

Effects of leptin on neurocognitive and motor functions in juvenile rats in a preterm brain damage model

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Abstract. Preterm infants face lifelong disabilities, including learning disorders, as well as visual, auditory and behavioral problems. Recent studies have demonstrated that leptin, an adipocytokine encoded by a gene associated with obesity and expressed in adipose tissue, affects neurocognitive and motor function; however, the mechanisms of brain damage in preterm infants are unclear. In the present study, the neuroprotective effects of leptin in a rat model of preterm hypoxic-ischemic brain damage were investigated. Rats (2-days-old) were subjected to brain damage (ligation of the common carotid artery followed by exposure to 6% oxygen for 2 h) and treated with vehicle (control) or leptin. Spatial memory was analyzed in the present study using the Morris water maze test 19 days following ligation. Over the 24-day post-surgical observation period, capture-resistance test, forelimb suspension and open field tests were conducted to evaluate motor function and anxiety-associated behavior. Treatment with leptin did not affect survival rate or body weight. Treatment with leptin increased the number of platform crossings in rats with premature brain damage in the Morris water maze test, which was used to assess spatial memory. Multivariate analysis revealed that leptin reduced the latency to finding the platform location, independent of gender and weight. In the capture-resistance, forelimb suspension and open field tests, there were no differences between animals administered leptin and the sham group. Collectively, the results of the present study suggested that leptin may alleviate spatial memory impairment resulting from premature brain damage, independent of gender or weight. These results may improve understanding of the neuroprotective effects exhibited by leptin in infants with preterm brain damage.

Introduction

Leptin, an adipocytokine encoded by an obesity-associated gene expressed in adipose tissue, affects feeding behavior, thermogenesis and neuroendocrine status via leptin receptors distributed in the brain, particularly in the hypothalamus (1). Recently, studies have investigated the role of leptin in non-hypothalamic areas, including regions associated with learning and memory, and cognitive function (2-7). An indicator that leptin may affect cognitive function is that the leptin receptor is expressed throughout the brain (2). Leptin receptors, particularly M-type leptin receptors, are highly expressed within the inner regions of the hypothalamus; however, they are also expressed in other areas of the brain, including regions associated with learning and memory, such as various cortical regions and the hippocampus (3-7). A potential regulatory role of leptin within these brain regions is that it may be associated with the effects of diet and obesity on cognitive function (8,9). Alterations in caloric intake or dietary composition are associated with the dysregulated gene expression profiles of the hippocampal and cortical areas, involved in glycolysis, protein deacetylation, PGC-1 α and mTor pathways, suggesting that these brain regions may be associated with changes in nutritional and metabolic status (8). Additionally, changes in nutritional status can also alter cognitive function; obesity has been associated with cognitive decline (9).

A number of studies investigating the role of leptin in a variety of brain regions and signaling systems have provided notable support for a neuroprotective role of the hormone (10-14); however, the underlying mechanisms remain unclear. Previous studies have demonstrated that leptin affects synaptic function at the molecular and cellular levels, as well as neural structures, suggesting that the peptide serves important diverse roles in the brain (10). Numerous investigations into the effects of leptin on the structure and function of the hippocampus, cortex and other areas of the brain have been conducted; such research has mainly focused on the hypothalamus (3-7). Few studies have examined the role of leptin signaling and resistance in non-hypothalamic regions. Furthermore, a small number of studies have mainly focused on adult neurocognitive diseases (11-14); the neuroprotective effects of leptin on brain damage resulting from premature development remain unknown.

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Across 184 countries, the rate of preterm birth (<37 weeks' gestation) ranges from 5 to 18% of total births, with an estimated 15 million babies born preterm every year; this number is increasing annually (15). Complications arising from preterm birth are the leading cause of mortality among children under 5 years of age, and have been associated with almost 1 million cases of mortality in 2013 (15). A total of 75% of these babies may be saved with current, cost-effective treatments; however, numerous survivors endure lifelong disabilities, including learning disorders, and visual and hearing issues (15). According to the U.S. Centers for Disease Control and Prevention, very early preterm births (<32 weeks' gestation) account for 16% of the total number of preterm births, in the USA (16). It has been reported that ~10% of the preterm births with gestational ages <32 weeks and birth weights <1,500 g exhibit defects in locomotion, whereas ~60% of infants possess neurocognitive disabilities and/or behavioral problems (16,17). The most common defect in preterm infants is periventricular leukomalacia (PVL) and the incidence of PVL in preterm infants has been reported to be >50% (18,19). At present, the mechanisms underlying premature brain damage are unclear, yet hypothermic, stem cell-associated and other types of therapeutic strategies have been reported (20); however, the majority of these applications lack efficacy. Therefore, it is important to develop novel, safe and effective treatment methods.

Hypoleptinemic patients have been reported to exhibit impaired cognitive flexibility and decreased visuospatial abilities when compared with their healthy counterparts (21-23). Individuals with early Alzheimer's disease or mild cognitive impairment with low plasma leptin levels may benefit from leptin replacement therapy (24). Elevated circulating leptin has been consistently detected in childhood neurodevelopmental disorders, including autism spectrum disorders and Rhetts disorder (1). Leptin treatment of neonates may reverse the hippocampal and frontal cortical changes that occur in rats as observed within a maternal deprivation model (25); however, it remains unknown whether these biological effects of leptin also manifest in the premature brain, or whether the preterm brain requires leptin. The umbilical cord blood leptin can be detected at the earliest 18 weeks of pregnancy and increase sharply during the 34 weeks of pregnancy. The development of fetal adipose tissue and the storage of fat are the determinants of leptin levels in the fetus (26,27). The development of adipose tissue in preterm infants may be delayed compared with in term infants, potentially resulting in leptin deficiency. Veselá *et al* (28) reported that the median cord blood concentration of leptin was 3.07 $\mu\text{g/l}$ and the median cord blood concentration of leptin in infants at 32-33 weeks' gestation was 2.89 $\mu\text{g/l}$, which was lower than the 3.13 $\mu\text{g/l}$ observed in preterm infants at 34-36 weeks' gestation. Nagasaki and Ohta (29) revealed that the median serum concentration of leptin at 33-38 weeks' gestation was 2.3 ng/ml (range: 1.0-3.9 ng/ml).

To investigate the potential protective effects of leptin in the developing brain, the present study investigated the neurocognitive and motor functional effects of leptin treatment in a rat model of premature brain damage. In addition, Oomura *et al* (30) reported that leptin possesses an inverted-U-shaped dose-effect associated with spatial learning and memory tasks with an optimal dose of 50 $\mu\text{g/kg}$. Therefore, this particular dose was utilized in the present study.

Materials and methods

Animals. The present study was approved by the Institutional Animal Care and Use Committee of Southeast University (Nanjing, China). Pregnant Sprague Dawley rats (days 18-19 of the estrous cycle) were obtained from Nanjing Medical University of China (Nanjing, China) and allowed to deliver (10-14 pups per dam). The pups' birth weight was 5.5-7 g, with an average of 6.5 g. A total of 41 pups were employed in the present study. The pups were housed with their mothers under a constant 12-h dark/light cycle with free access to food and water. Room temperature at 18~26°C, relative humidity 40-70%, without noise, ammonia concentration below 20 ppm, ventilation 8-12 times/h. Pups of each litter were randomly assigned to all experimental groups, with 13 in sham group, 14 in model and 14 in leptin group. A total of 36 rats were survival to 21-days-old and used in the behavioral experiments, with 6 male rats and 6 female rats in each group, while 5 of them perished following the model establishment.

Preterm brain damage model. Preterm human infants of 24-32 weeks' gestation are at high risk of developing PVL (31). The oligodendrocytes in the white matter 2-5 days following birth mainly constitutes late oligodendrocytes, similar to the peak period of PVL in human preterm infants (32). Therefore, the present study used postnatal day 2 rat pups for the subsequent experiments. The 2-day-old pups underwent permanent ligation of the right common carotid artery under a shadowless lamp. Following anesthesia via isoflurane inhalation for 1-2 min, rats were fixed on the operating table, a midline incision length 1.0 cm was performed on the neck. For both model and leptin groups, ligation of the right common carotid artery was performed with a 5-0 suture, followed by suturing of the skin. Surgery was conducted for <10 min; animals with excessive bleeding were excluded. The pups in the model and leptin groups were then returned to their home cage with their dam for 2 h, and thereafter removed from their dam and exposed to hypoxia (94% N₂/6% O₂) for 2 h in a sealed chamber partially submerged in a 37°C water bath (31). This procedure induced a major lesion in the periventricular white matter. By the end of the hypoxic treatment, pups were returned to their dam for recovery. In sham-operated rats, the same surgery procedure was performed without ligation or exposure to hypoxia.

Drug treatment. Following exposure to hypoxia, at the end of the ventilation period that ensured hypoxia, leptin was administered to the pups (2-5 days old) once daily as an intraperitoneal injection of recombinant murine leptin (50 $\mu\text{g/kg/day}$, diluted with normal saline, up to 0.1 ml/g; PeprTech EC Ltd., London, UK) for 4 days. The model group was administered an equal volume of saline following exposure to hypoxia, without leptin treatment. In addition, the sham group was treated with an equal volume of saline at the same time as the other two groups.

Observation of survival and monitoring of body weight development. The survival rate was observed to 21-days-old. Weight was measured on 0, 2, 3, 4, 5, 7, 10, 14, 17, 21 days of age at 8:00 a.m., prior to the change of the padding materials and feeding them. The weight was measured by the body

weight meter, accurate to 0.10 g, calibrated and zeroed before the measurement.

Evaluation of neurocognitive motor function. These experiments were conducted on postnatal days 27-30, as described below.

Resistance to capture. With sterile gloves, a researcher manually captured the rats individually, in a gentle manner to observe its reaction. The behavior of the rats (postnatal days 27-30) was then subsequently scored. The scoring scale employed was as follows: 0, easy to grab; 1, screaming or avoidance; 2, screaming and avoidance; 3, escape; 4, escape and screaming; 5, bite or attempt to bite the gloves; and 6, active jumps and attacks. The purpose of this procedure was to observe the emotional behavior of the animals.

Suspension test. This experiment was designed to assess the forelimb grip of the rats. The rats (postnatal days 27-30) were allowed to catch a level metal rod (diameter, 0.5 cm, length, 50 cm) with both forelimbs, and the rod was then raised 45 cm above the ground. The duration of grasp for each rat was then recorded.

Open field test. This experiment was performed to evaluate exploratory behavior and anxiety in a new environment. The experimental setup comprised a 45x45x45 cm carton without a lid. The base of the box was divided into a grid of nine equal regions, marked with black ink. Rats were placed in the central square and covered with a small paper box. Subsequently, following 30 sec, the box was lifted, and the rats were allowed to move freely for 90 sec. The frequency that more than half of the body was in an adjacent square or when the animal was standing up on its hind legs was recorded. In-house scoring criteria: More than half of the body in an adjacent square, 1 point; every incidence of standing up on hind legs, 1 point; and grooming and defecation, 1 point each. The total score was then calculated for each animal.

Morris water maze test. The Morris water maze test was performed when the rats were 21-28 days old and was used to evaluate spatial memory learning ability. The Morris water maze test is the most objective method of assessing learning and memory function currently available (30). The maze consists of a circular water tank and an automatic photographing and analysis system. The automatic image acquisition and processing system is comprised of a camera, computer and image monitor. As soon as the animals are placed in the water, the monitoring device that records the path of animal movement is activated; the analysis of the relevant parameters is automatic. The experimental procedures included the following: i) Place navigation, which was used to measure the learning and memory ability of rats in the water maze (this experiment lasted 5 days and included training the animals to find the platform four times a day at a fixed time); and ii) a spatial probe test, which was used to assess memory retention of the spatial location following learning to find the platform. At the end of the navigation experiment, the platform was removed, and the animal was placed at the same point of entry into the water, and the time of first arrival at the platform

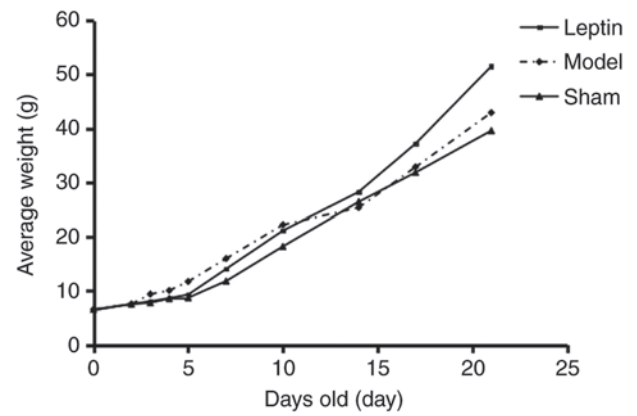


Figure 1. Weight development of neonatal rats within 21 days of age in the three groups. The average weight of the model group within 10 days of age was higher than that of the leptin-treated group. No significant differences across the three groups within 2 days of age were observed. From 3-5-days-old, the weights of leptin-treated rats were lower than that of the model group; however, following 5 days of age, the weight of leptin-treated rats increased. In 10-14 days-old leptin-treated rats possessed greater average weights than the model and sham group rats.

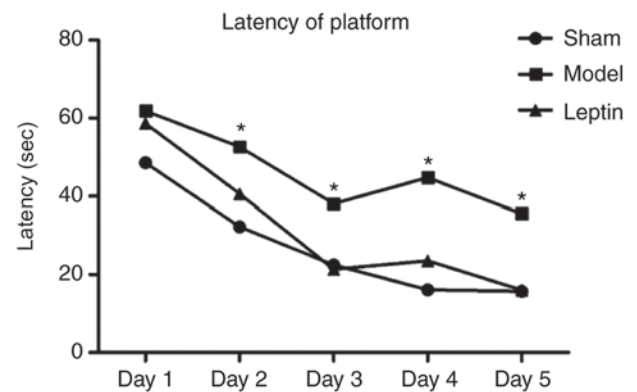


Figure 2. Latency of navigation training in the Morris water maze in three groups. The average latency in the model group was longer than of the sham group from day 2 and longer when compared with the leptin-treated and sham groups. No significance differences were observed between the leptin-treated and sham groups. *P<0.05 vs. Leptin.

and the number of crossings of the original platform location were recorded.

Statistical analysis. SPSS 19.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses conducted in the present study. Numerical data were presented with the mean \pm standard and were analyzed using one-way analysis of variance, followed by a Student-Newman-Keuls (SNK-q) test, for pairwise comparisons. For categorical data, the χ^2 test was conducted for analysis; multivariate linear regression was used for multivariate analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

General health observations. A total of 36 rats survived following the establishment of the preterm brain damage model, with 12 in

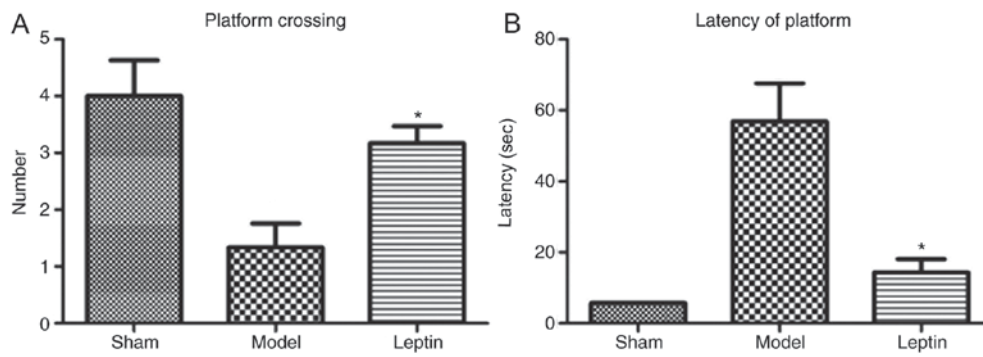


Figure 3. Results of the Morris water maze test within 90 sec in three groups. The distribution difference was statistically significant among the three groups ($P < 0.01$). (A) By using Student-Newman-Keuls test for pairwise comparison, the average frequency of crossing the platform in the leptin-treated group seemed higher than that of the model group, but this was not statistically significant. (B) Average latency of finding the platform in the leptin-treated group was shorter than that of the model group ($P = 0.015$), while compared with the sham group, the difference was not statistically significant. * $P < 0.05$ vs. Model.

each group. The mortality rate was 12.20% (36/41). Body weight was examined up to 21 days of age. One-way analysis of variance was used to compare developmental differences in body weight among the groups. The average weight was higher in the model group when compared with leptin treatment and the sham group, prior to 10 days of age (Fig. 1). The differences among the three groups prior to 2 days of age were not significant. From 3-5 days of age, the weight in the leptin-treated group decreased; however, after 5 days of age, weight in the leptin-treated group increased. Additionally, at 10-14 days, the average weight was higher in the leptin-treated group than in the remaining groups (Fig. 1). It may be suggested that leptin intervention could control the growth of body weight after HI, and the growth accelerated following the withdrawal of drug, leptin intervention had no adverse effect on the long-term weight growth.

Morris water maze test

Latency and platform crossings. The average latency of finding the platform was significantly longer in the model group than in the other groups from day 2. The difference between the leptin-treated and the sham groups was not significant ($P > 0.05$) as determined by the SNK-q test for pairwise comparisons (Fig. 2). The number of platform crossings was 1.33 ± 0.41 in the model, 3.17 ± 0.32 in the leptin-treated and 4.00 ± 0.61 in the sham groups. The difference between the three groups was statistically significant ($F = 8.306$, $P = 0.004$; Fig. 3A). The latency for finding the platform was 56.84 ± 26.454 sec in the model group, 14.27 ± 9.167 sec in the leptin-treated group and 5.67 ± 0.279 sec in the sham group. The difference between the three groups was statistically significant ($F = 17.238$, $P < 0.0001$; Fig. 3B). Additionally, the typical trajectories of the three groups are presented in Fig. 4. Using the SNK-q test for pairwise comparisons, the average number of crossings of the platform in the leptin-treated group was significantly higher than that of the model group ($P = 0.015$; Fig. 3A). The average latency to the platform was shorter in the leptin-treated group than that of the model group ($P < 0.05$; Fig. 3B). The number of platform crossings and latency to the platform in the leptin-treated group were not significantly different when compared with in the sham group.

Multivariate analysis. Regarding the latency to the platform as the dependent variable, and including gender, treatment, weight

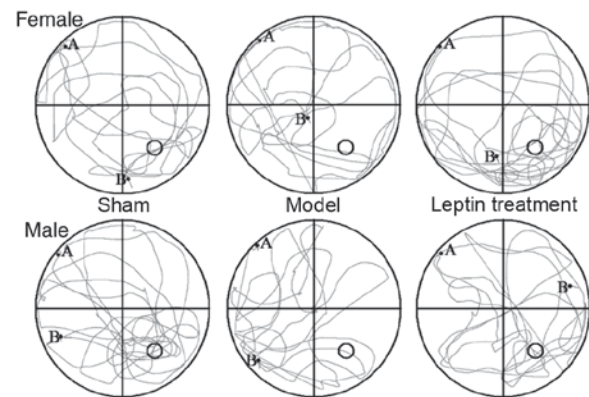


Figure 4. Representative typical trajectory between the three groups. The trajectory of the leptin-treated group was similar to that of the sham group, but notably different with that of the model group. In the leptin-treated group, the number of platform crossings within 90 sec was higher and the average latency of time spent finding the platform was shorter; the distance spent in the platform quadrant was greater compared with in the sham and model groups. Point A, water entry point, located opposite the platform; Point B, cutoff point, which varied in each experiment animal; O indicates the platform.

on day 1 and age, multivariable linear regression analysis was performed with an inclusion criterion of 0.05 and an exclusion criterion of 0.10. Latency was significantly correlated with treatment and age ($P < 0.05$). Latency to the platform was decreased in response to leptin treatment and age (Table I). It could be suggested that training could improve the performance of the model group, but still lag behind the sham group, intervention strategies, including drug therapy, is needed to promote brain injury rehabilitation. Early intervention of leptin and training can promote the recovery of brain injury and make them tend to be normal.

Capture-resistance, suspension and open field tests. In the capture-resistance test, the differences in capture-resistance scores among the three groups were not statistically significant ($F = 2.174$, $P = 0.131$; Fig. 5A); in the forelimb suspension test, the differences in grasp times among the three groups were also not significant ($F = 2.267$, $P = 0.120$; Fig. 5B). In the open field test, the differences in defecation among the three groups were also not significant ($F = 2.741$, $P = 0.080$; Fig. 6A). The differences in grid crossings among the three groups were

Table I. Multivariate linear regression of latency of rats in the platform.

Variable	Assignment method	Standard coefficient	t-score	P-value	Non-standard coefficient (95% CI)
Treatment	1=Model 2=Intervention ^a 3=Sham	-0.607	-3.854	0.001	-13.201 (20.325, -6.077)
Days old	Actual value	-0.903	-5.734	<0.0001	-9.208 (-12.548, -5.868)
Constants	-	-	6.216	<0.0001	315.338 (209.847, 420.829)

^aIntervention represents the leptin-treated group.

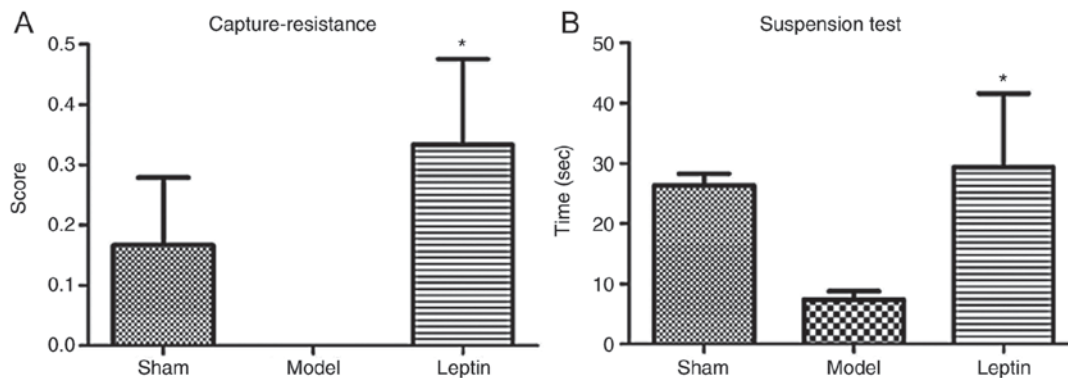


Figure 5. Score of capture-resistance and the time of suspension in three groups. (A) The distribution difference was not statistically significant among the three groups; however, following further pairwise comparisons, the average score of capture-resistance in the leptin intervention group was significantly higher than that of the model group (SNK-q analysis, $P=0.046$). (B) Duration of suspension in the leptin-treated group was longer than that of the model group (SNK-q analysis, $P=0.05$). * $P<0.05$ vs. Model. SNK-q, Student-Newman-Keuls.

also not significant ($F=2.265$, $P=0.121$; Fig. 6B). In addition, the differences in grooming ($F=2.008$, $P=0.151$; Fig. 6C), standing posture ($F=1.172$, $P=0.323$; Fig. 6D) and in total scores ($F=0.373$, $P=0.692$; Fig. 6E) among the three groups were not statistically significant.

The majority of the investigated parameters among the three groups were not statistically significant; however, similarities were observed in capture-resistance scores, duration of suspension and grid crossings across the leptin-treated and sham groups. Therefore, the SNK-q test for further pairwise comparison. This analysis revealed that the average capture-resistance scores were significantly higher in the leptin-treated group than in the model group ($P=0.046$; Fig. 5A); the duration of suspension was longer in the leptin-treated group when compared with the model group ($P=0.055$; Fig. 5B). In addition, the number of grid crossings was significantly higher in response to leptin treatment when compared with the model group ($P=0.045$; Fig. 6B). It may be suggested that early intervention of leptin may result in the recovery of forearm grasping ability, even could close to normal, resulting to more positive emotional reactions, more avoidance and exploratory behavior.

Discussion

With advances in perinatal technologies, the survival rates of premature infants, have increased; however, cognitive impairments and behavioral issues still exist (15). The main pathological feature of brain injury in preterm infants is

periventricular leukomalacia, particularly in infants born at 24-32 weeks' gestation (32). The model used in the present study was based on the hypoxic-ischemic brain damage model developed by Rice *et al* (31). In this model, necrosis of the white matter was reported and was greater ipsilaterally, originating and spreading from myelinogenic foci, similar to the features of brain injury in preterm infants (31). In the present study, 2-day-old neonatal Sprague Dawley rats were selected to establish the preterm brain damage model as brain development in 2-day-old rats is highly similar to that in severely preterm human infants (24-32 weeks' gestation). Rats that are 21-days-old (approximately equivalent to human childhood) are able to live without their mother; at this point, the establishment of memory and emotional responses is initiated (33).

The present study reported that leptin-treated rats had lower weights during treatment, but their weights increased following leptin withdrawal following the 4-days treatment, and the leptin-treated group gained more weight. Leptin regulates energy intake and expenditure (34). The results of the present study suggested that leptin may serve a role in growth control; however, leptin did not notably affect weigh. This finding is consistent with those of Nagasaki and Ohta (29), in which serum concentrations of leptin were not correlated with body weight at any time point in infants.

The protective effects of leptin on spatial memory appeared to be more significant than the effects on emotion and motor function. Differences in latency of navigation training between the three groups were statistically significant

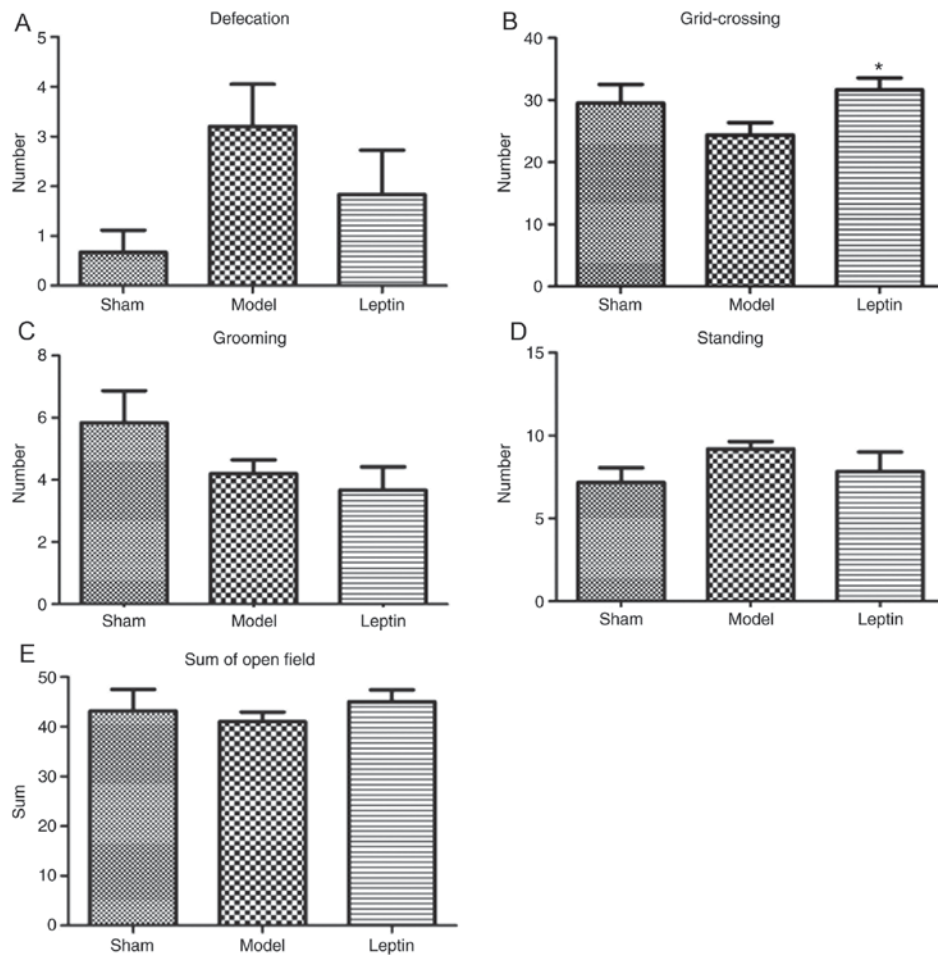


Figure 6. Results of open field test between the three groups. The results of (A) defecation, there was no statistical significance among the three groups, (B) grid-crossing, there was no statistical significance among the three groups, but a trend (Student-Newman-Keuls analysis, $P=0.045$), (C) grooming, with no statistical significance among the three groups, (D) standing, with no statistical significance among the three groups and (E) sum of the open field tests across the three groups, with no statistical significance among the three groups. * $P<0.05$ vs. model.

from the second day. In the water maze test, leptin significantly increased the number of platform crossings and reduced the platform latency when compared with the model group; the observations of leptin were similar to those of the sham group. In addition, multivariate analysis demonstrated that the spatial memory-enhancing effect of leptin was independent of gender or weight, but was correlated with age; however, whether spatial memory is similarly affected by leptin in adult rats is unknown. Childhood and adolescence are important periods for learning and memory development. Disruptions during these periods may result in notable life-long adverse effects (33). These results are consistent with reports indicating that leptin treatment can improve cognitive abilities (30,41); however, Oomura *et al* (30) observed this within 6-8-week-old rats without any disease. The effects between 50 $\mu\text{g}/\text{kg}$ leptin treatment and the control group were significant from day 2 (30). Rats treated with 50 $\mu\text{g}/\text{kg}$ leptin exhibited a significantly shorter duration in the goal area on test day, compared with the group that received the vehicle control (30).

In the capture-resistance, suspension and open field tests, there was significant differences between leptin and model group, and the majority of tested parameters revealed similar values of the leptin-treated and sham groups. This suggested that leptin treatment partially, but not completely, alleviates

motor impairment. Furthermore, leptin may induce a more positive emotional state (34). Diabetic rats consistently exhibit greater anxiety-associated behavior and lower leptin levels in the blood (35). Chronically stressed rats exhibit reduced leptin levels and depression-like symptoms, which may be reversed upon treatment with leptin (36). In present study, the leptin-treated group had more evasive, anxious and even aggressive behavior than the model group. These observations supported the findings of the capture-resistance test.

Numerous studies on the role of leptin in various brain regions and signaling systems provide strong support for a neuroprotective role of leptin (37-43). There is evidence that leptin promotes neurogenesis in the adult hippocampus *in vitro* and *in vivo* (37-39). Furthermore, the early use of leptin has been reported to promote the proliferation of astrocytes in the hypothalamus (40). The leptin antagonist L39A/D40A/F41A inhibits leptin-induced alterations in somatostatin receptors, leptin signaling and cyclic adenosine monophosphate response element binding protein activation (41). Chronic leptin treatment has been reported to reverse $\text{A}\beta$ -induced deficits in learning and memory, and contributes to the maintenance of late phase-long term potentiation (42). Leptin levels are decreased in the neonatal hypoxic-ischemic rat brain, and leptin treatment may improve neuronal density

and decrease apoptosis in newborn rats with hypoxic-ischemic brain injury (43).

The intracellular signaling mechanisms involved in leptin signaling include the Janus kinase 2, signal transducer and activator of transcription, phosphoinositide 3-kinase, protein kinase B, mammalian target of rapamycin and extracellular signal-regulated kinase signaling pathways (1). The mechanisms underlying the effects of leptin on mood, psychiatric disorders and memory are unclear; however, they may be associated with serotonin signaling, via the inhibition of nitric oxide synthase (NOS) (44). The various isoforms of NOS may affect learning and memory via different sites of action, such as learning rate that is correlated positively with neuronal NOS (nNOS) and negatively with inducible NOS (iNOS) level. It may also be involved in the neuroprotective mechanism of leptin, which could serve as a direction for further research on mechanism. (44). However, it has been reported that the overall levels of hippocampal NOS were higher in rats with reduced learning abilities due to increases in memory errors (44). Thus, the inhibitory action of leptin on NOS may promote memory within the hippocampus; further investigation is required as numerous reports have revealed that NOS and NO activities may promote learning and memory, and deficient NOS and NO activities may be associated with Alzheimer's disease and poorer memory function, underlying these observations may be the vasodilating effects of NO, rather than the direct effects on brain tissue (45,46).

Leptin has been demonstrated to be involved in the organization and maturation of the nervous system (47). For instance, leptin may promote the differentiation of glial cells in the brain during development, as mice lacking leptin possess fewer functional glial cells later in life due to improper differentiation during development (47). In addition, leptin also stimulates the proliferation of neuroblastoma and prevents apoptotic cell death via the regulation of apoptotic enzymes, including caspase-10 and the tumor necrosis factor-associated apoptosis-inducing ligand, which are critical for brain development (48). Additionally, in obese mice that lack leptin, myelination is impaired as it occurs less frequently and myelination density is reduced; myelination only partially recovers with post-natal leptin treatment and is not fully restored to that of the wild type (49). However, accumulating evidence has revealed that leptin serves an important role in mood, and other cognitive and behavioral disorders (34). Furthermore, leptin has been reported to be critical for normal brain growth, development and developmental maturation; however, the underlying mechanism of leptin in these processes requires further investigation. Additionally, whether the observed effects of leptin on cognition and behavior are mediated by an effect within discrete brain areas, including the hippocampus and cortex, or involve more global alterations in neural structure or synaptic function and plasticity is unknown. Future studies may be conducted to improve understanding within these fields of research.

The present study demonstrated that leptin may alleviate impairments in spatial memory resulting from premature brain damage. The results reported in the present study suggested that leptin may have therapeutic potential for the treatment of preterm infants with brain damage, and alleviate neurocognitive impairments and behavioral problems. However, prior to clinical application, further understanding of the underlying

mechanisms of neuroprotection associated with leptin is required, as well as determination of the optimal drug dose and administration protocol.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

LJ conceived and designed the study, and approved the final version of the manuscript. ECF performed the experiments, analyzed the data, and wrote the manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Animal Care and Use Committee of Southeast University (Nanjing, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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