Scientific Reports

https://doi.org/10.1038/s41598-026-40163-9

Article in Press

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Received: 18 July 2025

Accepted: 10 February 2026

Published online: 21 February 2026

Cite this article as: Kariminejad-Farsangi H., Kariminejad-Farsangi H., Rajizadeh M.A. *et al.* Intranasal insulin ameliorates prenatal LPS-induced learning and memory impairments in adolescent male rats: A behavioral, electrophysiological, and molecular study. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-40163-9

Hadis Kariminejad-Farsangi, Haniyeh Kariminejad-Farsangi, Mohammad Amin Rajizadeh, Mahdiyeh Abbaspoor, Lily Mohammadipoor-Ghasemabad, Sara Joushi & Vahid Sheibani

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Intranasal Insulin Ameliorates Prenatal LPS-Induced Learning and Memory Impairments in Adolescent Male Rats: A Behavioral, Electrophysiological, and Molecular Study

Hadis Kariminejad-Farsangi[1], Haniyeh Kariminejad-Farsangi[1], Mohammad Amin Rajizadeh[2], Mahdiyeh Abbaspoor[1], Lily Mohammadipoor-Ghasemabad[1], Sara Joushi[1]*, Vahid Sheibani[1]*

1 Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.

2 Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.

*Corresponding Authors at Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran. email addresses: s.joushi@gmail.com s.joushi@kmu.ac.ir, vsheibani2@yahoo.com, vsheibani@kmu.ac.ir

* first corresponding author: sara joushi

second corresponding author: vahid Sheibani

Abstract:

Prenatal stress, including maternal immune activation (MIA), affects cognitive performance in the offspring. Since insulin could improve cognitive function in several aspects, we hypothesized that intranasal insulin would attenuate MIA-induced learning and memory deficits. In the present study, the pregnant Wistar rats received lipopolysaccharide (LPS, 250 µg/kg) intraperitoneally on gestational day 15. Intranasal insulin (2 IU, 7 days) was administered to male pups from PND 34-47. During late adolescence, the Morris Water Maze and in vivo electrophysiological recording were performed in male rats to assess spatial learning and memory and long-term potentiation (LTP), respectively. Also, the hippocampal expression of BDNF and PSD-95 was evaluated using real-time PCR. Our results demonstrated that MIA impaired spatial learning and memory in the male pups. Hippocampal synaptic plasticity was also impaired in the adolescent male rats. However, intranasal administration of insulin could overcome MIA-induced impairments and improve learning, memory, and synaptic plasticity in the male pups. Although BDNF and PSD-95 levels were not altered in the hippocampus of MIA pups, intranasal insulin increased PSD-95 expression. Taken together, these findings suggest that intranasal insulin promotes cognitive performance in MIA-exposed pups during adolescence; however, the underlying molecular mechanisms remain to be elucidated.

Keywords: maternal immune activation (MIA); intranasal insulin; long term potentiation (LTP); learning and memory; BDNF; PSD-95

Introduction:

Maternal immune activation (MIA) describes the maternal immune system's response to infectious or non-infectious stimuli during pregnancy, which may adversely affect fetal brain development (1). Inflammatory mediators can cross the placental barrier, trigger downstream signaling cascades, and ultimately disrupt fetal brain development (2). Numerous epidemiological studies indicate that maternal infection during pregnancy is associated with an increased risk of various neuropsychiatric diseases, including schizophrenia and autism spectrum disorder (ASD) in children (3–6).

Studies have shown that maternal immune response due to infectious or non-infectious events can adversely affect brain growth, functional connectivity (7), neurogenesis (8), neuronal migration, and synaptic development in the offspring (9). Commonly used models include the administration of polyinosinic:polycytidylic acid (Poly I:C), which mimics viral infection, and lipopolysaccharide (LPS), which mimics bacterial infection (10). However, it is challenging to compare the results due to the wide variation in immunogen type, dose, and timing in different studies (11,12). The gestational day (GD15) and LPS dose were selected based on previously published pilot studies, in which these parameters were systematically evaluated to induce long-term behavioral alterations in the offspring while minimizing pregnancy-related complications, including spontaneous abortions, stillbirths, and maternal sickness behavior (13). Collectively, a large number of studieshave shown that offspring exposed to MIA exhibit increased anxiety-like behavior (14), impaired social interaction (15), cognitive deficits (16), and repetitive behavior (9). Moreover, offspring exposed to MIA often demonstrate difficulties in tasks related to spatial learning and memory, such as those assessed by the Morris Water Maze (MWM) (17–19). Recent systematic evidence indicates that maternal immune activation induces robust learning and memory deficits in rodent offspring, with several studies reporting sex-dependent variability in the magnitude and timing of these effects (20). Such cognitive deficits are linked to impaired hippocampal neurogenesis (21), as well as specific molecular changes in the brain, including alterations in the levels of neurotrophic factors (15,22,23) and synaptic proteins (24–26), which are essential for proper neuronal function and plasticity.

Synaptic plasticity, defined as the ability of synapses to strengthen or weaken over time, is fundamental for learning and memory (27,28). Long-term potentiation (LTP) is a crucial form of synaptic plasticity that underpins learning and memory formation (29,30). Several proteins are known to contribute to synaptic plasticity. For example, Brain-derived neurotrophic factor (BDNF) affects plasticity by promoting dendritic outgrowth, spine formation, and synaptogenesis. It also regulates gene expression and protein synthesis, which are crucial for maintaining synaptic plasticity. BDNF enhances synaptic function by promoting dendritic growth and regulating gene expression, primarily through TrkB signaling (31,32). Postsynaptic density protein-95 (PSD-95), a vital scaffolding protein, organizes key components of the postsynaptic density that are essential for synaptic signaling, development, and survival (33). Abnormal PSD-95 activity may be a key role in the synaptic abnormalities observed in neurodevelopmental disorders like autism (34). Moreover, MIA, via immune-related molecules, may directly or indirectly alter the expression level of PSD-95 and BDNF in the offsprings (11,35).

Given these MIA-induced neurodevelopmental deficits, there is a strong need to explore therapeutic strategies to mitigate long-term cognitive and synaptic impairments in offspring. Insulin is a 51-amino acid peptide hormone produced by pancreatic β cells. Although its most widely known function is peripheral glucose homeostasis, it plays a critical role in the brain as a neurotrophic factor (36). By binding to its receptors in all brain regions, particularly the olfactory bulb, hypothalamus, cortex, cerebellum, and hippocampus, insulin could contribute to regulating various neuronal functions such as growth, metabolism, plasticity, survival, and cholinergic function, which are needed for learning and memory (37). Intranasal insulin administration is a non-invasive method that allows insulin to reach the brain directly without causing peripheral hypoglycemia (38). In clinical settings, intranasal insulin has also been reported to improve memory performance in both healthy individuals and patients with mild cognitive impairment (39–43). Intranasal insulin also alleviates cognitive impairment in the rat model of Parkinson’s disease (44), Alzheimer’s disease (37,45), offspring of diabetic mothers (46), stroke (47). Therefore, insulin could be a promising option for improving cognitive impairment in the affected children.

Hence, in the present work, we aimed to assess the effects of intranasal insulin administration on spatial learning and memory performance in adolescent male rats in an LPS-induced model of MIA. Hippocampal synaptic plasticity was also evaluated during late adolescence in MIA-exposed pups.

Results

Effect of Intranasal insulin and MIA on spatial learning and memory.

Male pups born to LPS- or saline-treated dams received intranasal insulin or saline every other day from PND 34 to 47, for a total of 7 doses. In order to assess the spatial learning and memory, MWM was used (29) on PND 50. In this experiment, learning was quantified by a decrease in swimming path length and escape latency to locate the hidden platform during training blocks (n = 8). In the first step, a statistical analysis among saline groups and LPS groups was performed. To determine the appropriate LPS dose (between 100 and 250 μg/kg) for inducing probable cognitive impairments, a statistical analysis was conducted on the behavioral results of groups 1, 2, 4, 5. Our results indicated that 250 μg/kg LPS could induce significantly greater cognitive impairments than 100 μg/kg in male pups. So, LPS 250 µg/kg was selected for the rest of the experiments (The data are provided in supplementary data, Figure S1).

A repeated-measures two-way ANOVA showed significant differences among groups in the path length [F (3, 28) = 12.38, p<0.01]. Total distance moved by LPS (250)+saline pups was increased compared to the saline+saline group in the 2nd and 3rd blocks of the learning phase (p=0.005). In addition, intranasal insulin administration significantly reduced the total distance moved by the LPS (250)+insulin rats compared to the LPS(250)+saline group (p=0.02). No significant difference was observed between the LPS (250)+insulin and the saline+saline group (Fig. 2a).

A repeated-measures two-way ANOVA indicated significant differences among groups in the escape latency [F (3, 28) = 5.528, p=0.004]. LPS (250)+saline rats showed a higher escape latency compared to the saline+saline group in the 2nd (p=0.0017) and 3rd (p=0.02) blocks of the learning phase. As depicted in Fig. 2b, the escape latency was significantly reduced in the LPS (250)+insulin rats compared to the LPS (250)+saline (p=0.02) in the 3rd block. There was no significant difference between LPS (250)+insulin and saline+saline group.

Learning trends of the individual groups during the training phase are shown in supplementary data, Figure S2. Our results revealed that there was no significant change in total distance or escape latency in the LPS (250)+saline group, while the LPS (250)+insulin group, like saline+saline and saline+insulin groups, showed a significant decrease in total distance during spatial learning.

The probe test was carried out 2 hours after the last training trial. The mean percentages of the time and distance in the target quadrant were considered as parameters of the spatial memory retention. A one-way ANOVA showed a significant difference in the distance percent among groups [F (3, 28) = 10.27, p<0.0001]. The distance percent in the target quadrant by LPS (250)+saline rats was decreased compared to the saline+saline group (p=0.04). As shown in the fig. 2c, intranasal administration of insulin could significantly enhance the traveled distance in the target quadrant compared to LPS (250)+saline (p=0.01). These results were also observed in the time percent in the target quadrant [F (3, 28) = 18.86, p<0.001]. In detail, LPS (250)+saline rats spent less time in the target quadrant compared to the saline+saline group (p=0.002). However, intranasal insulin in the LPS (250) rats increased the time spent in the target quadrant compared to the LPS (250)+saline group (p=0.002) (Fig. 2d).
Swimming velocity was increased in the 2nd block in the LPS group compared to the saline+saline group [F (3,28) = 15.41, p=0.0005). This result was observed in the 3rd block (p=0.03) (Table 1). Although the swimming velocity of LPS was increased, the swimming path length and escape latency to find the hidden platform were not reduced; this effect suggests a hyperactive phenotype (48).

Effect of Intranasal insulin and MIA on hippocampal LTP.

To confirm the MWM findings, in vivo electrophysiological recordings of fEPSPs from the CA1 region were conducted. High-frequency stimulation of the Schaffer-collateral pathway induced early long-term potentiation (E-LTP), characterized by a significant rise in the slope of the field excitatory postsynaptic potential (fEPSP), which was assessed for 2 hours following high-frequency stimulation, representing the maintenance phase of LTP. The mean fEPSP slope was measured among groups after baseline recording and following HFS induction up to 120 minutes. Following the application of HFS, the mean fEPSP slope in the LPS(250)+saline group was markedly reduced compared to the saline+saline group (Fig. 3a, F (3, 16) = 34.08, p<0.001, statistical differences are indicated by asterisks in the graph). However, in these groups, after an initial increase in fEPSP slope, the fEPSP slope approximately declined to the baseline during 2 h (Fig. 3a, p<0.001) and at the end of 2 hours (Fig. 3a, P<0.01), compared to the saline+saline group. Moreover, the current results showed that following HFS, treatment with insulin in the LPS(250)+insulin group could enhance the mean fEPSP slope compared to the LPS(250)+saline group (Fig. 3a, p<0.05, statistical differences are indicated by # in the graph). No significant differences in LTP magnitude or maintenance between the two saline+saline and saline+insulin groups were observed. Although LTP magnitude and maintenance were improved in the LPS (250)+insulin group relative to the LPS (250)+saline group, they did not fully reach the levels observed the saline+saline and saline+insulin groups. However, this difference between these groups was insignificant (n = 5 in each group).

To assess the impact of LPS and/or insulin on baseline synaptic function, input-output (I–O) curves were constructed, depicting alterations in the slope of the field excitatory postsynaptic potential (fEPSP) in relation to escalating stimulus intensities. Overall, no significant difference was observed in the input/output relationship among all rats (Fig. 3b). Moreover, no substantial changes were made in the PPF ratios of any of the groups. (Fig. 3c). (n = 5 in each group).

Effect of Intranasal insulin and MIA on BDNF and PSD-95 expression.

To investigate the molecular mechanisms of intranasal insulin, the expression levels of BDNF and PSD-95 were assessed using real-time PCR. As shown in Figure 4a, there were no significant differences in hippocampal BDNF expression among groups. Intranasal insulin increased the PSD-95 expression in the MIA pups compared to the LPS (250)+saline group (F (3, 12) = 8.347, p=0.007). However, there was no significant difference in the levels of PSD-95 among in LPS (250) +saline group and the saline group (F (3, 12) = 0.8022, P=0.51, Fig. 4b).

Discussion

This study aimed to evaluate the effects of intranasal insulin on learning and memory in adolescent male rats exposed to MIA. Intranasal insulin administration was used as a treatment to reduce the probable detrimental effects of MIA in adolescent pups. In this study, independent batches of rats were used for behavioral, electrophysiological, and molecular assessments. MWM test was conducted to evaluate spatial learning and memory in the male pups on PND 50. Moreover, in vivo electrophysiological recording and molecular studies were carried out to assess LTP and the expression level of BDNF and PSD-95 in the hippocampus, respectively. Our results demonstrated that a single dose of LPS during mid gestation could disrupt learning and memory in the male pups. We also found that intranasal administration of insulin could mitigate the cognitive impairments in the MWM and LTP. Furthermore, PSD-95 expression in the hippocampus was significantly increased following intranasal insulin treatment, while BDNF expression levels remained unchanged.

Fetal brain development is highly sensitive to maternal environment (49). Infection during pregnancy is increasingly recognized as a critical factor that can disrupt the developing brain, with significant implications for synapse formation and function (11). Learning and memory are closely tied to the proper development and function of synaptic networks in the brain (50,51). Previous research in rodent models of MIA has shown that prenatal immune challenge leads to persistent alterations in offspring neurobiology, including changes in brain structure and function that underlie cognitive impairments (12). A study conducted by Hao et al. revealed that the LPS-induced MIA in the adult Sprague–Dawley rats led to spatial learning and memory deficits in the MWM test (52). This result was also replicated in the research in which the C57BL/6J mice offspring born to LPS-treated dams exhibited a marked increase in duration and distance to find the hidden platform during training phase. Moreover, the memory deficit was observed in these rats (17). Consistent with the previous studies, we also found that prenatal LPS exposure led to spatial learning and memory impairments during late adolescence.

LTP involves an enhancement in synaptic response following repeated electrical stimulation and is considered a cellular mechanism for long-term memory in the hippocampus (28,53). Prenatal immune activation using Poly I:C has been shown to impair hippocampal LTP in male offspring (19). These impairments were associated with reduced hippocampal neurogenesis and downregulation of VEGFA and its receptor VEGFR2. In line with these findings, our results showed that LPS-induced MIA impaired hippocampal LTP in male offspring. Our electrophysiological findings support the behavioral deficits observed in the MWM test.

To explore the molecular alterations following LPS-induced maternal immune activation, we evaluated the hippocampal expression levels of BDNF and PSD-95. PSD-95 is a key scaffolding protein found in the postsynaptic density of excitatory synapses, where it plays an essential role in maintaining synaptic structure and plasticity (54,55). Our real-time PCR result showed that MIA did not change the expression level of PSD-95 in the hippocampus. Consistent with our finding, PSD-95 expression did not change in the prenatal activation with Ploy I:C on PND 21 (56). Importantly, some studies that used direct LPS administration also found no change in PSD-95 expression, which is consistent with our results. For instance, even though the rats showed signs of cognitive problems after receiving LPS, their PSD-95 levels stayed the same (57). Similarly, in the study by Das S, working memory was affected by LPS, but PSD-95 gene expression did not change. This suggests that memory problems can happen even when PSD-95 levels are not reduced (58). In contrast, Zhang et al. (17) reported reduced PSD-95 expression in the LPS-induced MIA pups at PND 95, suggesting that age may influence PSD-95 regulation. It is also possible that while mRNA levels remained unchanged, PSD-95 protein expression was decreased. On the other hand, some studies have revealed that MIA could reduce the BDNF mRNA in the hippocampus of offspring. For instance, LPS-induced MIA could reduce the BDNF levels in the hippocampus of adult mice (17). Consistent with this study, a reduction in the BDNF levels was observed in the hippocampus of LPS-induced MIA mice on the PND 28 and 60 (15). Moreover, it has been demonstrated that LPS injection on GD 15, 16 and 17 could decrease the expression of BDNF in the hippocampal microglia of adult mice (22). However, our data showed no significant changes in BDNF mRNA levels across experimental groups. This finding is consistent with Hemmerle et al. (59), who reported that prenatal Poly I:C exposure did not alter hippocampal BDNF expression in juvenile rats but affected BDNF and TrkB levels in the cortex and striatum at PND 14 and PND 60. Similarly, Han et al. (60) reported no significant differences in BDNF-TrkB signaling in the hippocampus of mice prenatally exposed to Poly I:C, although alterations were observed in the prefrontal cortex. These findings suggest that although BDNF mRNA expression remained unchanged in the hippocampus, BDNF-TrkB signaling may still be impaired either locally or in other brain regions, contributing to MIA-induced cognitive deficits. Furthermore, the absence of transcriptional changes does not rule out functional synaptic alterations, which may arise from mechanisms beyond gene expression regulation. A study conducted by Cieślik et al. showed a molecular cascade in which prenatal LPS exposure induces selective neuroinflammatory changes (elevated IFN-γ with M2 microglial polarization), leading to mitochondrial dysfunction and oxidative stress in hippocampal synapses. This mitochondrial pathology connects to synaptic alterations through Cdk5 activation, which phosphorylates and inhibits GSK-3β, causing hypophosphorylation of microtubule-associated protein tau (MAPT) and accompanying changes in synaptic proteins including altered synaptophysin, synapsin, PSD-95, and VAMP1 expression (61). Finally, we cannot exclude the possibility that our study was underpowered to detect subtle molecular changes. Future investigations should examine both mRNA and protein levels across developmental time points to more fully elucidate the impact of prenatal immune activation on synaptic architecture.

Intranasal insulin has emerged as a promising area of research in the treatment of cognitive impairment (62). Insulin modulates hippocampal synaptic plasticity by activating the PI3K/Akt signaling pathway, which facilitates changes in synaptic strength and supports long-term potentiation induction at CA3–CA1 synapses in the hippocampus. Specifically, intra-hippocampal insulin application induces synaptic LTP and increases levels of PI3K, Akt, and GSK-3β mRNA, indicating that insulin-sensitive signaling directly engages intracellular cascades underlying synaptic enhancement independent of canonical neurotrophin transcription (63–65). For example, administering insulin for three days prior to anesthesia effectively prevented spatial learning and memory deficits in the MWM, suggesting a potential strategy to reduce postoperative cognitive decline (66). Moreover, intranasal insulin administration for 6 weeks in the APPswe/PS1dE9 transgenic mice, a common AD model, improved cognitive function (45). Consistent with previous studies, we found that intranasally administered insulin for seven times during the mid adolescent period could ameliorate the spatial learning and memory deficits in the male pups exposed to MIA. Moreover, our results indicated that intranasal insulin enhanced LTP following MIA. In line with our study, other studies in this field have demonstrated that insulin could rescue the deficits in hippocampal synaptic plasticity (67,68). At the molecular level, an increased expression of PSD-95 was observed in this study following intranasal insulin administration. PSD-95 expression was evaluated as a molecular correlate of synaptic integrity rather than a direct functional measure of synaptic plasticity. Also, substantial variability in the LPS+insulin group and emphasized that the observed increase in PSD-95 should be interpreted cautiously. Consistent with our study, a previous study has reported that intranasal insulin administration could enhance synaptic function by upregulating the expression of PSD-95 (38). As for BDNF, it has been demonstrated that insulin could increase the BDNF expression (69,70). In contrast, our finding showed that intranasal insulin did not alter the levels of BDNF expression in the hippocampus. It is possible that the observed insulin effects are mediated through a BDNF-independent pathway or by affecting other brain regions. For example, a study conducted by Kellen Ugioni Simon et al. revealed that intranasal insulin did not increase the hippocampal BDNF, but increased the nerve growth factor-β (NGF-β) in the cortex of a low-grade inflammation mouse model (71). NGF-β exerts its effect through PI3K/Akt signaling pathway (72). This cascade is associated with neuronal survival, neuroplasticity and synaptic transmission maintenance (72,73). Previous studies indicated that insulin can modulate synaptic plasticity through various intracellular signaling cascades, such as the PI3K/Akt pathway, independent of BDNF signaling (74,75). Therefore, it is suggested that insulin could exert its effect on the synaptic plasticity in a way independent of BDNF signaling. Further investigations are needed to elucidate the molecular pathways following intranasal insulin administration in MIA offspring.

Taken together, our findings suggest that prenatal immune activation impairs spatial learning, memory, and hippocampal synaptic function in adolescent male rats. Intranasal insulin attenuated these deficits and was associated with improvements in electrophysiological measures and changes in synapse-related gene expression. However, the molecular findings should be interpreted as supportive rather than definitive evidence of enhanced synaptic plasticity. Future studies incorporating protein-level analyses, larger sample sizes, and pathway-specific investigations are required to clarify the mechanisms underlying insulin’s effects in the context of MIA. Moreover, studies could examine whether combining insulin with other treatments that target BDNF signaling might produce synergistic effects in MIA models.

Materials and methods

Animals

In the present work, Wistar rats (animal farm of Kerman University of Medical Sciences) were housed under a controlled 12-hour light-dark cycle, with a stable temperature maintained at 23 ± 2°C. They had ad libitum access to food and water.

All experimental procedures were conducted in accordance with institutional and international guidelines for animal care, as approved by the Institutional Animal Research Ethics Committee of Kerman University of Medical Sciences (Ethics code: IR.KMU.AEC.1402.84).

Maternal immune activation (MIA) protocol

Two female rats were randomly put in the cage to mate with a male rat. The following morning, gestational day 0 (GD0) was confirmed via vaginal smear examination indicating the presence of sperm. The pregnant rats randomly received an intraperitoneal injection of LPS (250 µg/kg; Escherichia coli, serotype 026:B6; L-3755, Sigma, St. Louis, MO) or vehicle (sterile saline) on GD15 (13).

All intraperitoneal (i.p.) injections were conducted at 10-12 AM local time. Following weaning on postnatal day 21, four male pups were randomly housed in in separate cages.

Intranasal insulin administration

Male pups received 2 IU (68) of regular human insulin (1 IU/10 µl and 10 µl/nostril, Loghman Pharmaceutical Co., Iran) or vehicle (normal saline) on PND 34-47 every other day (7 days totally). A total volume of 20 µl insulin or saline was administered in 5 µl droplets using a micropipette every two minutes. Following the method described by Sara Joushi et al. and Marks et al, rats were gently restrained, and the solution was applied bilaterally to the rhinarium, defined as the glabrous skin around the nostrils, an area highly innervated by free nerve endings (76,77). The 20 µl solution was evenly distributed onto the squamous epithelium of both the left and right rhinarium using a 10-µl pipette, while avoiding direct application into the nostrils. After administration, the rats were returned to their home cages (76).

Exprimental groups

Six groups (n=8) were designed for behavioral test, as follows:

1. Saline: the male pups that their dams have received saline on GD15.

2. Saline + Saline: the male pups that their dams have received saline on GD15. Then, during mid adolescence, these pups have received intranasal saline on PND 34-47 every other day (7 days totally).

3. Saline + Insulin: the male pups that their dams have received saline on GD15. Then, during mid adolescence, these pups have received intranasal insulin on PND 34-47 every other day (7 days totally).

4. LPS (100) + Saline: the male pups that their dams have received LPS (100 µg/kg) on GD15. Then, during mid adolescence, these pups have received intranasal saline on PND 34-47 every other day (7 days totally).

5. LPS (250) + Saline: the male pups that their dams have received LPS (250 µg/kg) on GD15. Then, during mid adolescence, these pups have received intranasal saline on PND 34-47 every other day (7 days totally).

6. LPS (250) + Insulin: the male pups that their dams have received LPS (250 µg/kg) on GD15. Then, during mid adolescence, these pups have received intranasal insulin on PND 34-47 every other day (7 days totally).

Independent batches of rats were used for behavioral (n=8), electrophysiological (n=5), and molecular (n=4) assessments. Animals were randomly selected from different dams, and experimenters were blinded. A timeline diagram is shown in Figure 1.

MWM

The Morris Water Maze (MWM) test was conducted on postnatal day 50 (PND50) to assess spatial learning and memory capabilities in rats. This rats were obtained from the different dams. The experimental setup included a circular tank with a diameter of 160 cm and a height of 80 cm, filled with water to a depth of 40 cm. The pool was virtually divided into four quadrants (North, South, East, and West) of equal size. A hidden square platform measuring 10 cm per side was positioned 1.5 cm below the water surface, centered in the northeast quadrant. To support spatial navigation, distinct geometric cues were placed on the surrounding walls, and the lighting in the room was dimmed to minimize reflection on the water (78). Behavior was recorded and analyzed using the Noldus EthoVision XT software (version 7.1, Netherlands). The water temperature was maintained at 25 ± 2 ºC throughout the experiment. Each rat underwent three training blocks, with a 30-minute interval between blocks. In each block, animals completed four trials, each initiated from a different starting point, facing the tank wall. Each trial lasted up to 60 seconds, with a 60-second inter-trial interval. The platform’s location remained constant throughout the training phase. If the rat located the platform, it was allowed to rest on it for 20 to 30 seconds before being returned to its home cage for another 20 to 30 seconds, after which the next trial began. Escape latency and distance swum to reach the platform were recorded for analysis (79).

To assess spatial memory, a probe trial was carried out two hours after the last training session. During this 60-second trial, the platform was removed, and the time spent and distance covered in the target quadrant, the one that previously contained the platform, were recorded as indicators of memory retention. Following the probe trial, a visible platform test was performed to evaluate the animal’s visual acuity, motor function, and motivation. In this test, the platform was raised 2 cm above the water surface and covered with aluminum foil to make it easily visible (78,79).

Electrophysiological study

The electrophysiological study was conducted from PND 48 to PND 50. In vivo recordings of field excitatory postsynaptic potentials (fEPSPs) in the CA1 region of the hippocampus were performed following the protocol described by Rajizadeh et al. (80). Wistar rats were anesthetized with urethane (1.2 g/kg; Sigma–Aldrich) and placed in a stereotaxic frame. After exposing the skull, two small holes were drilled under sterile conditions based on coordinates from the Paxinos and Watson atlas (2006) to allow insertion of the stimulating and recording electrodes. Body temperature was maintained at 36.5 ± 0.5 °C using a heating system (Harvard Apparatus). To stimulate the Schaffer collateral pathway, a concentric bipolar electrode (0.125 mm, stainless steel, Advent, UK) was positioned ipsilaterally (AP: 3 mm; ML: 3.5 mm; DV: 2.8–3 mm). The recording electrode was placed in the stratum radiatum of the CA1 area (AP: 4.1 mm; ML: 3 mm; DV: 2.5 mm) (81). Stimuli were delivered through a constant-current stimulator, and signals were recorded via an amplifier. After a 30-minute stabilization period, input-output (I/O) curves were obtained by gradually increasing stimulus intensity and measuring corresponding fEPSP responses (82). Field potentials were amplified and band-pass filtered (1 Hz–3 kHz). A baseline was established by delivering test pulses every 10 seconds for 20 minutes, using an intensity that evoked approximately 50% of the maximum fEPSP response. Before inducing long-term potentiation (LTP), paired-pulse facilitation (PPF) was evaluated using ten consecutive paired-pulse stimulations at inter-pulse intervals of 20, 50, 70, and 100 ms (0.1 Hz) (83). PPF was expressed as the ratio of the second fEPSP slope to the first (fEPSP2/fEPSP1). Early-phase LTP (E-LTP) was induced via high-frequency stimulation (HFS; 10 pulses at 400 Hz for 7 seconds, repeated for 70 seconds) (84). LTP maintenance was monitored for 2 hours post-HFS by delivering test pulses every 10 seconds. Each data point represented the mean slope of ten consecutive fEPSPs. Data acquisition and stimulation were controlled using Neurotrace software (version 9) and Electromodule 12 (Science Beam Institute, Tehran, Iran). Response analysis was performed using Potentialise software from the same institute (80).

RNA extraction, cDNA synthesis, and real time PCR

Euthanasia was performed by CO_2 inhalation followed by decapitation, in accordance with the ethical guidelines approved by the Institutional Animal Research Ethics Committee of Kerman University of Medical Sciences. Hippocampal tissue samples were randomly collected from male pups on PND48 and stored at −80 °C until homogenization (n = 4). According to the protocol of Mohammadipoor-Ghasemabad (85), RNA extraction was conducted using RNX-plus reagent. For cDNA synthesis, equal amounts of isolated RNA were used. Random hexamer and oligo (dT) primers were utilized to synthesize cDNA for evaluating the PSD-95 and BDNF, respectively. cDNA synthesis was performed by incubating RNA with primers and DEPC-treated water at 70 ◦C for 5 minutes, followed by addition of dNTPs, reverse transcriptase, buffer, RNasin, and incubation under specified conditions.

Real-time PCR was performed in 20-µl reaction volumes. Each reaction contained 10 µl of qPCR master mix, 1 µl each of forward and reverse primers, 2 µl of cDNA, and 6 µl of RNase-free water. The sequences of primers from 5' → 3' were as follows: BDNF-forward: AAATAAAAGGAGCCCCATCAC, BDNF-reverse: GAAACAGAACGAACAGAAACAG, PSD-95-forward: CACACCTCCCACCTTTTTC, PSD-95-reverse: TGTCTGTCTCTTCCTTTCACTC, β-Actin-forward: CCCAGAGCAAGAGAGGCATC, and β-Actin-reverse: GCCTTAGGGTTCAGAGGGGC.

Statistical analysis

The results were expressed as mean ± SEM. Given that the parameters had a normal distribution, parametric statistics were employed. A repeated-measures two-way analysis of variance (ANOVA) was employed to assess the differences in learning among groups in the Morris water maze (with groups and blocks as variables) and to evaluate overall differences in LTP across time points (with group and time as factors). One-way ANOVA was employed to assess the MWM probe experiment and molecular data. When significant differences were found, Tukey's multiple-comparison test was used as a post-hoc analysis. Differences among groups were considered statistically significant at $P < 0.05$. Statistical analyses were conducted using Graph Pad Prism 9.5.1 (Graph Pad Software, Inc., San Diego, CA).

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Acknowledgements This study was supported by Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.

Funding

In this project, funding experiments was provided by Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.

Author's contributions

Hadis.K. and Haniyeh.K. contributed to modeling, performed the behavioral experiments and molecular assessments, analyzed the data and wrote the manuscript. M.R. contributed to electrophysiological experiment. M.A. contributed to modeling and behavioral experiments. L.M. contributed to molecular assessments. S.J. designed and supervised the project and contributed to interpretation of the results, and revised the manuscript. V.Sh. supervised the project.

Ethics declarations

All experiments were done in accordance with the ARRIVE guidelines and National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996). All experimental procedures were conducted in accordance with institutional and international guidelines for animal care, as approved by the Institutional Animal Research Ethics Committee of Kerman University of Medical Sciences (Ethics code: IR.KMU.AEC.1402.84).

Conflict of interest The authors declare no competing interests.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Figure legends:

Fig. 1 Timeline diagram. Pregnant dams received an intraperitoneal injection of either LPS or saline on GD 15. Male offspring were raised under standard conditions until PND 34. From PND 34 to PND 47, intranasal insulin or saline was administered every other day (seven doses in total). Electrophysiological recordings and molecular analyses were performed on PND 48. Spatial learning and memory were assessed using the Morris water maze on PND 50. Independent cohorts of animals were used for behavioral, electrophysiological, and molecular experiments.

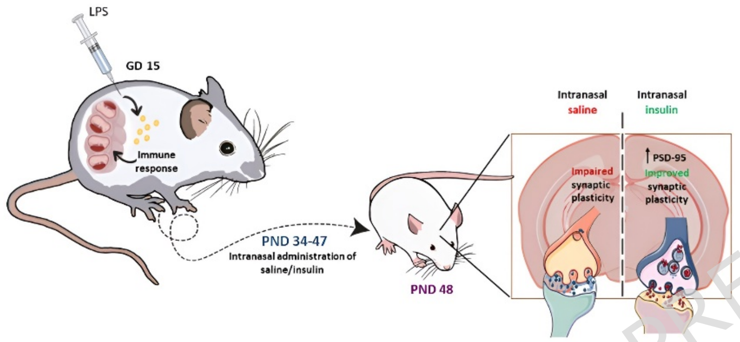
Fig. 2 The effects of intranasal insulin on MIA on MWM parameters. Total distance moved (a) and also escape latency onto the hidden platform (b) were increased in LPS(250)+Saline group (2 way ANOVA, Rereated measures). Distance (c) and time (d) percent in target quadrant were decreased in LPS (250)+ Saline group and they were increased in LPS(250)+Insulin group. The data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 compared to saline+saline group (n = 8).

Fig. 3 The effects of intranasal insulin on MIA-induced LTP impairments. (a) fEPSP slope (% of baseline) comparison among groups. (b) Input/output curve. Constant current stimulation was used and the stimulus intensity is represented in units of microamperes. There was no significant difference in the input/output curve among all the groups (p < 0.05). (c) paired pulse facilitation (PPF). PPF ratio was not significantly different among groups. (d) Sample records of fEPSP for all groups. The data are presented as mean ± SEM. *p < 0.05, **p < 0.01, compared to saline+saline group. #p < 0.05 compared to LPS (250)+Saline group. Calibrations (500 mv/5 ms) were applied throughout the recordings (n = 5).

Fig. 4 The effects of MIA and intranasal insulin on the expression levels of BDNF and PSD-95. (a) BDNF expression, there was no significant difference among groups. (b) PSD-95 expression, PSD-95 mRNA level was increased in LPS(250)+insulin group compared to LPS and saline groups. The data are presented as mean ± SEM. **$p < 0.01$, compared to saline+saline group. ##$p < 0.01$ compared to LPS (250)+Saline group ($n = 4$).

Figures:

Graphical abstract



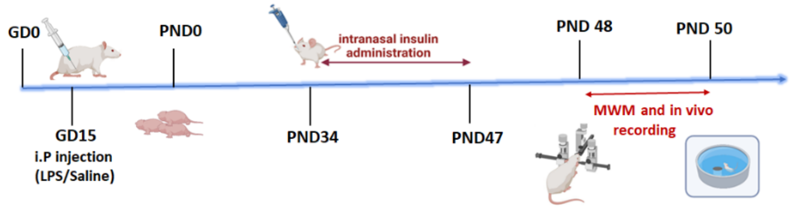
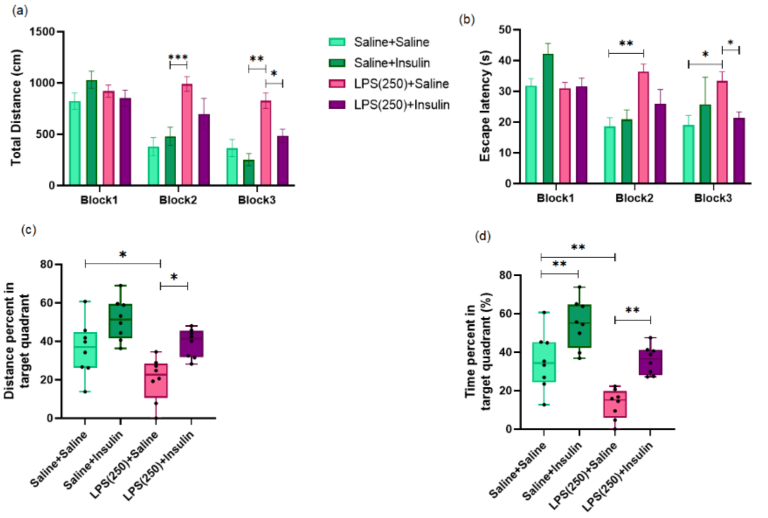


Fig. 1


(a)
1500
1000
500
0
Total Distance (cm)
Block1
Block2
Block3
***
**
*
Saline+Saline
Saline+Insulin
LPS(250)+Saline
LPS(250)+Insulin
(b)
50
40
30
20
10
0
Escape latency (s)
Block1
Block2
Block3
**
*
*
(c)
80
60
40
20
0
Distance percent in target quadrant
*
*
Saline+Saline
Saline+Insulin
LPS(250)+Saline
LPS(250)+Insulin
(d)
80
60
40
20
0
Time percent in target quadrant (%)
**
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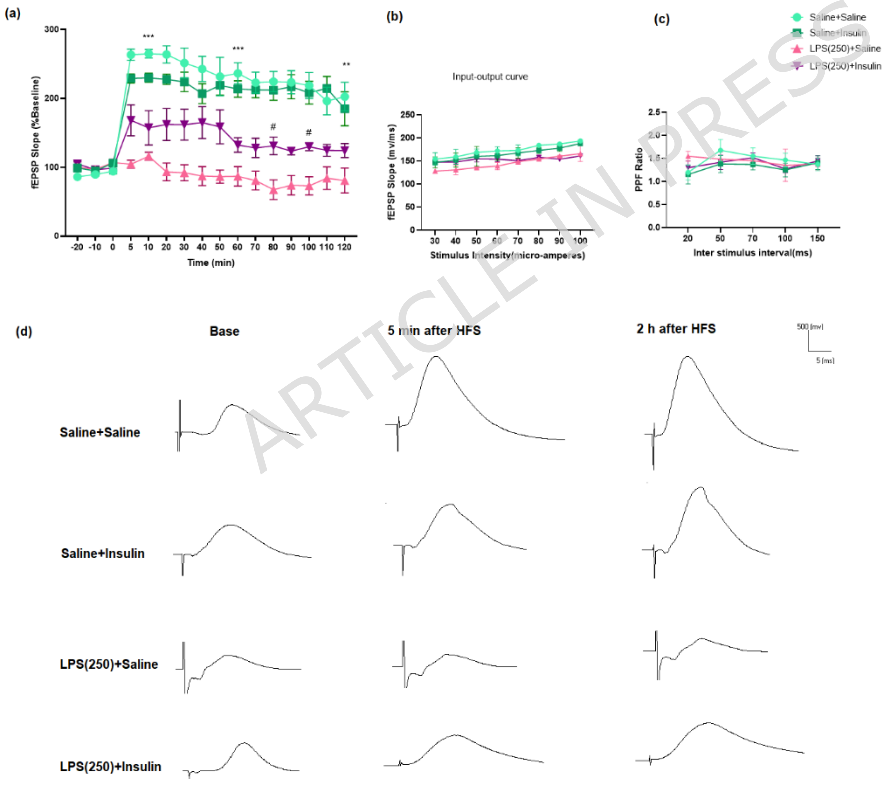
**
Saline+Saline
Saline+Insulin
LPS(250)+Saline
LPS(250)+Insulin

Fig.2


(a)
300
200
100
0
fEPSP Slope (%Baseline)
***
***
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#
#
-20 -10 0 5 10 20 30 40 50 60 70 80 90 100 110 120
Time (min)
(b)
Input-output curve
250
200
150
100
50
0
fEPSP Slope (mv/ms)
30 40 50 60 70 80 90 100
Stimulus Intensity(micro-amperes)
(c)
Saline+Saline
Saline+Insulin
LPS(250)+Saline
LPS(250)+Insulin
2.5
2.0
1.5
1.0
0.5
0.0
PPF Ratio
20 50 70 100 150
Inter stimulus interval(ms)
(d)
Base
5 min after HFS
2 h after HFS
500 (mv)
5 (ms)
Saline+Saline
Saline+Insulin
LPS(250)+Saline
LPS(250)+Insulin

Fig. 3

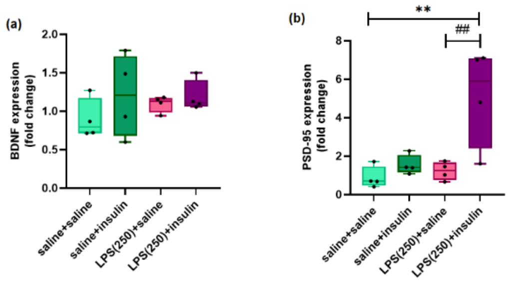

Fig. 4

Tables:

Table. 1. Swimming velocity (cm/s) of experimental groups (Mean±SEM)

Blocks	Saline+Saline	Saline+insulin	LPS(250)+Saline	LPS(250)+insulin
1st	26.79 ± 1.44	25.11 ± 0.67	32.68 ± 1.50	28.12 ± 1.05
2nd	18.18 ± 1.06	21.57 ± 1.41	25.96 ± 0.99^{***}	24.17 ± 1.85
3rd	17.37 ± 1.39	15.27 ± 1.07	23.13 ± 1.16^{*}	21.22 ± 1.47

The data are presented as mean ± SEM. *p < 0.05, ***p < 0.001, compared to saline+saline group.