THE EFFECTS OF ASTROCYTIC S100B PROTEIN AND ITS ANTIBODY ON ACQUISITION AND CONSOLIDATION OF MEMORY IN MALE RATS

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Summary

The effects of intra-hippocampal infusion of S-100B protein and antibody against this protein were evaluated on acquisition and consolidation of memory in male rats. Experiments were performed on male Wistar rats weighing 200-250 g. Seven days after stereotaxic surgery the rats were trained in a step-down passive avoidance task (0.5mA, 100Hz, 5sec) and immediately after training, infused bilaterally with a 0.5 µl S-100B (5 , 50, 500, or 5000 ng); or antibody against S-100B (1/50; 1/100; 1/500; 1/5000; 1/100000 ng). In order to evaluate the acquisition phase the effective dose of protein (5ng) and its antibody (1/50ng) were administrated Fifteen minutes before training. Control groups received the same volume of saline.

The results showed that low doses of both S-100B protein and anti S-100B led to a significant improvement in memory consolidation; whereas high doses of S-100B and its antibody significantly decreased it. We suggest that neural processing of information involved in memory condolidation needs an optimum levels of S-100B protein. This study reveals the importance of astrocytes in neural plasticity in addition to their supportive roles in nervous system .

Keywords: S-100B, learning, memory, astrocytes, rat

Introduction

Astrocytes are the most abundant cell type in the central nervous system, which play important roles in various brain functions. One of these functions is secretions of a protein called S-100B. This protein is a calcium-binding protein with a number of intracellular and extracellular effects (1, 2). It has been known that exogenous S-100B stimulate the survival of neurons (3) and neuritis extension (4).

Recently it has been known that S-100B is able to affect on learning and memory in a paracrine, autocrine and endocrine manner (2). It seems to play an important role as a mediator of glial-neuronal interactions, which is important during brain development and also modulation of synaptic transmission in adulthood (5), probably through the G-proteins coupled receptor(6). The extracellular action of S-100b includes an increase in the intracellular calcium concentration by opening voltage- dependent Ca-channels (7), or by depletion of internal Ca stores (6).

Previous studies in humans revealed that, S-100B and its receptor RAGE (receptor for advanced glycation end products) are increased in various neurodegenerative disorders like Alzheimer, down syndrome, trauma (2, 8) and brain injury (9-11) which consequently have cognitive impairments.
On the other hand, Kleindienst et al., (2005) showed that intraventricular S-100B infusion induces neurogenesis within the hippocampus, which can be associated with an enhanced cognitive function following experimental trauma (10). Donato et al. 2008, also showed an increase in the level of S-100B by environmental factors (9). It seems that S-100B might implicate neural activity in the processing of information (12-13). There are more controversies in the literatures, showing facilitation of long-term memory by S-100B infusion (12), impairment of memory and exacerbation of brain damage in S-100B –over expressed transgenic mice (14, 15), inhibition of long-term memory in chicks (16); disturbance of defensive behavior (17) and possessing the antidepressant activity in rats after S-100B infusion (18).

Notwithstanding the wide diversity of S-100B functions, the role of this protein in the CNS is still a matter of debate. This study was designed to investigate the effect of both S-100B protein and its antibody infusion into CA1 region of hippocampus on acquisition and consolidation of memory in a passive avoidance task in male rats.

Methods

Animals

Experiments were performed on adult male Wistar rats weighing 200–250 g from our breeding. Animals were housed five to a cage with a free access to water and food, under a 12-h light/dark cycle (lights on at 7:00 AM) at a temperature of 24±2 °C.
All groups consisted of eight animals. All animal manipulations were approved by the Ethical Committee of Guilan University of Medical Science. All measurements were conducted in the daytime (9.00-15.00). Animals were handled 5 min/day for three consecutive days in experimental box, in order to decrease the effects of environmental stress.

Surgery

The animals were anesthetized intraperitoneally by a mixture of ketamine and xilazine (100 and 10 mg/kg, respectively). After being fixed in the stereotaxic apparatus (David Kopf Instruments, USA) with flat-skull position, the rat's scalp was cut, a small craniotomy was drilled and cannulae (22-gauge diameter) were bilaterally implanted into the CA1 region of hippocampus, at coordinates AP: −3 mm from bregma, L: ±2 mm from midline and V: −2.8 mm from the skull surface (19).

Microinjection procedure

The animals were gently restrained by hand; the stylets were removed from the guide cannulae and replaced by 27-gauge injection needles (1mm below the tip of the guide cannulae), that were connected by polyethylene tubing to 10-µl Hamilton microsyringe. The injection solutions were administered in a total volume of 1 µl/rat (0.5 µl of S-100B or its antibody or saline in each side) over a 60 s period. Injection needles were left in place for an additional 60 s to facilitate the diffusion of the drugs. The drugs used in the present study were S-100B protein and antibody against to S100B (SIGMA, USA) which was dissolved in sterile 0.9% saline. Drugs and saline injected intra-CA1 bilaterally.

Step-down passive avoidance task

1. Training

A one-trial step-down passive avoidance task was used to determine the effect**.** This task was performed in a 40×30×40 cm box, whose floor consisted of parallel 3.0-mm stain-less steel bars spaced 1.0 cm apart. The animal was placed on a Plexiglas platform (12 ×10, height 7 cm) in the central of the apparatus. Each rat was gently placed on the platform. When the rat stepped-down from the platform and placed all 4 paws on the grid floor, a single trial electric foot shock (0.5 mA, 100Hz, 5sec) was applied to the grid. Then the animals were immediately withdrawn from the training apparatus. This training procedure was carried out between 9: 00 and 15: 00 h.

2. Retention test

The animals were tested 24 h and seven days after training. In test sessions, each rat was again placed on the platform, without any shock. The step-down latency was taken as a measure of retention. Time of the rat's descent from the platform and total time spent on platform was recorded. An upper cutoff time of 300 s was set.

Experimental procedure

1. Effect of S-100B protein on memory consolidation

The aim of this experiment was to assess the effect of immediately post-training infusions of S-100B protein into the CA1 region of hippocampus on memory consolidation in passive avoidance task. For this purpose forty rats were divided into four experimental groups and one control group (n = 8). Immediately after the training, animals (experimental groups) received 0.5 µl infusion of different doses of S-100B (5, 50, 500, or 5000 ng) or saline (control group), bilaterally. Rats were tested for memory retention at two times, 24 h and 7 days after training to measure memory retention. Step-down first latency and total time spent on platform were measured as learning memory indices.

2. Effect of S-100B protein on memory acquisition

The aim of this experiment was evaluate the effect of pre-training infusions of S-100B protein into the CA1 region of hippocampus on memory acquisition in passive avoidance task.. Fifteen minutes before training, animals (n = 8) received 0.5 µl infusion of one effective dose of S-100B (5 ng) or saline (control group), bilaterally. Passive avoidance performance was tested 1 and 7 days after training**.** Step-down first latency and total time spent on platform were measured as learning memory indices.

3. Effect of antibody against to S-100B protein on memory consolidation

In this experiment we assess the effect of post-training infusions of antibody to S-100B protein on consolidation of passive avoidance task. Rats were divided into four experimental groups and one control group (n = 8). The antibody was aliquot in saline and store in -20^{oc} for further usage. Immediately after the training, animals (experimental groups) received 0.5 µl infusion of a dose of antibody to S-100B (1/50; 1/100; 1/500; 1/5000; 1/100000 ng) or saline (control group), bilaterally. Step-down first latency was measured as learning memory indices.

4. Effect of antibody against to S-100B protein on memory acquisition

The aim of this experiment was to assess the effect of pre-training infusions of antibody to S-100B protein on acquisition of passive avoidance task. Rats were divided into two experimental and control group (n = 8). Fifteen minutes before training experimental groups received 0.5 µl bilaterally infusion of a dose of antibody to S-100B (1/50) or saline respectively. Step-down first latency was measured as learning memory indices.

Histology

After the end of the behavioral procedure all animals were deeply anesthetized and 1 µl of a 4% methylene-blue solution was bilaterally infused into the CA1 (0.5 µl/ 1 side), as described in the drug section, then decapitated and their brains were dissected and stored in formaldehyde (10%) for histological evaluation of cannula placements. Then the brains were sliced and the sites of injections were verified according to Paxinos & Watson, 1997. Data from the animals with the injection sites located outside the CA1 were not used in the analysis (Fig. 1)

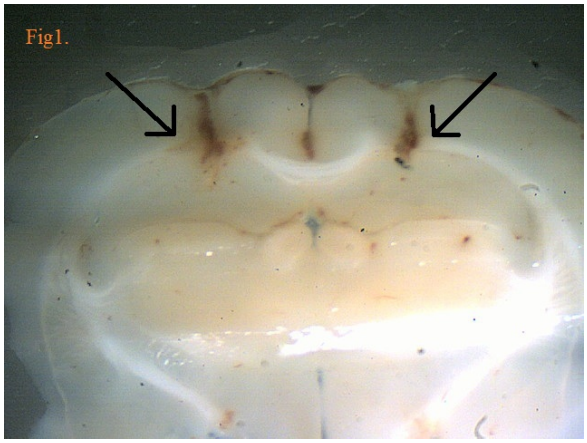

Fig.1. A rat brain section showing the extension of the area reached by infusions into the hippocampus in the animals with correct infusion placements.

Statistics

The step-down latencies are expressed as the median and interquartile range. After assaying the normality of data with kolmogorov - smirnov test, comparison of data among five groups was performed using the one-way analysis of variance with Tukey's post-test (Post Hoc tukey), while comparison of data among two groups was performed using the student t-test. When the P-value was <0.05, the difference was considered to be significant.

Results

In our experiment, step down first latency and total time spent on platform were measured as learning memory indices. Cut off time set at 300 seconds. In training session, latent period of descent from the platform in both control and experimental groups on average was about 5 s.

Experiment 1: Effect of S-100B protein on memory consolidation

Fig. 2 shows the effects of post-training intra-CA1 infusion of S-100B protein, in both indices of step-down latency; total time spent on platform and step down first latency. There was a significant difference on step-down first latency [$f(4,35) = 24.02$; $P< 0.001$](Fig. 2A) and total time spent on platform [$f(4,35) = 41.82$; $P< 0.001$] in test 24h after training, as compared to control group.

Immediately post-training infusion of S-100B protein (5ng) showed significant increase in step-down first latency ($p<0.05$), and total time spent on platform ($p<0.01$), compared with saline-treated animals. Whereas strong significant decrease ($p<0.001$) were observed in both indices on the test performed 24h after training, in groups received 500 and 5000 ng of S-100B, relative to control group. There were no significant differences in memory indices in animals infused with 50ng of S-100B compared to saline. Thus according to these data our results showed that S-100B protein, in doses of 500 and 5000 ng impairs, while in a dose of 5 ng, facilitates consolidation of passive avoidance memory. In addition, 7days after training, retention test was carried out again. There was a significant difference on step-down first latency [$f(2, 21) = 57.303$; $P< 0.001$] (Fig. 2B) and also total time spent on platform [$f(2, 21) = 10.308$; $P< 0.01$] in groups receiving 5 ng S-100B compared to control group.

Fig.2A

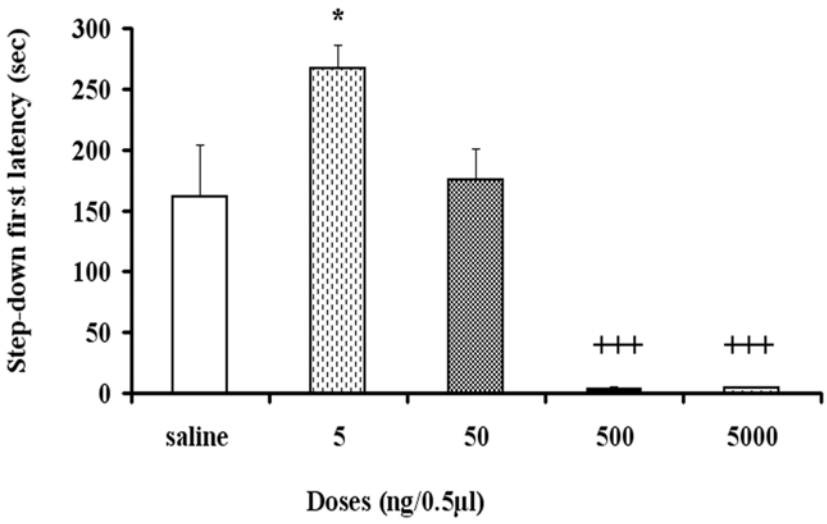

Fig. 2A The effects of S100B post training infusion on memory consolidation in passive avoidance learning. Columns show mean ± S.E.M. of step-down first latency of retention test which was performed 24 h after training. *P < 0.05, +++$p<0.001$, indicates significant difference vs. control (saline) group.

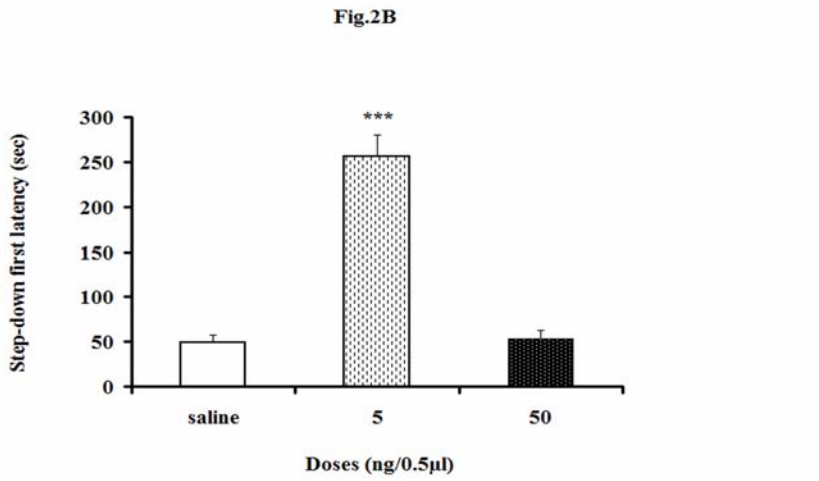

Fig. 2B The effect of intrahippocampal infusion of S100B immediately after training on passive avoidance memory consolidation. Columns show mean ± S.E.M. of step-down first latency of retention test which was performed 7 days after training. ****P* < 0.001 indicates significant difference vs. control group.

Experiment 2: Effect of S-100B protein on memory acquisition

Fig.3 shows the effects of pre-training intra-CA1 infusion of S-100B protein in step-down first latency. There was a significant difference ($P< 0.05$) on step-down first latency in group receiving (5ng, l side) S-100B Fifteen minutes before training in test 24h and 7 days after training ($P< 0.05$), as compared to control(Fig. 3A). In addition, the total time spent on platform showed significant difference between per trained S-100B receiving group compare to saline ($P< 0.05$) (Fig. 3B).

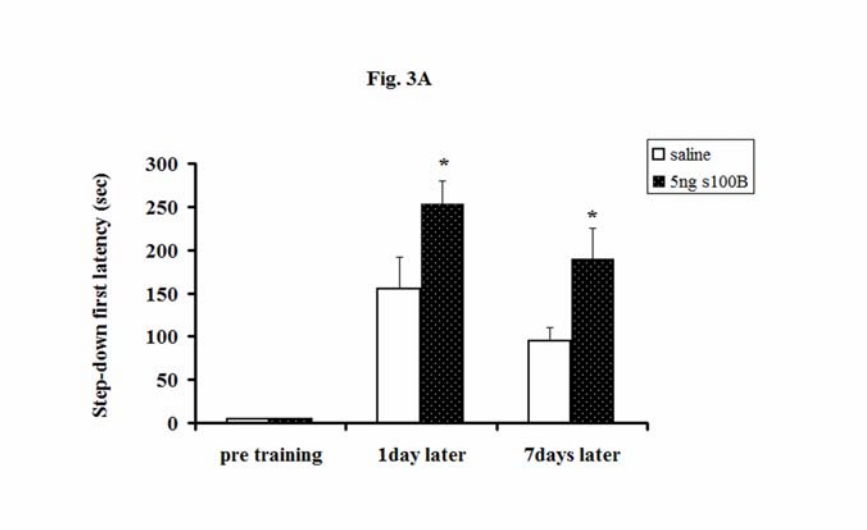

Fig. 3A The effects of pre-training infusion of S100B protein on memory acquisition in passive avoidance learning. Each bar shows the mean ± S.E.M. of step-down first latency of retention test which was performed 24 h and 7 days after training. **P* < 0.05 vs. control group. (n=8)

Fig. 3B

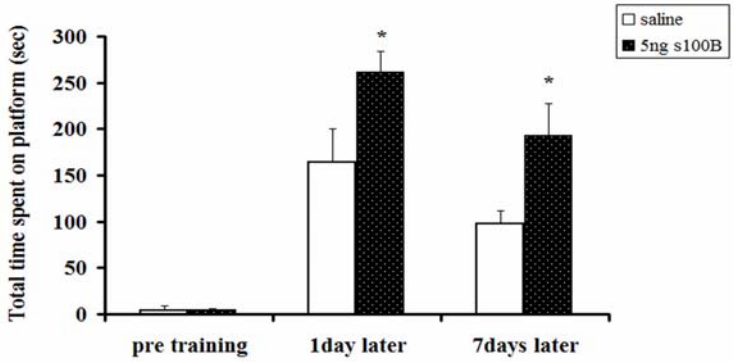

Fig. 3B The effects of pre-training infusion of S100B protein on memory acquisition in passive avoidance learning. Each bar shows the mean ± S.E.M. of total time spent on platform of retention test which was performed 24 h and 7 days after training. **P* < 0.05 vs. control group. (n=8)

Experiment 3: Effect of antibody against to S-100B protein on memory consolidation

Fig. 4 shows the effects of post-training intra-CA1 infusion of antibody to S100B protein on step-down latency. Immediately post-training administration of antibody to S100B protein showed strong significant decrease ($p < 0.001$) in both indices on the test performed 24h after training, in groups received 1/50, relative to control group. Animals receiving doses of 1/100, 1/500, antibody against S 100B also showed impairment of memory ($p<0.05$, $p<0.01$ respectively). Instead, antibody in ultra low doses of 1/1000, 1/1000000 showed better memorization compared to groups receiving high doses of antibody.

Fig 4

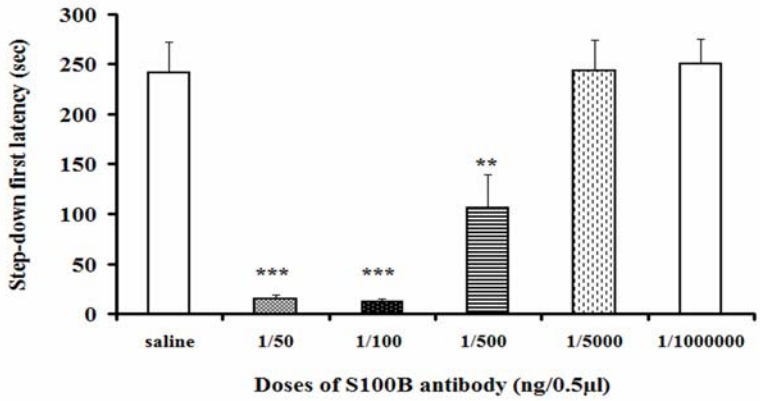

Fig.4 The effects of post - training infusion of different doses of antibody against S100B protein on memory consolidation in passive avoidance learning. Each bar shows the mean ± S.E.M. of step-down first latency of retention test which was performed 24 h after training. ****P* < 0.001, **p<0.01 vs. control group. (n=8)

Experiment 4: Effect of antibody against to S-100B protein on memory acquisition

In this experiment we were able to use one effective dose of antibody (1/50) in order to evaluate the pre training effect of antibody on acquisition of memory. Fig. 5A and Fig 5B indicates the effects of pre-training intra-CA1 infusion of antibody to S100B protein on step-down latency. Pre-training administration of antibody to S100B protein, fifteen minutes before training, disrupted acquisition of memory in step-down first latency $p < 0.001$. (fig. 5A) and total time spent on platform ($p < 0.001$); (Fig 5B) compared with saline-treated animals, on the test performed 24h after training.

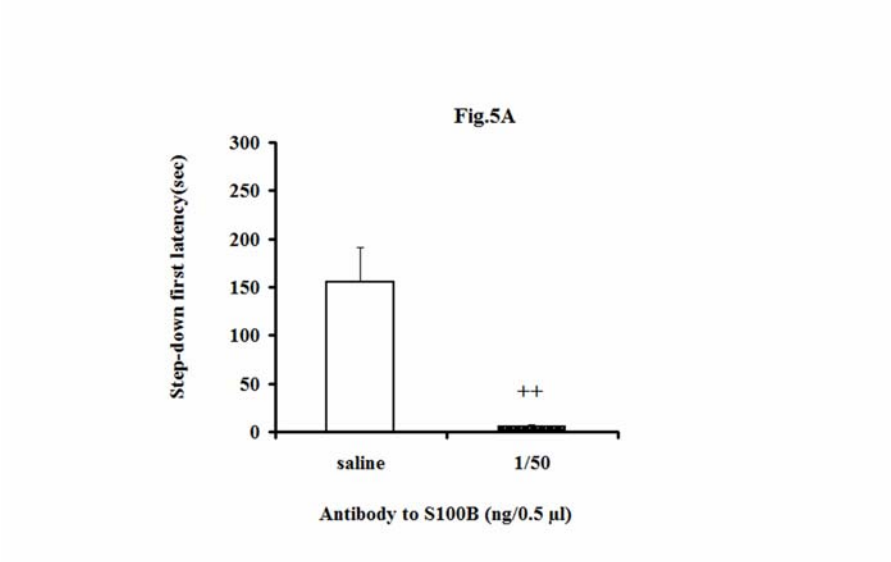

Fig.5A The effects of pre training infusion of antibody against S100B protein on consolidation of memory. Each bar shows the mean ± S.E.M. of step-down first latency of retention test which was performed 24 h after training. ++ $P < 0.01$ vs. control group. (n=8)

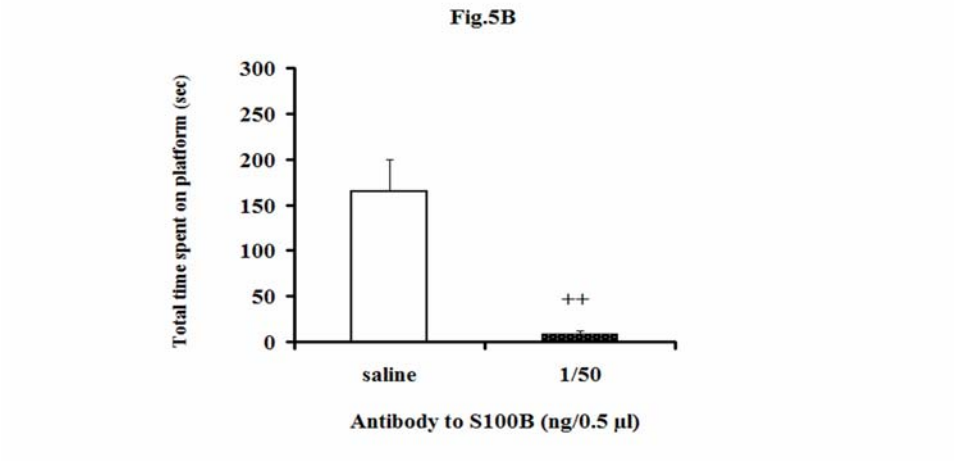

Fig.5B The effects of pre training infusion of antibody against S100B protein on total time spent on safe platform in passive avoidance learning. Each bar shows the mean ± S.E.M. of step-down first latency of retention test which was performed 24 h after training. ++$P < 0.01$ vs. control group. (n=8)

Discussion

The results of present study suggest that infusion of S-100B and its antibody after training have dose dependent effects on memory. Intra-CA1 infusion of S-100B protein and its antibody in high doses impaired memory while, infusion of both drugs in a ultra low doses facilitates memory consolidation in passive avoidance task.

Consistent to our data in the study on transgenic mice has been shown that, high levels of S-100B, impaired spatial learning in T-maze and water maze (14), as well as habituation to novelty (20). Also Sherstnev et al. (2003) reported that infusion of S-100B into the vermis of the cerebellum in doses of proapoptotic impair, while in antiapoptotic dose facilitate acquisition of two forms of defensive behavior(17). Memory-enhancing effect of S-100B in ultra low doses observed in our study is in agreement with neurotrophic effect of this protein (2, 12, 21, 22). On the other hand, memory impairment in micromolar levels resembles the apoptotic and pro-inflammatory effects of S 100B (8, 23). However, there is only one study (24), showing that S-100B infusion in high concentration of micro molar into the rat hippocampus not only doesn't disrupt memory, but also facilitates long-term memory for an inhibitory avoidance task. The contrariety in these two studies might be related to different protocols and design of studies. They had two times infusion of S100B with one-week interval betwee open field and passive avoidance task, however, we had a single injection. In order to determine whether the activity of S-100B protein is important for learning and memory formation we injected different doses of S-100B antibody into the CA1 region of hippocampus.

The results of second part of our study revealed that animals infused with low concentrations of antibody against S-100B, showed a significant improvement in cognitive function over that observed in vehicle-infused animals. While animals infused with low concentrations of antibody against S-100B showed impairment in the consolidation of memory. This finding has been confirmed in the study of Pavlov 2007 (18), even though they studied per oral administration of ultra low dose of antibody on the task of contact with sucrose solution. In addition, the block of long-term sensitization following administration of S-100B antibody in the terrestrial snail which has been reported by Andrianov et al. (2009) confirms the role of this antibody in memory formation (25).

In our study animal learned to avoid footshook stimulus in a single trial avoidance learning. It seems this kind of associative learning is modulated by S-100B protein in the hippocampus. This finding with the results of earlier studies showing increase in S-100B levels after some behavioral tasks (26-27) reinforce the hypothesis of the involvement of astrocytes in data processing of neurons. Our interpretation of these findings is that S-100B probably involves in the early stage of memory formation such as early phase of LTP ،rapid enhancing the intracellular Ca concentrations (10, 28), or even modulating glutamate transmission (29).). Whether or not s100B receptor, RAGE (30-31) is involved in the mediating of neural plasticity on memory needs more investigations.

It seems, the rapid increase in memory, does not certainly involve the formation of new neurons, or enhancing hard ware of data processing in hippocampus, which was postulated from apoptotic effects of this protein from previous studies. Moreover the finding that S-100B blockage by antibody inhibits the learning and memory, indicates that antibody blockage might disrupts the processing of engram in early phases.
On the contrary, low doses of antibody might enhance the protein effect, possibly through releasing of S-100B from glial cells (32) or direct stimulation of second messengers like cAMP (33) which leads to LTP induction. Generally, our data indicates that memory formation needs an optimum level of S-100B, and any increase or decrease in the level of this protein can disturb consolidation of memory. Therefore involvement of this protein cannot be limited to plastic phenomena of the physiological (memory-like) function, but also to pathological ones (34).

Thus from clinical point of view these results explain why elevation in the brain concentration of S-100B following some situations like trauma, Alzheimer and stroke, leads to behavioral disturbances and cognitive dysfunctions. Although S-100B elevation might be part of a protective effect of microglia in brain homeostasis (30). Moreover, this could provide compelling evidence for the theraupeutic potential of S-100B in improving functional recovery. Future studies targeted toward a more complete understading of mechanisms of S-100B on neurons are required.

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