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Memory-enhancing effects of the leptin hormone in Wistar albino rats: sex and generation differences

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Abstract: Leptin is a hormone secreted by adipose tissue that informs the brain about the fat stores of the body. In this study, we examined the effects of timed leptin injections on spatial memory performances of adult and juvenile male and female Wistar rats with the Morris water maze test. We applied the injections and conducted the training trials for 4 days. On the fifth day after leptin injections, the experiments were performed. The activities were recorded and analyzed with the Noldus Ethovision Animal Video Tracking System. The results show that the spatial memory performance of the rats was improved by the leptin injections since they shortened the latency to find the platform and elongated the time spent in the correct quadrant. The age of the rats was found to be important since the adults found the platform faster than the juveniles. There was an interaction effect between sex and generation in all parameters examined except for the time spent in the correct quadrant. The results of the present study suggest that leptin administration via timed injections strengthens spatial memory in Wistar albino rats, but this effect depends upon the sex and the generation of the rats.

Key words: Leptin, Morris water maze, spatial memory

1. Introduction

Leptin, the protein product of the obese gene, is a circulating hormone produced primarily by adipose tissue and is known to play a key role in regulating feeding and body fat stores (1,2). It is involved in various physiological events including reproduction, hematopoiesis, angiogenesis, wound healing, insulin secretion, and neuroprotection (3). The release of leptin exhibits a 24-h rhythm that varies in timing between different laboratory rodent species (4). In mice and rats, serum leptin concentrations and mRNA content of leptin in adipose tissue decrease in the light phase and increase in the dark phase (5–7). The stored fat in the white adipose tissue informs the central nervous system via leptin about the amount of energy available for different purposes, such as reproduction.

Synaptic plasticity and behavioral performance related to learning and memory may be modulated by the hormone leptin (8). There is growing evidence that ob-rb, the long form of the leptin receptor, is expressed in the other central nervous system regions such as the hippocampus, amygdala, and neocortex, which are known to be associated with memory and cognitive functions (8). Leptin receptors are found in the hippocampus and amygdala (8). Leptin exists in the hippocampus to facilitate long-term potentiating, which is important for the operating process of memory. Although a low dose of leptin increases long-term potentiating and behavioral performance, a high dose of leptin impairs them (8). In rodents, leptin receptor deficiency may lead to an impairment of spatial memory function (9).

Sex differences have been reported in terms of the amount of circulating leptin levels (10,11). Females generally have a greater percentage of body fat than males of similar body weight or body mass index; therefore, leptin levels are greater in females than in males of equivalent fat mass (12). There are 2 plausible explanations for this difference. First, females have more subcutaneous fat than males, and leptin secretion has been reported to be greater in subcutaneous adipocytes than omental ones for the same subject (13,14). The second is the endogenous hormonal milieu, with estrogens generally reported to increase leptin production (15) and testosterone or androgens to suppress leptin levels (16–18). Despite these endeavors in the literature, the effect of leptin with sex on the spatial memory still is not known. Given the fact that females have a higher level of leptin than males and leptin has a beneficial effect on learning, it can be hypothesized that females are better at spatial learning than males.

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Serum leptin concentration is highly correlated with age and adipose tissue mass (19,20). Rats tend to gain weight as they age. Total leptin mRNA levels in rats increase with age, peaking at 24 months, and are correlated with adiposity. Serum leptin levels increase with age, also peaking at 24 months, and are correlated with total leptin mRNA. The rate of increase in serum leptin is greater than the increase in adiposity with age, suggesting contributions from both the increase in leptin expression per unit of white adipose tissue (WAT) and the increase in WAT depot size (21). Given the literature finding that leptin facilitates learning, adults should show better learning performance than juveniles since the leptin levels are higher in adults than juveniles. However, such a suggestion has not yet been confirmed in the literature since there is no research that examines the effect of leptin with age on spatial memory.

Even though there have been some studies that examined effects of leptin, sex, and generation on different learning models, these studies did not investigate the effects of these variables in a single research paradigm that enables researchers to compare the effects of these variables together. Given the considerations mentioned above, the present study was conducted to investigate the effects of leptin treatment (leptin injection and saline injection), sex (male and female), and generation (juveniles and adults) on the spatial memory performance of rats in a Morris water maze.

2. Materials and methods

2.1. Animal care

Adult male Wistar rats (200–250 g) were obtained from our laboratory colony maintained at Abant İzzet Baysal University (AİBU). They were exposed from birth to 12 h of light and 12 h of darkness, with lights off at 1800 hours. Animals were maintained in plastic cages (16 × 31 × 42 cm) with pine shavings used as bedding. Food pellets and tap water were accessible ad libitum. The procedures in this study were carried out in accordance with the institution's scientific procedures for animals and was approved by the Institutional Animal Care and Use Committee. All lighting was provided by cool-white fluorescent tubes controlled by automatic programmable timers. Ambient temperatures in the animal facilities were held constant at 22 ± 2 °C in air-ventilated rooms.

2.2. Experimental protocol

In the present study, a total of 120 rats were used: 60 male (n: 28) and female (n: 32) adult rats (6 months old), and 60 male (n: 28) and female (n: 32) juvenile rats (1 month old). They were randomly divided into either the leptin injection or the saline injection group. The spatial memory of the Wistar rats was measured at 1800 hours by means of a Morris water maze test.

2.3. Leptin administrations

Leptin (Sigma) was dissolved in ethanol and further diluted in saline; it was injected in a dose of 10 μg/kg at 1700 hours intraperitoneally for 5 days. Saline was injected with the same amount of leptin.

2.4. Morris water maze

To test spatial memory, the performances of the rats in the Morris water maze were evaluated. The experiments were carried out in a circular, galvanized steel maze (1.5 m in diameter and 60 cm in depth), which was filled 40 cm deep with water kept at 28 °C and rendered opaque by the addition of a nontoxic, water soluble dye. The maze was located in a large, quiet test room, surrounded by many visual cues external to the maze (e.g., the experimenter, ceiling lights, rack, pictures), which were visible from within the pool and could be used by the rats for spatial orientation. The locations of the cues were unchanged throughout the period of testing. A video camera fixed to the ceiling over the center of the maze was used for recording and monitoring the movements of the animals. There were 4 equally divided quadrants in the pool. In one of the quadrants, a platform (1.0 cm below the water surface, 10 cm in diameter) was submerged centrally and fixed in a position that was kept constant throughout the acquisition trials. The rats performed 4 trials per day for 4 consecutive days (16 trials). In the swimming trials, each individual rat was released gently into the water at a chosen quadrant, except for the one that contained the hidden platform, to face an extra maze cue. The rats swam and learned how to find the hidden platform within 60 s. Escape latency, which is the time elapsed before the rat reaches the platform in each trial, is a measure of the acquisition of spatial navigation. After reaching the platform, the rat was allowed to stay on it for 15 s and was then taken back into the cage. During the intertrial intervals, the animals were kept in a dry home cage for 60 s.

A platform (10 cm in diameter) was placed in 1 of the 4 maze quadrants (the target quadrant) and submerged 1.5 cm below the water surface. The platform remained in the same quadrant throughout the experiment. The rats were required to find the platform using only distal spatial cues available in the testing room. The cues were maintained constant throughout the testing. The rats underwent 4 consecutive daily training trials. Video camera recordings were obtained on the fifth day of the experiment. Each rat had to swim until it climbed onto the platform submerged underneath the water. The escape platform was kept in the same position relative to the distal cues. The time to reach the platform (latency in seconds), total distance travelled, time passed in the correct quadrant, entrance frequency to the correct quadrant, and immobility were measured as the indexes of the spatial memory. In this experiment, a video camera (Gkb CC-28905S, Commat LTD. ŞTİ.,

Ankara, Turkey) was mounted above the arena, recording behavior into the Ethovision Video Tracking System (Noldus Ethovision, Version 6, Netherland; Commat LTD.ŞTİ. Ankara/Turkey), which provided a variety of behavioral measures including distance, time on the edge, time in the center, frequency on the edge, frequency in the center, and immobility among the different areas of the arena. All animals were then returned to the breeding and exhibition colonies.

2.5. Statistical analyses

Data were analyzed using SPSS 15.0. Data were examined by a means of 2 (the presence of treatment: leptin injection and control (sham) group) × 2 (sex: male and female) × 2 (generation: juveniles and adults) between ANOVA design. Values were considered statistically significant at $P < 0.05$. Data are presented as mean ± standard error after back transforming from ANOVA results. Nonsignificant findings were not reported in this article due to limited space.

3. Results

3.1. Total distance travelled in the Morris water maze

The main effect of leptin administration was significant on the total distance travelled in the Morris Water maze, $F(1, 112) = 4.41$, $P = 0.04$, $\eta^2 = 0.04$. Leptin-administered subjects (M = 296.99) travelled less distance than saline-injected subjects (M = 402.59) (Figure 1).

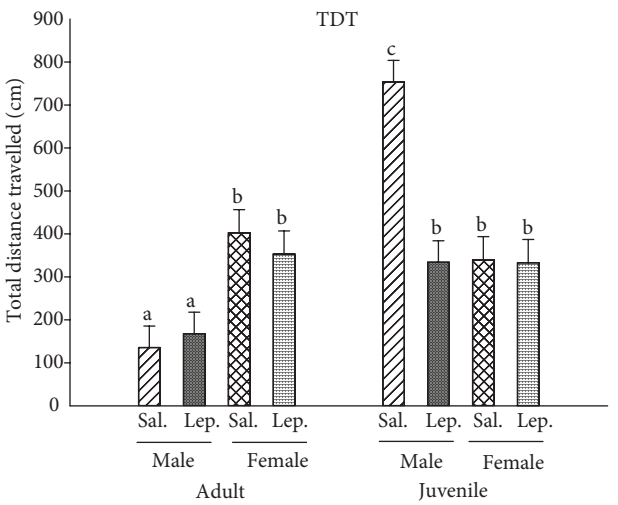

Figure 1. The total distance travelled (TDT) in the Morris water maze is represented. The right-striated bar represents saline injection (Sal.) for adult and juvenile males and the black bar represents leptin injection (Lep.) for adult and juvenile males. The cross-striated bar represents saline injection (Sal.) for adult and juvenile females and the brick-striated bar represents leptin injection (Lep.) for adult and juvenile females. Data are presented as means ± standard error. Different letters indicate the statistically different groups (at least all $P < 0.05$). The statistical values of the groups can be seen in the relevant text.

The main effect of generation was significant on the total distance travelled in the Morris water maze, $F(1, 112) = 11.03$, $P = 0.001$, $\eta^2 = 0.09$. Adults (M = 273.40) travelled less distance than juveniles (M = 419.15).

The interaction effect between generation and treatment was significant, $F(1, 112) = 3.76$, $P = 0.05$, $\eta^2 = 0.04$. Even though the difference between leptin-administered adults (M = 260.54) and saline-injected adults (M = 269.04) was not significant in terms of distance travelled, leptin-administered juveniles (M = 333.46) travelled significantly less distance than saline-injected ones (M = 546.66).

The interaction effect between generation and sex was also significant, $F(1, 112) = 16.87$, $P = 0.0001$, $\eta^2 = 0.13$. In adults, males travelled (M = 151.72) less distance than females (M = 377.86), whereas in juveniles, females (M = 336.30) travelled significantly less distance than males (M = 543.82).

3.2. Time spent to find the platform

The main effect of leptin administration was significant on the time spent to the find the platform on the Morris Water maze, $F(1, 112) = 4.19$, $P = 0.04$, $\eta^2 = 0.04$. Leptin-administered subjects (M = 13.36) spent less time than saline-injected subjects (M = 17.75) (Figure 2).

The main effect of generation was also significant on the time spent to the find the platform in the Morris water maze, $F(1, 112) = 13.37$, $P = 0.0001$, $\eta^2 = 0.11$. Adults (M = 11.96) spent less time to find the platform than juveniles (M = 18.85).

The interaction effect between generation and treatment was significant, $F(1, 112) = 5.45$, $P = 0.02$, $\eta^2 = 0.05$. Even though the difference between leptin-administered adults (M = 11.95) and saline-injected adults (M = 11.33) was not significant in terms of time spent

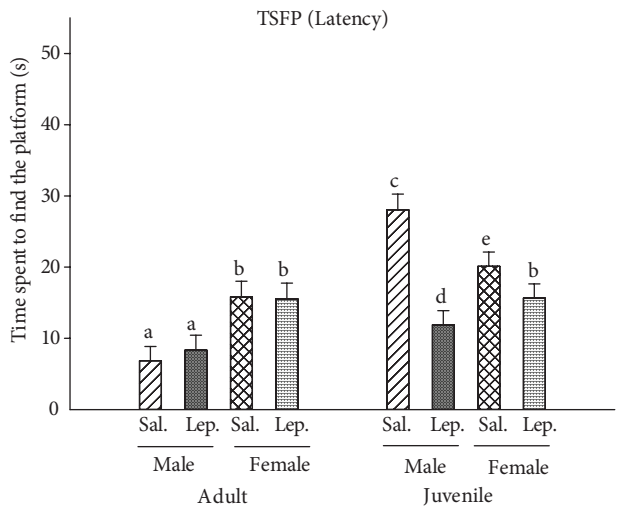

Figure 2. The time spent to find the platform (TSFP) or latency in the Morris water maze is represented. The bar explanations appear under Figure 1.

to find the platform, leptin-administered juveniles (M = 14.76) spent significantly less time than saline-injected ones (M = 24.09).

Furthermore, the interaction effect between generation and sex was also significant, F (1, 112) = 6.90, P = 0.01, η^2 = 0.06. In adults, males spent (M = 7.60) less time than females (M = 15.69), whereas in juveniles, females (M = 17.88) spent significantly less time than males (M = 20.98).

3.3. Time spent in the correct quadrant

The interaction effect between sex and treatment was significant, F (1, 112) = 5.695, P = 0.02, η^2 = 0.05. Leptin-administered males (M = 0.10) spent more time in the correct quadrant than saline-injected males (M = 0.07), whereas saline-administered females (M = 0.09) spent more time in the correct quadrant than leptin-administered females (M = 0.07) (Figure 3).

3.4. The entrance frequency to the correct quadrant

The main effect of generation was significant on the entrance frequency to the correct quadrant in the Morris water maze, F (1, 112) = 10.96, P = 0.001, η^2 = 0.09. Juveniles (M = 2.03) entered the correct quadrant more frequently than adults (M = 1.50) (Figure 4).

The interaction effect between sex and generation was significant, F (1, 112) = 10.96, P = 0.001, η^2 = 0.09. Adult females (M = 1.69) entered more frequently than adult males (M = 1.25), whereas juvenile males (M = 2.46) entered more frequently than juvenile females (M = 1.69).

3.5. Mobility time

The main effect of the leptin administration was significant on mobility, F (1, 112) = 21.89, P = 0.0001, η^2 = 0.16. Leptin-administered subjects (M = 0.01) were less mobile than saline-injected subjects (M = 0.03) (Figure 5).

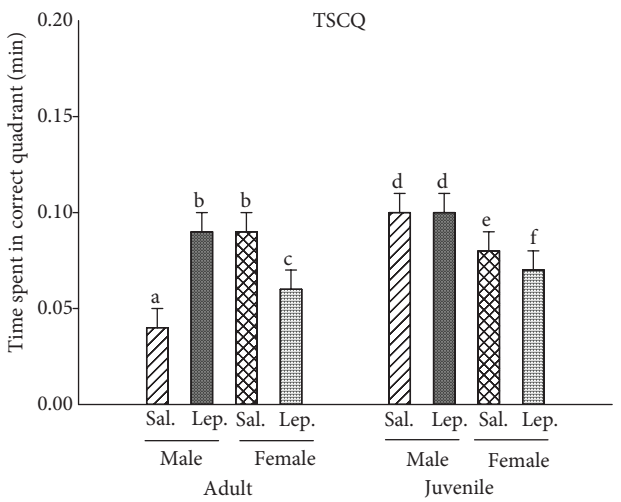

Figure 3. The time spent in the correct quadrant (TSCQ) of the Morris water maze is represented. The bar explanations appear under Figure 1.

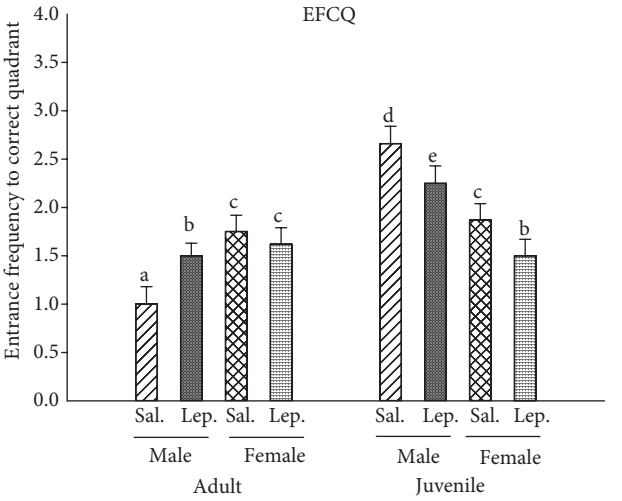

Figure 4. The entrance frequency to the correct quadrant (EFCQ) in the Morris water maze is represented. The bar explanations appear under Figure 1.

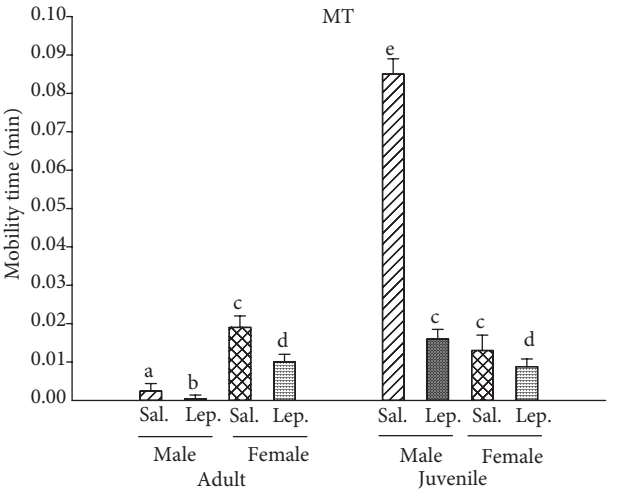

Figure 5. The mobility time (MT) in the Morris water maze is represented. The bar explanations appear under Figure 1.

The main effect of generation was significant on mobility, F (1, 112) = 26.12, P = 0.0001, η^2 = 0.19. Juveniles (M = 0.03) were more mobile than adults (M = 0.009).

The main effect of the sex was significant on the mobility, F (1, 112) = 8.52, P = 0.004, η^2 = 0.07. Males (M = 0.02) were more mobile than females (M = 0.01).

The interaction effect between generation and treatment was significant, F (1, 112) = 12.18, P = 0.001, η^2 = 0.10. Leptin-administered adults (M = 0.006) were less mobile than saline-injected adults (M = 0.01), and leptin-administered juveniles (M = 0.01) were less mobile than saline-injected ones (M = 0.05). The difference was greater in juveniles.

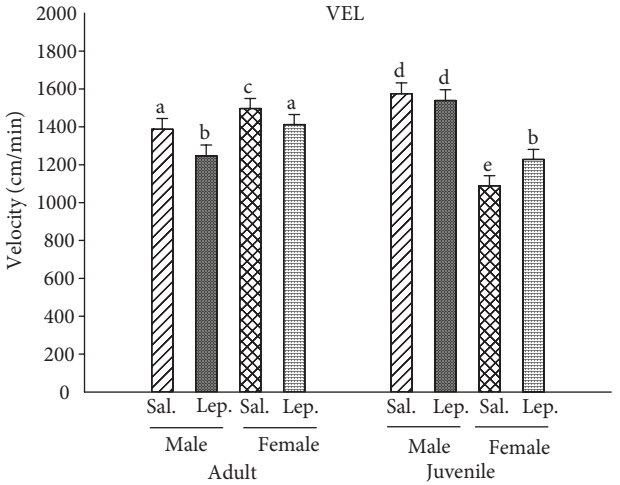

Figure 6. The velocity (VEL) in the Morris water maze is represented. The bar explanations appear under Figure 1.

The interaction effect between treatment and sex was significant, F (1, 112) = 10.04, P = 0.002, η^2 = 0.08. Leptin-injected males were less mobile (M = 0.01) than saline-injected males (M = 0.05), whereas there was no significant difference between leptin and saline-administered females (M = 0.01), which travelled significantly less distance than males (M = 0.02).

The interaction effect between generation and sex was also significant, F (1, 112) = 34.99, P = 0.0001, η^2 = 0.24. Adult males were less mobile (M = 0.001) than adult females (M = 0.02), whereas juvenile females (M = 0.01) were less mobile than juvenile males (M = 0.05).

3.6. Velocity

The main effect of sex was significant on velocity, F (1, 112) = 5.47, P = 0.02, η^2 = 0.05. Males (M = 1431.30) were faster than females (M = 1307.13) (Figure 6).

The interaction effect between generation and sex was significant, F (1, 112) = 23.15, P = 0.0001, η^2 = 0.17. Juvenile males (M = 1557.80) were faster than juvenile females (M = 1159.06); however, adult males (M = 1317.37) and females (M = 1455.20) were not significantly different from each other.

4. Discussion

The aim of the present study was to investigate the effect of leptin hormone injection on the spatial memory performance of Wistar albino rats in the Morris water maze. The Morris water maze is one of the most suitable tests for the investigation of spatial memory (22). In this test, the animal is trying to find a hidden platform that is found below the water level by using cues around the experimental area. The time taken to find the platform, the time spent in the correct quadrant, and the entrance frequencies to the correct and incorrect quadrants are used as the parameters representing the spatial memory and learning performance of the animals. Since our focus was on spatial memory, we used the Morris water maze test.

In sum, the results of this study indicated that (a) leptin injection enhanced spatial memory performance in terms of distance travelled, time spent to find the platform (latency), and mobility; (b) adults showed better spatial memory performance in terms of distance travelled, time spent to find the platform, frequency of entrance to the quadrant of the platform, and mobility; and (c) males were more mobile and faster than females.

Leptin receptors are found in the hippocampus and the amygdala (8). There is evidence that, in rodents, leptin receptor deficiency impairs spatial memory function (23). In the present study, leptin injection may have had a direct effect by binding to these receptors and thereby increasing the activities of these brain regions. Since these brain regions are responsible for memory, such activation may improve spatial memory. In addition to this direct effect, leptin may have also indirect effects on learning and memory by at least 2 mechanisms. First, leptin facilitates long-term potentiation, which is important for the operating process of memory (9). Long-term potentiation is one form of activity-dependent synaptic plasticity (24–26), which is a part of the neurophysiological basis of learning and memory (27). Thus, the memory-enhancing properties of the leptin may be attributed to the long-term potentiation facilitated by the leptin. Second, there is evidence that leptin decreases anxiety in mice (28). The anxiolytic effects of leptin may produce facilitatory effect on searching for the platform in the correct quadrant.

There is evidence that leptin levels rise in neonatal mice, and this may regulate the postnatal development of the neuroendocrine axis (5,29). Leptin deficiency has recently been found to reduce brain weight, neuronal soma size, and the synthesis of synaptic proteins and of glial fibrillary acidic protein in the hippocampus and neocortex. It also leads to deficiencies in brain myelin (30). Therefore, in the current study, leptin implementation may have helped to enhance the synthesis of synaptic proteins and synaptic communication among the neurons in hippocampus and neocortex, which are important components for the spatial memory. We implemented 10 μg/kg of leptin, which is considered to be a low level. Our findings are consistent with the previous research findings (8).

In addition to these main effects, there were also interaction effects between the investigated variables. Specifically, leptin-administered juveniles travelled significantly less distance than saline-injected ones; leptin-administered juveniles spent significantly less time than saline-injected ones; leptin-administered females spent

less time in the correct quadrant than saline-administered females; and adult females entered more frequently than adult males, whereas juvenile males entered more frequently than juvenile females. In terms of the interaction effect between leptin treatment and generation, our data suggest that in adults a high level of endogenous leptin is more effective than exogenous leptin administration. In the present study, we did not find significant evidence for the beneficial effect of the leptin over the saline condition on the spatial memory performance of adults. Rats tend to gain weight as they age. Along with this increase in weight, the total leptin mRNA levels in rats also increase with age (19,20). Therefore, this finding of our study could be attributed to the fact that the leptin receptors may lose their sensitivity to exogenous leptin in adults since they already have high levels of endogenous leptin.

We also found that both leptin-administered adults and juveniles were less mobile than saline-injected adults and juveniles. This can be attributed to the modulation of the hypothalamic feeding and energy expenditure circuits by leptin (19,31,32). Leptin suppresses food intake but stimulates energy consumption (33). In the present study, exogenous administration of the leptin hormone may have increased the rate of basal metabolism. Since energy storage is limited, animals may adapt to this new condition (elevation of basal metabolism) by decreasing mobility in the arena of the water maze. Future research should examine the plausible interaction effects between leptin treatment and generation on memory and their underlying mechanisms for such effect in a greater detail.

Moreover, the present study showed that in adults, males travelled less distance than females, whereas in juveniles, females travelled significantly less distance than males; in adults, males spent less time than females, whereas in juveniles, females spent significantly less time than males; adult males were less mobile than adult females, whereas juvenile females were less mobile than juvenile males; and juvenile males were faster than juvenile females. In other words, in adults, males learned better than females, whereas in juveniles, females learned better than males. The present finding indicating that, in juveniles, females learned better than males is consistent with the recent research finding that female rats performed significantly better than male rats in a recognition task (34). This consistent evidence was also confirmed by the research finding in human studies that females showed better recognition performance than males (35). This consistent finding can be explained from 3 perspectives: different leptin levels between males and females, selectivity, and estrogen differences between males and females.

First, females generally have a greater percentage of body fat than males of similar body weight or body mass index; therefore, leptin levels are greater in females than in males of equivalent fat mass (12). Second, the selectivity hypothesis, which was developed by Meyers-Levy et al. (36), proposes that females tend to process information comprehensively, processing all available information, both relevant and irrelevant. In contrast, males tend to process information in a more selective fashion, focusing only on what is relevant to the task or to themselves. Our results supported this hypothesis, which has also been reported to be particularly relevant to observed sex differences in cognition when it involves recognition or location memory (35,37,38).

Third, estrogen differences between males and females may have contributed to the present findings. The hormone estrogen can play an important role in regulating spatial memory. Initial studies using the spatial radial arm maze suggest that estradiol exerts differential dose-dependent effects on spatial working memory processes (39–42). Low levels of estradiol reduce working memory errors, whereas high levels of estradiol increase the number of working memory errors relative to ovariectomized control rats (42,43). Moreover, estrogen may have an indirect effect on learning by increasing leptin levels. There is some evidence that an endogenous hormonal milieu with estrogens generally increases leptin production (15).

On the other hand, this hormonal explanation is not valid for males' spatial memory performance in adults since they showed the best learning performance. There are at least 2 plausible explanations for this effect. First, males tend to lose their selectivity (focusing only on what is relevant to the task or to themselves) to their environment and may increase in processing all information related to their surroundings from their recognition memory as they age. Second, males may be more assertive in searching for food and females in their environment as they mature. On the other hand, females tend to spend less time searching their environment because they must take care of their offspring for at least some period of time. This may put some restriction on the spatial memory performance of females.

In conclusion, the results suggest that leptin may have a beneficial effect on spatial memory performance. It should be kept in mind that the memory-enhancing effects of leptin also depend on sex and generation in Wistar albino rats.

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