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*Full Length Research Paper*

# Evaluation of the effect of intrahippocampal injection of leptin on spatial memory

**Mohaddes Gisou[1, 3], Rasi Soheila[1] and Naghdi Nasser[2]***

[1]Drug Applied Research Centre of Tabriz University of Medical Sciences. Iran.
[2]Department of Physiology and Pharmacology, Pasteur Institute of Iran.
[3]Tabriz university of Medical Sciences. Tabriz, East Azarbaijan Province, Iran.

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**Leptin is a peptide hormone secreted by adipose tissue. Some studies have suggested that leptin may affect learning and memory. The hippocampus has been implicated in many learning and memory functions including spatial memory. The present study is scheduled to investigate the effect of intrahippocampal (IH) injection of different doses of leptin on spatial memory formation. 40 male rats were divided into 4 groups in our experiment: (1) sham (saline treated animals), and (2), (3), (4) intrahippocampal injection of 0.1, 0.5 and 1 µg doses of leptin respectively. All groups were trained in Morris water maze for two consecutive days. Learning parameters were compared among groups. Our results showed, there were significant differences of learning parameters between sham group and test groups in spatial learning. Lower dose of leptin improved spatial learning better than high dose. In conclusion, our findings indicate that leptin in the hippocampus is involved in memory processing in rat and suggests also that low levels of leptin may perform better.**

**Key words:** Hippocampus, spatial memory, leptin, rat.

## INTRODUCTION

Leptin is a hormone that regulates body weight and energy homeostasis via its actions on specific hypothalamic nuclei (Harvey et al., 2006). Leptin is the product of the obese (ob) gene that is synthesized predominantly, although not exclusively, by white adipose tissue (Harvey et al., 2003). Adipocytes secrete leptin into the blood. As it circulates through the cerebrovasculature, transporters for leptin carry it across the blood brain barrier (BBB) to enter the interstitial fluid of the brain (Banks et al., 1996; Schwartz et al., 1996). Leptin functions are thought to occur through the leptin receptors mainly in the hypothalamic nuclei. However, leptin receptors exist throughout the brain including the hippocampus (Farr et al., 2006). Immunoreactivity for leptin receptors has been found in the hippocampus especially in the dentate gyrus and CA1 (Wayner et al., 2004). Moreover, it has been diffe- demonstrated that leptin receptor-deficient animals' show impaired Long Term Potentiation (LTP) in CA1 and poor spatial memory. In the Morris water-maze test, their poor performances in the invisible-platform situation may suggest a spatial memory deficit in both Zucker fatty rats and db/db mice (Li et al., 2002).

The hippocampus has a well-documented role in spatial memory acquisition (Naghdi et al., 2005). It has been also determined that hippocampus has an essential role in rodent spatial memory and navigation (Morris, 1981). Lesion of the CA1 subfield in rat spatial learning has been evaluated by the MWM previously (Modo et al., 2000). Hippocampal lesions produce memory deficits, particularly spatial memory (Lathe, 2001).

Some studies have been conducted on leptin effect on rent type of learning and memory. Farr and colleagues reported the role of leptin in learning and memory using an animal model. They found that mice demonstrated navigated a maze better after they received leptin. Their research indicated that administration of leptin to mice improved retention of T-maze footshock avoidance and step down inhibitory avoidance (Farr et al., 2006). Re-cently, Oomura et al. showed a facilitation effect on learning and memory performance in passive avoidance and Morris water maze task after daily intravenous injection of leptin (50 ug/

*Corresponding author. E-mail: nnaghdiir@yahoo.com, naghdi@pasteur.ac.ir. Tel:/Fax: +982166968854

kg) in rats (Paulus et al., 2005).

Another study also suggested that leptin applied directly into the dentate gyrus; enhanced normal LTP at 1.0 µM but inhibited LTP at lower and higher doses in the Morris water maze in urethane anesthetized rats (Wayner et al., 2004). Finally, Just one experiment reported that leptin exhibit no effect on memory processes (Paulus et al., 2005).

The purpose of the current study is to examine the effect of different doses of leptin on memory processing in the hippocampus in normal adult male rats.

## MATERIALS AND METHODS

### Animals and substances

Adult male Wistar rats (220 - 250 g, aged 12 week) were obtained from colony of Tabriz university of Medical Sciences. They were housed in a temperature (22 ± 2°C) and humidity-controlled room. The animals were maintained under a 12:12-h light/ dark cycle, with lights off at 8:00 p.m. Food and water provided ad libitum except for the periods of behavioral testing in Morris Water Maze (MWM). The behavioral testing was done during the light phase. All experimental procedures were approved by the Regional Ethics Committee of Tabriz University of Medical Sciences. Leptin was purchased from Peprotech Pharmaceutical Company and was Solved in phosphate buffer (Niewiarowski et al., 1999) and then diluted in sterile 0.9% saline.

### Surgery

Rats were implanted with bilateral canula aimed at the dorsal hippocampus. Before surgery, animals were anesthetized with IP injections of ketamine (60 mg/kg body weight) and xylazine (12 mg/kg body weight) (Mohaddes et al 2009). The animals were mounted into a stereotaxic frame used to position the 22-gauge stainless steel guide canula in the dorsal hippocampus (AP -3.8, L ± 2.2 and DV-2.7). Coordinates were chosen based on a rat brain atlas (Paxinos et al., 1986). The internal canula was 0.5 mm longer than guide canula. The canula was anchored to the skull using stainless steel screws and acrylic cement.

### Apparatus

The water maze was a black circular pool with a diameter of 136 cm and a height of 100 cm, filled with 20 ± 1°C water to a depth of 60 cm. The maze was divided geographically into four equal quadrants and release points that were designed at each quadrant as N, E, S, and W. A hidden Square platform (10 cm each side), was located in the center of the southwest quadrant, submerged 1.5 cm beneath the surface of the water. Fixed, extra maze visual cues were present at various locations around the maze (i.e., computer and signs). A video camera was mounted above the center of the maze so the animal motion can be recorded and sent to the computer. A tracking system was used to measure the escape latency, traveled path and swimming speed.

### Microinjection procedure

The microinjections were made using a 5 µl Hamilton syringe through a short piece of polyethylene tube. The needle was inserted 0.5 mm beyond the tip of the canula. Saline or leptin (0.1, 0.5 and 1 µg) was injected (0.5 µl) into bilateral CA1 region du ring 2 min and the needle was left in the place for 1 min following the the microinjections to minimize the flow back of the solution. Thirty minutes after the intrahippocampal injection of leptin or saline, water maze training was started.

### Behavioral procedure

One week after surgery, the rats were trained in the water maze. The single training session consisted of eight trials (in two blocks) with four different starting positions that were equally distributed around the perimeter of the maze. The task requires rats to swim to the hidden platform guided by distal spatial cues. After mounting the platform, the rats were allowed to remain there for 20 s, and were then placed in a holding cage for 30 s until the start of the next trial. Rats were given a maximum of 60 s to find the platform and if it failed to find the platform in 60 s, it was placed on the platform and allowed to rest for 20 s. Latency to platform and distance traveled were collected and analyzed later. After completion of the training, the animals were returned to their home cages until retention testing 24 h later. The probe trial consisted of 60 s free swim period without a platform and the time swum in the target quadrant was recorded (Moosavi et al., 2006).

In order to assess the possibility of drug interference with animal sensory and motor coordination or the animal motivation, the capability of rats to escape to a visible platform was tested in this study. The trained rats were given four trials for visuo-motor coordination on the visible platform.

### Experimental groups

The aim of this experiment was to evaluate the effect of intrahippocampal leptin injection on memory. The intrahippocampally injected rats were randomly divided into four groups (ten rats in each): saline treated and leptin with doses 0.1, 0.5 and 1 µg. Saline or leptin was injected intrahippocampally 30 min before training. The retention testing was done 24 h later as a 60 s probe trial (leptin or saline were injected 30 min before probe trial.)

### Statistical analysis

Data are expressed, as means ± S.E.M. The statistical analysis of the data was carried out by one-way ANOVA-followed by Turkey test. In all comparisons, $P < 0.05$ was considered significant.

## RESULTS

### The effect of pre-training intra CA1 leptin administration on memory

In this experiment different doses of leptin (0.1, 0.5 and 1 µg) or saline (sham operated) were injected 30 min before training to test the effect of leptin on memory. Short escape latency and short traveled distance indicate more rapid learning in locating the hidden platform.

During acquisition, the performance of all groups improved with subsequent block of training. The difference of traveled distance between block 1 and block 2 was extremely significant ($p < 0.001$) in sham and 0.5 µg dose of leptin. It was also significant ($p < 0.01$) in 0.1 µg and ($p < 0.05$) in 1 µg groups (Figure 1A). In comparison of block 1 and block 2, escape latency was also significantly ($p < 0.01$) different in 0.1 and 0.5 groups (Figure 1B).

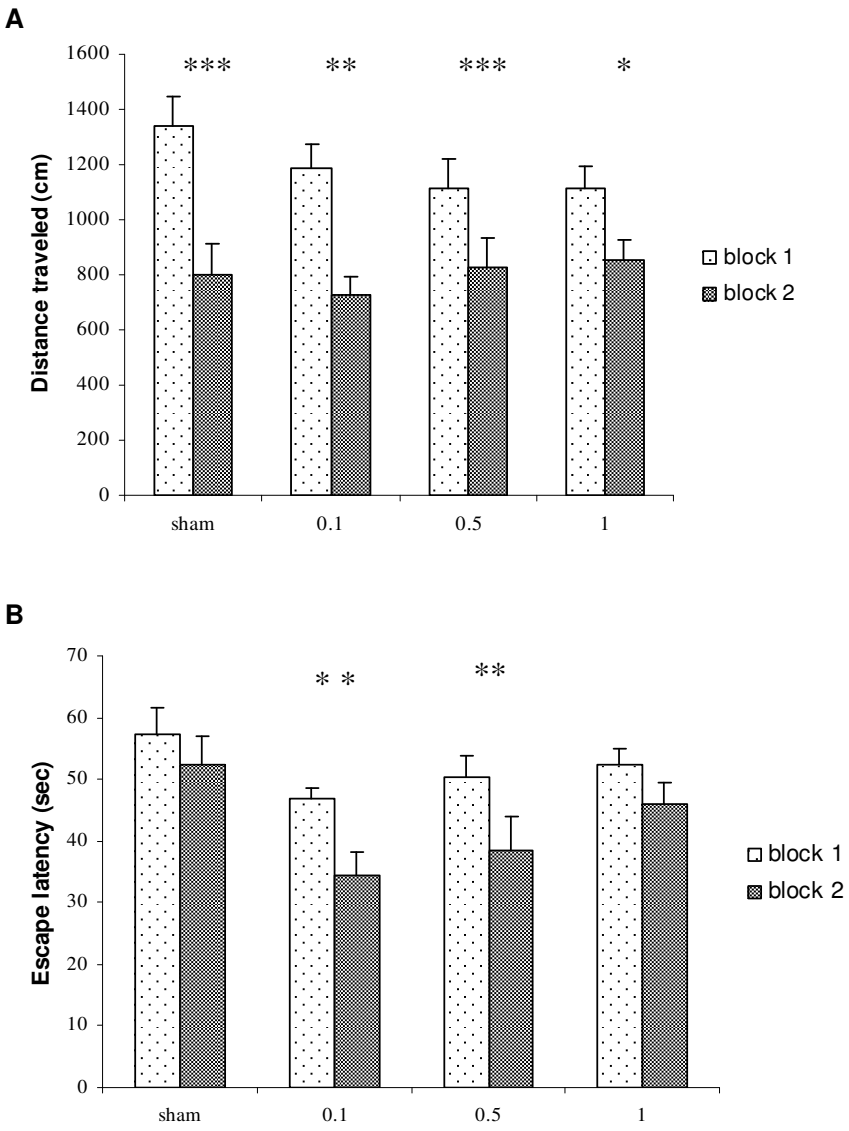


**Figure 1. A)** The effects of different doses of leptin on the distance traveled between block 1 and block 2. **B)** The effects of different doses of leptin on the escape latency between block 1 and block 2. All leptin doses improved acquisition, , * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The one-way ANOVA of the escape latency of the first day revealed significant differences between groups. Injection of 0.1 µg of leptin demonstrated better ($p < 0.01$) spatial learning than that of the saline treated ani-mals. Animals treated with higher doses of leptin (0.5 and 1 µg) did not show any significant difference on water maze acquisition (Figure 2).

Probe test data were compared between groups. One-way ANOVA of the distance traveled in the target quadrant revealed significant differences between groups. Pre-training injection of 0.1 µg leptin significantly ($p < 0.01$) increased the distance traveled in target quadrant (Figure 3). Leptin in 0.5 and 1 µg had no significant effects on spatial learning parameters.

As there is no difference in training of different groups, the changes in probe trial can be attributed to the effect of leptin.

### The effect of leptin on visible platform performance

Bilateral intra CA1 leptin injection 30 min before visual trial (visible platform) also showed no difference in escape latency (Figure 4) to find the visible platform, compared to the sham group.

## DISCUSSION

We demonstrated that microinjection of leptin to CA1 re-

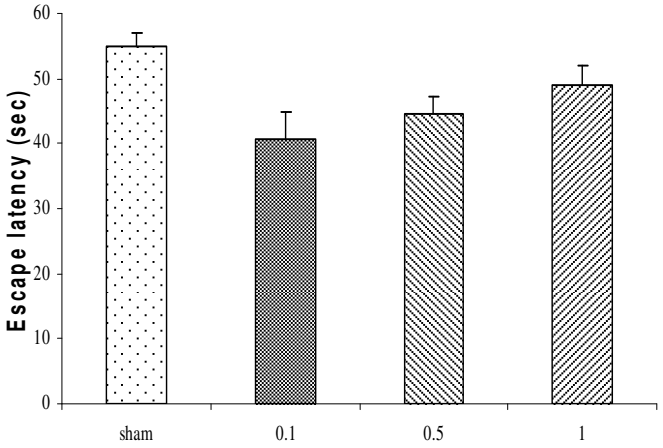


**Figure 2.** The effects of pre-training intrahippocampal administration of 0, 0.1, 0.5 and 1 µg leptin on acquisition in Morris Water Maze. Average escape latency of the first day between sham and test groups is shown. Leptin 0.1 µg improved acquisition, ** $p < 0.01$.

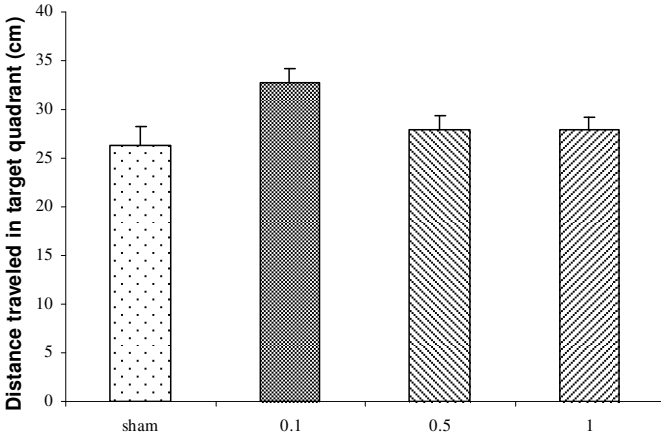


**Figure 3.** Probe test after withdrawing the platform. The 0.1 µg leptin group traveled longer distance in the target quadrant than the vehicle group on probe test day (second day), whereas other leptin groups showed no significant difference, ** $p < 0.01$.

gion of hippocampus have a discrepant effect on learning and memory. Intrahippocampal injection of different doses of leptin improved spatial learning and memory. However, the improving effect of lower dose was stronger than the improving effects of higher doses.

Leptin enters areas throughout the brain by a system that is partially saturated at endogenous blood levels of leptin (Banks et al., 2000). Transporters for leptin carry it across the BBB to enter the interstitial fluid of the brain (Banks et al., 1996; Schwartz et al., 1996) and Choroid plexus plays a key role in regulating leptin entry into the CSF under physiological conditions (Zlokovich et al., 2000). Leptin also acts within areas such as the cortex and hippocampus to influence neuronal function and pro-

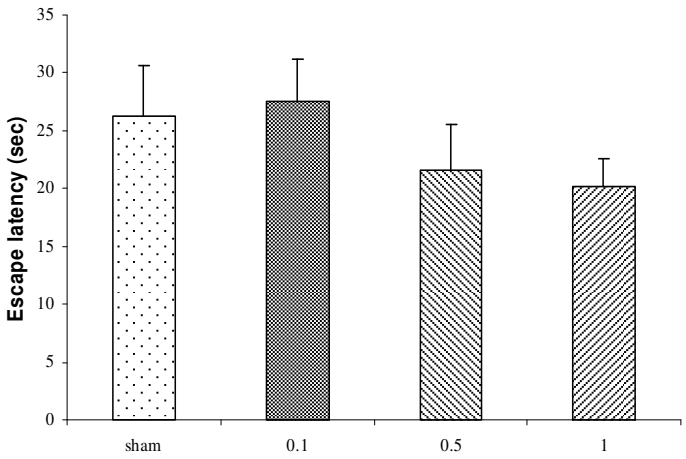


**Figure 4.** Visible test shows there is no significant difference to find platform between groups. Leptin did not affect visuo-motor and motivational factors in animals.

mote cognition (Christopher, 2009). The hippocampus specially has been shown to be critically involved in learning and memory processes (Alonso et al., 1998; Isgor et al., 1998; Naghdi et al., 2001).

Our data support previous results on leptin effects on different type of memory, such as in a water maze performance (Li et al., 2002; Oomura et al., 2006), T-maze footshock avoidance (Farr et al., 2006) and passive avoidance tasks (Oomura et al., 2006). Oomura's rat study shows that intravenous injection of leptin facilitated learning and memory in the Morris water-maze test, enhanced CA1 LTP maintenance, attenuated LTD, and led to increased CaMK II activity in the CA1 area (Oomura et al., 1998). In addition, a close association between enhanced hippocampal LTP and facilitated learning and memory has been demonstrated (Manabe et al., 1998; McKernan et al., 1997; Rogan et al., 1997; Silva et al., 1992a; Silva et al., 1992b; Tsien et al., 1996). Farr and colleagues assessed the role of leptin in memory processing using two different avoidance paradigms. Their results indicate the leptin improves memory processing for T-maze footshock avoidance in SAM-P8 male mice (Farr et al., 2006).

On the other hand, Paulus explored the effect of intrahippocampal administration of leptin on spatial memory formation following a radial maze task and leptin receptor expression in different areas of the hippocampus in rats. Spatial memory formation was found unaltered following the application of leptin (Paulus et al., 2005).

Our previous study on peripheral dose-response effect of leptin on spatial memory (Rasi et al., 2008) was consistent with Oomora's study. Oomura showed spatial learning and memory performance was unchanged with 0.5 and 5 mg/kg leptin, enhanced with 50 mg/kg leptin, and impaired with 500 mg/kg (Oomura et al., 2006). Application of peripheral leptin in behavioral experiments demonstrated that leptin shows an inverted-U dose

related function in terms of its effects on learning and memory. In the present behavioral study intrahippocampal injection of leptin improved spatial learning and memory. However, the improving effect of dose (0.1 µg) was stronger than the improving effects of other doses. Leptin with higher doses (0.5, 1 µg) had weaker effects on water maze task, which indicates there is an optimal dose for memory.

Collectively, it is possible that higher doses of leptin trigger other types of receptors or other intracellular signaling pathways. For example, it may be related to the effect of leptin on internalization of AMPA receptors in hippocampal CA1 neurons (Shanley et al., 2001). AMPA receptor-mediated synaptic transmission in the hippocampus is critical for encoding and consolidation of spatial (Riedel et al., 1999), aversive (Cammarota et al., 1998) and recognition memory (Winters et al., 2005; Broadbent et al., 2004). It has also shown that leptin inhibits rat hippocampal neurons by increasing a $K^+$ conductance (Shanley et al., 2002). Maybe; higher doses of leptin inhibit hippocampal cells through AMPA receptor down-regulation or increasing of $K^+$ conduction.

Other findings show that leptin; at concentrations comparable with those circulating in the plasma (Caro et al., 1996) can modulate hippocampal synaptic plasticity, by conversion of short term potentiation (STP) into LTP. A key process underlying this effect is the enhancement of NMDA responses; a process not only requiring activation of PI 3-kinase, but also MAPK and Src tyrosine kinases. A crucial intracellular process regulating NMDA receptor function is phosphorylation (Mac Donald et al., 1989), and both serine– threonine and tyrosine phosphorylation regulate NMDA receptor function. In particular, Src tyrosine kinases can directly phosphorylate

NMDA receptor NR2A (Lau et al., 1995) and NR2B (Moon et al., 1994) subunits. Functionally this may be important in hippocampal synaptic plasticity because it has been hypothesized that during LTP induction, Src is rapidly activated leading to enhanced NMDA receptor function (Salter et al., 1998). Possibly the improving effect of the lower dose of leptin in this study acted through these mechanisms. Taken together, our results and previous studies indicate that the same peptide could possibly have different modulator post synaptic effects in different hippocampal synapses dependent upon different types of post synaptic receptors (Farr et al., 2006; Wayner et al., 2004).

In summary, we found that leptin improves memory and it has better effect with a low dose. It is possible that alterations in nutritional status can alter cognitive function.

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