water Maze Test (MWM) rats underwent swimming stress condition, this task performed with different set of animals.

2.4 Behavioral assessments

2.4.1 Open field test

The open-field test was used to evaluate exploratory, locomotor activity and anxiety-like behaviors in the dams. Apparatus consists of a cubic arena [90*90*30 cm] surrounded by high walls. The distance moved and the number of rearing and grooming behaviors were used to assess activity and anxiety-like behavior. A video camera at the top of the field recorded the test session and animals' performance was evaluated by Noldus Ethovision system, version 7.1. Number of rearing behavior was recorded by the experimenter (S. Joushi, Esmaeilpour, Taherizadeh, Taheri, & Sheibani, 2020). The apparatus was cleaned with ethanol 70% between each 5 minutes session.

2.4.2 Elevated plus maze (EPM)

The mothers were tested in the elevated plus maze (EPM) to measure anxiety-like behavior. The apparatus consisted of a plus-shaped maze placed 50 cm above the floor with two opposite open arms (50 cm×10 cm) and two closed arms (50 cm × 10 cm with 40 cm walls). At the beginning of the test, each rat was placed onto the central area (10 cm×10 cm) of the maze facing a closed arm and was allowed to explore the maze freely. During 5 min exposure to EPM, the number of entries and the time spent in each arm were video recorded. An entry was defined as the placing of two forepaws into the arm. Subsequently, the time spent in the open and closed arms and the number of entries into each arm was recorded. After each test, the apparatus was cleaned with 70% ethanol to eliminate the remaining odors (Masoumi-Ardakani et al., 2017).

2.4.3 Morris water maze (MWM) test

The maze is a black circular pool (160 cm in diameter and 80 cm in height) that is filled to a depth of 40 cm with water and kept at room temperature. A black square platform (10 cm in diameter) was centered in northeast quadrant of the pool and submersed 1.5 cm into the water so that it was imperceptible at water level. The experiments were done in a dimly lit room with spatial cues attached to the walls around the maze at different points. The animals' performance was recorded by Noldus Ethovision system, version 7.1.

For evaluating learning, the animals were tested in four subsequent days. Each block consisted of four sequential trials each taking 60s (Inter-trial interval=30s). Rats were released randomly in the tank while facing the wall of each quadrant. After each rat found the platform, it was allowed to stay there for 20s followed by relocating to its cage to rest for 10s before starting the next trial. In case a rat did not find the

platform in 60s, it was guided to the platform by the experimenter. The time spent and distance moved to find the hidden platform as well as velocity of movement were analyzed later. A single probe trial was given 2 h after the last training trial to test the short-term spatial memory in the water maze. In this trial the platform was removed and the rat was allowed to swim for 60 seconds. The time and distance spent in the target quadrant were analyzed as a measure of spatial memory retention (Taheri, Sepehri, Sheibani, & Esmaeilpour, 2017; C. V. Vorhees & M. T. Williams, 2006).

Following the probe trial, rats had to complete a visible platform test, so the ability of animals to escape to a visible platform was evaluated while the platform was raised 2 cm above the water and was visible with an aluminum foil. This test was performed to determine any possibility of treatment interference with sensory and motor coordination or motivation in the dams (Taheri et al., 2017).

2.5 Statistical analysis

The data was presented as mean $\pm$ SEM. Parameters were examined for normality with Shapiro-Wilk normality test. As the majority of parameters did not show a normal distribution, non-parametric statistics were used. For the open field, elevated plus maze and probe test in MWM, comparisons between the experimental groups were made by using an Unpaired t test. Repeated measures two-way analysis of variance (ANOVA) was used to compare mean differences between groups in Morris water maze (separation and learning days as the factors). For statistical significance between groups, Tukey's multiple-comparison post test was performed. Differences among groups were considered to be significant at a P value of <0.05 . Statistical analysis was performed with Graph Pad Prism 8.0 (Graph Pad Software, Inc., San Diego, CA) (Sara Joushi et al., 2021).

Results

3.1 Effect of MS on exploratory and anxiety-like behaviors of mothers in open field test

To assess exploratory, locomotor activity and anxiety-like behaviors, the animals were tested in the open field. An Unpaired t test showed that distance moved in the open field was not significantly different between MS-24h and CTRL-24h [$p = 0.85$]. There was also no significant difference between MS-1week and CTRL-1week [$p = 0.06$]. However, movement distance was reduced in the MS-1month group compared to CTRL-1month group [Mann Whitney test, $p < 0.001$] (figure1. A).

A Mann Whitney test [$p < 0.001$] revealed that the velocity of movement was also decreased in MS-1month group compared to CTRL-1month group. The velocity of movement was not significantly different between MS-24h and MS-1week and their corresponding CTRL groups [t test, MS-24h vs CTRL-24h: $p = 0.21$ and MS-1week vs CTRL-1week: $p = 0.06$] (figure1. B).

Although rearing behavior was not significantly different between MS-24h and MS-1week groups and their corresponding CTRL groups [t test, MS-24h vs CTRL-24h: $p = 0.11$ and MS-1week vs CTRL-1week: $p =$

0.63], it was lower in MS-1month group compared to CTRL-1month group [Unpaired t test, $p = 0.003$] (figure1. C).

Grooming behavior was higher in MS-1week compared to CTRL-1week group [Unpaired t test, $p = 0.04$]. MS-1month group also showed more grooming behavior compared to CTRL-1month group [Mann Whitney test, $p = 0.003$]. However, it was not significantly different between MS-24h group and its corresponding CTRL group [MS-24h vs CTRL-24h: $p = 0.19$] (figure1. D).

Summary: MS induced less locomotor activity and more anxiety in MS-1month mothers.

3.2 Effect of MS on anxiety-like behaviors of mothers in the EPM

To measure anxiety-like behaviors, the mothers were tested in the elevated plus maze (EPM). An Unpaired t test showed that entries into the open arm decreased in MS-24h and MS-1week groups compared to their corresponding CTRL groups [MS-24h vs CTRL-24h: $p = 0.02$ and MS-1week vs CTRL-1week: $p = 0.002$]. Entries into the open arm was also significantly different between MS-1month and CTRL-1month group [Mann Whitney test, $p = 0.002$] (figure 2.A). Entries into the close arm was increased in MS-1week and MS-1month groups compared to their corresponding CTRL groups [MS-1week vs CTRL-1week: Unpaired t test, $p = 0.004$ and MS-1month vs CTRL-1month: Mann Whitney test, $p = 0.003$]. However, there was not statistically significant difference between MS-24h and CTRL-24h group [Unpaired t test, $p = 0.07$] (figure 2. B). An Unpaired t test [$p = 0.009$] revealed that time spent in open arm was decreased in MS-1month group compared to CTRL-1month group. However, this parameter was not significantly different between MS-24h and MS-1week groups and their corresponding CTRL groups [Unpaired t test, MS-24h vs CTRL-24h: $p = 0.19$ and MS-1week vs CTRL-1week: $p = 0.07$] (figure 2. C). Time spent in close arms was also higher in MS-24h, MS-1week and MS-1month groups compared to their corresponding CTRL groups [Unpaired t test, MS-24h vs CTRL-24h: $p = 0.04$, MS-1week vs CTRL-1week: $p = 0.005$ and MS-1month vs CTRL-1month: $p < 0.001$]. (figure 2.D).

Summary: MS induced more anxiety-like behavior in all dams.

3.3 Effect of MS on learning and memory of mothers in MWM test

MWM was used to assess spatial learning and memory (Charles V. Vorhees & Michael T. Williams, 2006). Learning in the MWM test was recorded as a reduction of the swimming path length and escape latency to find the hidden platform during training. A two-way ANOVA revealed that there was no significant difference between MS and CTRL mothers at 24h and 1week time points after weaning [MS-24h vs CTRL-24h: $F(1, 12) = 0.5445$, $p = 0.47$ and MS-1week vs CTRL-1week: $F(1, 12) = 0.2222$, $p = 0.64$]. However, total distance moved by MS-1month mothers was increased compared to CTRL-1month mothers in all three blocks of the learning phase [$F(1, 12) = 34.01$, $p < 0.001$, 1st block: 0.03, 2nd block: $p = 0.001$ and 3rd block: $p = 0.010$]. (Fig. 3.A).

A two-way ANOVA showed that escape latency was not significantly different between MS and CTRL mothers at 24h and 1week time points after weaning [MS-24h vs CTRL-24h: $F(1, 12) = 0.0007633$, $p=0.98$ and MS-1week vs CTRL-1week: $F(1, 12) = 2.280$, $p=0.16$]. However, there was a higher escape latency in MS-1month mothers compared to CTRL-1month mothers [$F(1, 12) = 29.38$, $p<0.001$, 2nd block: $p= <0.001$, 3rd block: $p= 0.01$]. (Figure 3.B).

A probe test was conducted 2 hours after the last training trial and the mean percentage of distance and time in target quadrant were analyzed to assess spatial memory retention. There was no significant difference in distance percent in target quadrant in MS-24H and MS-1 week mothers compared to their corresponding CTRL group [Unpaired t test, MS-24h vs CTRL-24h: $p= 0.90$ and MS-1week vs CTRL-1week: $p=0.51$] (Figure 4. A). Moreover, time percent in target quadrant was not significantly different between MS-24h and MS-1 week groups compared to their corresponding CTRL group [Unpaired t test, MS-24h vs CTRL-24h: $p= 0.95$ and MS-1week vs CTRL-1week: $p=0.58$] (Figure 4. B). However, distance percent and time percent in target quadrant was lower in MS-1month group compared to CTRL-1months group [Unpaired t test, distance percent: $p=0.03$ and time percent: $p=0.01$].

There was no significant difference in swimming speed among rats [F]. There was no significant difference in the latency to find the visible platform among experimental groups [F], so our manipulations did not induce any motor or sensory deficits on experimental animals (Table 1).

Summary: MS induced spatial learning and memory deficits only in MS-1month mothers.

Discussion

The present study was designed to investigate the effects of MS on mothers' anxiety, locomotor activity and also spatial learning and memory. Since most of the previous studies investigated mothers' behavior immediately after weaning (PND 21-22) (J. Aguggia et al., 2019; J. P. Aguggia et al., 2013) and few studies have investigated MS- induced changes on mothers' behavior sometime beyond weaning of the pups, we chose three time points to test whether MS effects are permanent and would last through weeks. Moreover, to the best of our knowledge, no study has ever investigated MS effects on mothers' spatial learning and memory in the MWM.

In the present study, MS mothers showed more anxiety-like behavior at 24h, 1-week and 1-month time points after weaning, since they entered less in the open arms of the EPM than control dams. Time spent in the open arms was lower only in MS-1month dams. Consistently, all MS dams spent more time in the close arms of the EPM. Entries to the close arm was higher in MS-1week and MS-1month groups. Moreover, MS-1month mothers showed less activity in the open field and showed more anxiety, as they showed less rearing and more grooming behaviors. Learning in the MWM was unaffected due to the separation in the MS-24h and MS-1month mothers. However, MS-1month mothers showed learning deficits. Memory also was impaired in MS-1month mothers, while MS-24h and MS-1week mothers had normal memory in the MWM. Regarding anxiety, the majority of the previous studies reported an increase

in anxiety-like behaviors. However, it seems there is controversy in the results (Alves et al., 2019). Mothers which experienced MS were more depressive and anxious than control ones four months after separation (Bousalham et al., 2013). While some studies have shown that dams were more anxious in the EPM (J. P. Aguggia et al., 2013; Bousalham et al., 2013; Maghami et al., 2018) and in the dark-light test (Alves et al., 2019), a study by Kalinichev and colleagues (Kalinichev et al., 2000) showed a reduction in the anxiety levels in both EPM and open-field tests. At 4-5 weeks after weaning, dams that experienced MS entered the open arms of the plus-maze more than twice as often as control dams and spent significantly more time on these arms compared to controls (Kalinichev et al., 2000). In a novel locomotor arena, MS dams also spent twice as much time in the center of the arena than control dams throughout three consecutive days of testing (Kalinichev et al., 2000). But the general locomotor activity in the plus-maze test or in a novel locomotor arena was unaffected by the separation experience (Kalinichev et al., 2000). Moreover, it seems that strain may be an accountable factor since a study showing reduced MS-related anxiety was conducted in the Long Evans rats (Kalinichev et al., 2000), a strain that is considered less anxious than Wistar rats (Shaw, Chuang, Shieh, & Wang, 2009).

Results of the present study reveals MS would induce behavioral alteration in dams that may not be obvious immediately after the end of separation, i.e., after weaning. Instead, MS effects on the dam's behavior would appear weeks after the end of separation. As we showed MS-1week and especially MS-1month group showed cognitive impairments. In the present study, we didn't report molecular assessments. However, molecular data in the literature concurs with the behavioral observations of the previous studies, showing that MS increases the glucocorticoid receptor gene expression in the hippocampus of dams (Alves et al., 2019; Orso et al., 2018), which is typically associated with higher vulnerability to persistent stress (Cheryl D. Conrad, 2008); and also c-Fos expression in the central amygdala that can be linked to fear responses (J. P. Aguggia et al., 2013; Alves et al., 2019). These results suggest that prolonged MS evokes emotional responses in the dams that could alter the activity of the hypothalamic-pituitary-adrenal axis (HPA), which is involved in anxiety and depression development (B. Aisa, Tordera, Lasheras, Del Río, & Ramírez, 2008). Previous studies have reported higher plasma corticosterone levels in dams that experienced both short MS (Eklund, Johansson, Uvnäs-Moberg, & Arborelius, 2009) and long MS (Maghami et al., 2018).

The only two studies evaluating memory alterations in the mothers after MS (J. P. Aguggia et al., 2013; Sung et al., 2010), both using the step-down inhibitory avoidance task, reported memory impairments after weaning (PND 21 (J. P. Aguggia et al., 2013) and 25 (Sung et al., 2010)).

In the present study, for the first time, we evaluated spatial learning and memory of dams in the MWM at three time points after weaning. We showed that spatial learning and memory was not impaired immediately after weaning, since MS-24h and MS-1week mothers showed normal learning and memory. Deficits of spatial learning and memory was evident only in MS-1month mothers.

Because of abundant expression of glucocorticoid receptors on hippocampus, this structure is a preferential target for the effect of stress hormones. Exposure to high levels of glucocorticoids have been

associated with learning and memory deficits as well as hippocampal atrophy (Sousa et al., 2014). The mechanisms underlying the effects of high corticosterone levels are not widely known. A possible reason is that high corticosterone levels suppresses BDNF expression that has been linked with memory function (Sousa et al., 2014).

Moreover, there is strong evidence that increased levels of glucocorticoids can cause insulin resistance and may alter levels of insulin in the brain, which seems to be correlated with changes in cognition involving complex insulin signaling pathways (Zhao & Alkon, 2001). Additionally, studies have been shown that reduced insulin signaling in forebrain regions is correlated with poorer cognitive performance in patients with diabetes or neurodegenerative diseases (Duarte, Moreira, & Oliveira, 2012). In this context, further research needs to be conducted to explore the functional consequences of excessive glucocorticoids in dysregulation of insulin-specific signaling in postpartum depression and related memory/cognition deficits.

Conclusion

Taken together, our results showed that MS induces changes in dams' behavior that may last for long periods after weaning. We demonstrated for the first time that MS could impair spatial learning and memory in the MWM that appeared 1 month after the end of separation. More investigations are needed to clearly determine the exact mechanisms underlying behavioral alterations of dams that experience MS.

Declarations

Ethics approval and consent to participate:

All experimental procedures were conducted in accordance with guidelines relevant to the care of experimental animals, as approved by the Institutional Animal Research Ethics Committee of Kerman University of Medical Sciences (Ethic code: KNRC-96-63).

Consent for publication:

Not applicable

Availability of data and materials:

The data that support the finding of this study are available from the corresponding author upon reasonable request.

Competing interests:

No conflict of interest was reported by the authors.

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Author contribution Statement:

Sara Joushi: performed the behavioral experiments, wrote the manuscript with input from all authors.

Zahra Taherizadeh: performed the molecular study contributed to sample preparation

Fatemeh Mohtashami Borzadaran: conceived of the presented idea, contributed to the interpretation of the results

José Francis-Oliveira: wrote the manuscript with input from all authors, contributed to the interpretation of the results

Khadijeh Esmaeilpour: supervised the project, contributed to the interpretation of the results, designed the experiments, derived the models and analyzed the data.

Vahid Sheibani: supervised the project, conceived of the presented idea, contributed to the interpretation of the results

All authors contributed to the final version of the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript

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Tables

Table 1. A. velocity and B. visible test data. There was no significant difference in swimming speed and in latency to find the visible platform among experimental groups.

A

Velocity (cm/s)	CTRL 24h	MS 24h	CTRL 1week	MS 1week	CTRL 1month	MS 1month
	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM
1 st Block	23.56± 0.77	21.60± 0.30	22.48± 1.32	18.98± 0.76	23.84± 1.04	22.73± 0.57
2 nd Block	20.80± 0.34	18.50± 0.68	19.83± 1.60	14.71± 0.52	24.04± 1.44	22.22± 1.18
3 rd Block	18.69± 0.80	17.11± 0.80	17.72± 1.91	13.94± 0.45	23.32± 0.79	22.38± 1.35

B

Time to find the platform (s)	CTRL 24h	MS 24h	CTRL 1week	MS 1week	CTRL 1month	MS 1month
	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM
	19± 1.98	18.07± 1.53	19.07± 2.53	18± 1.80	18.14± 1.79	17.64± 1.77

Figures

Figure 1

Locomotion and anxiety-like behavior of mothers in the open field. Movement distance was reduced in the MS-1month group compared to CTRL-1month group [Mann Whitney test, $p < 0.001$] (A). A Mann Whitney test [$p < 0.001$] revealed that the velocity of movement was also decreased in MS-1month group compared to CTRL-1month group. (A). Total distance moved and the velocity of movement was not significantly different between MS-24h and also MS-1week and their corresponding CTRL groups (B). Rearing behavior was lower in MS-1month group compared to CTRL-1month group [Unpaired t test, $p = 0.003$] (C). Number of grooming was also higher in MS-1week compared to CTRL-1week group [Unpaired t test, $p = 0.04$]. MS-1month group also showed more grooming behavior compared to CTRL-1month group [Mann Whitney test, $p = 0.003$] (D). The data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to CTRL group.

Figure 2

Effect of MS on anxiety-like behavior of mothers in the EPM. Entries into the open arm decreased in MS-24h, MS-1week and MS-1month groups compared to their corresponding CTRL groups [Unpaired t test, MS-24h vs CTRL-24h: $p = 0.02$ and MS-1week vs CTRL-1week: $p = 0.002$ and MS-1month vs CTRL-1month: Mann Whitney test, $p = 0.002$] (A). Entries into the close arm was increased in MS-1week and MS-1month groups compared to their corresponding CTRL groups [MS-1week vs CTRL-1week: Unpaired t test, $p = 0.004$ and MS-1month vs CTRL-1month: Mann Whitney test, $p = 0.003$] (B). Time spent in open arm was decreased in MS-1month group compared to CTRL-1month group (C). Time spent in close arms was also higher in MS-24h, MS-1week and MS-1month groups compared to their corresponding CTRL groups (D). The data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to CTRL group.

Figure 3

Performance of experimental groups in MWM. Total distance moved (A) and also escape latency onto the hidden platform (B) by MS-1month mothers was increased compared to CTRL-1month mothers in all three blocks of the learning phase (Two-way ANOVA, Repeated measures). Distance percent (C) and time percent in target quadrant (D) was lower in MS-1month group compared to CTRL-1months group [Unpaired t test, distance percent: $p=0.03$ and time percent: $p=0.01$]. The data are presented as mean $\pm$ SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to CTRL group.