eISSN 1303-1775 • pISSN 1303-1783

Neuroanatomy (2005) 4: 43–48

Original Article

The effect of low protein diet on thalamic projections of hippocampus in rat

Published online 29 November, 2005 © http://www.neuroanatomy.org

Mohammad BAYAT [1] ✦
Gholam Reza HASANZADEH [2]
Mitra BARZROODIPOUR [1]
Maryam JAVADI [3]

Department of Anatomy [1] and Department of Social Medicine [3], Qazvin University of Medical Science, Qazvin–Iran; Department of Anatomy [2], Tehran University of Medical Science, Tehran–Iran.

✦ Mohammad Bayat,
MS of Anatomy, Department of Anatomy, Qazvin University of Medical Science, Shahid Bahonar Boulevard, Qazvin–IRAN
☎ 98-281-333 60 01-6
98-281-332 49 70
mohamad_bayat@yahoo.com

Received 28 August 2005; accepted 24 November 2005

ABSTRACT

Recent investigations show that protein malnutrition alters the structure and function of some areas of the hippocampal formation. We investigated therefore the effect of protein malnutrition on thalamic projections to the CA1 hippocampal area.

In this study, the efferent projections from the thalamus to hippocampus in rat by horseradish peroxidase (HRP) neural tract tracing was investigated in two groups. The control group was fed with regular diet (18% protein) whereas the study group was fed with low protein diet (8% protein).

In the control group we found that the whole anterior thalamic nuclei and nucleus reuniens send projections to the CA1 hippocampal region. Among these nuclei, the anteroventral nucleus (AV) had the highest amount of labelled neurons which sent projection to the CA1 hippocampal region. Anterior thalamus projected to both hippocampus. Number of HRP labelled neurons in the contralateral thalamus were less than the ipsilateral thalamus. As a result of the influence of low protein diet, efferent projection from the anterior thalamus and nucleus reuniens to the CA1 region of hippocampus had decreased ($p<0.05$) in the study group.

The reason may be due to reduction of neuronal activity of thalamus and hippocampal formation under the influence of protein restriction or the affected progression of developmental programmes controlling synaptogenesis. ***Neuroanatomy; 2005; 4: 43–48.***

Key words [thalamus] [CA1] [hippocampal area] [protein malnutrition] [efferent projection]

Introduction

Hippocampal formation is a part of limbic system that consists of dentate gyrus, proper hippocampus, subicular complex and entorhinal cortex [1]. Proper hippocampus consists of three parts in transverse section: CA1, CA2 and CA3. Each part has three cellular layers. Middle layers consist of pyramidal cells that are the chief cells of hippocampus. Efferent projections from proper hippocampus are axons of these pyramidal cells [2]. Hippocampus converts short term memory into long term memory. Without hippocampus, symbolic type of long term memory is not stabilized [3]. Hippocampus has connections with the entorhinal area such that efferents of entorhinal area synapse with pyramidal cells of CA3. The entorhinal area connects with extensive areas of neocortex. In this manner all types of sensory inputs reach the hippocampal formation [2].

Hippocampal formation receives afferent fibers from septal area, supramammillary area cholinergic cells of medial septal and Broca nucleus, anterior thalamus, lateral dorsal and midline thalamic nuclei and medial part of pulvinar. Activity of hippocampal formation is modified with afferents that come from the brainstem [2, 4].

Thalamus is the largest part of diencephalon and contains numerous nuclei. Each thalamic nucleus except for the reticular nucleus, sends efferents to the cortex, so that each part of cortex has reciprocal connections with the thalamus. Internal medullary lamina consists of axons that enter or leave the thalamus, dividing it into three masses of gray matter: anterior, lateral and medial thalamic nuclear groups. Anterior thalamic nucleus is enclosed by the bifurcation of the lamina [5].

The principal afferent of anterior thalamus comes from mammilary bodies through the mammillothalamic tract. These nuclei also receive direct and massive fibers from pyramidal cells of the hippocampus. Cortical efferents of anterior thalamus are sent to the cingulate gyrus through the anterior limb of internal capsule [2]. Also, projections from anterior and midline thalamic nuclei and nucleus reuniens are sent to the hippocampus [6]. Connections between hippocampus and diencephalon have an important role in memory. An experimental study on monkeys showed that thalamic lesions cause disorder of recent memory [3].

Protein malnutrition is a common type of malnutrition troughout the world [7]. It is evident that this type of malnutrition has effects on some parts of the brain such as the hippocampal formation [8]. Protein malnutrition causes decrease in the number of synapses in some cortical areas and changes of behavior in animals. It can also reduce the serotonergic afferents of hippocampus [9]. Based on the physiological importance of connections between thalamus and hippocampus in memory and learning, and effects of protein malnutrition on hippocampal formation, we decided to study the effect of protein malnutrition on the thalamic efferent projections to the hippocampus.

Material and Methods

Thalamus is made up of several nuclei that individually form specific connections. In this experiment we used HRP to study the effects of protein malnutrition on the connections between thalamus and CA1 hippocampal area.

Following injection, HRP is absorbed by axonal endings and is transferred retrogradely to perikaria that send projections to the injection site. It accumulates in several vesicles, which are HRP labelled cells. In this study, after injection of HRP into CA1 hippocampal area, we counted the number of labeled cells and studied their topography in thalamic nuclei bilaterally both in study and control groups.

Wistar rats of male sex were used (103±2 gr; n=22). Prior to the study they were divided into two groups (study and control) in simple randomized manner. During a 7 months period the control group was fed with regular (18% protein) and the study group was fed with low protein (8% protein) diet. The rats were kept in a 12 hours light/dark cycle. All of the experimental procedures was approved by the Animal Ethics Committee of Qazvin Medical University. Animals were anesthesized after an intraperitoneal injection of ketamin (40 mg/kg) and zylazin (5mg/kg). We performed a stereotaxic injection of HRP (Sigma) enzyme into the CA1 hippocampal region of the two groups. After surgery, rats were allowed to recover for 48 to 72 hours and were then anesthesized deeply with ketamin and zylazin. The animals were perfused intracardiacly with fixative solution (glutaraldehyde 1.25% and paraformaldehyde %1 in 0.2 mol buffer phosphate at pH=7.4) followed by sucrose buffer 10%.

After removal, the rat brains were cut using freezing microtome (Cryocut 1800) in coronal sections at a thickness of 40 μm and stored in phosphate buffer. Sections were reacted with tetra methyl benzidin (Sigma, Mo.,USA) following the procedure of Mesolam et al [10]. Sections were then mounted onto gelatinized slides, air-dried and counterstained with neutral red.

After assessment of the injection site, we examined slides with light microscope and took digital photographs from all thalamic areas. Selected slices were traced with reference to the atlas of Paxinos and Watson (1986) and the injection site (Fig. 1) and retrogradely labeled cells were plotted with the use of a microprojectore. Topographical study on dispersion of labelled cells with HRP was performed by Adobe Photoshop 7.0 software; we used Image tool 2.0 and SPSS 11.0 (Mann-Whitney and t test) softwares for the analysis of findings.

Results

The results of our study are summarized in Chart 1.

Anterodorsal (AD) thalamic nucleus

In the control group we observed that the ipsilateral AD sends numerous projections to CA1, which appears with the presence of numerous labelled cells in the slices (Fig. 2). These cells were scattered throughout the nucleus.

The contralateral AD also send projections to CA1. The number of labeled cells in the contralateral nucleus was lesser than ipsilateral AD but the topography was similar.

In the study group meaningful difference ($P<0.05$) was present between study and control groups in the number of labeled cells in the ipsilateral AD with no difference in topography. There were no labeled cells in the controlateral AD of the study group (Fig 3).

Anteroventral (AV) thalamic nucleus

This nucleus is divided into ventrolateral (AVVL) and dorsomedial (AVDM) parts. In the control group ipsilateral AVVL sent numerous projections to CA1. In the anterior (rostral) part of AVVL, the labelled cells located in the dorsal part of the nucleus, and in the posterior (caudal) part the labelled cells were in the ventral part. In ipsilateral AVDM labelled cells had more density in rostroventral part of nucleus. The number of labelled cells in the contralateral AVVL and AVDM were less than those of the ipsilateral side, but the topography was similar.

In the study group that was fed with low protein diet we found that the amount of projections from AVVL and AVDM were lesser than ($P<0.05$) the control group. In the ventral parts of AVVL and AVDM there were no labelled cells with few labelled cells in the dorsal part.

Anteromedial (AM) and interanteromedial (IAM) thalamic nuclei

In the study group the lateral part of the AM sent projections to CA1 region of both sides. Fibers that reached the contralateral hippocampus were fewer. More labelled cells were seen in the middle sections of AM. In the study group, we observed that the number of projections sent to the hippocampus were reduced ($p<0.5$) and the density of labelled cells were more anterolaterally located.

The ipsilateral AMV (ventral part of AM) was found to send fibers to CA1 with no difference between the number and topography of labelled cells in the control and study groups.

In the rostral slices, AM nuclei of both sides were fused. In the control group we observed few labelled cells rostrally in the midline. There were no labelled cells in the IAM of the study group.

Lateral dorsal (LD) thalamic nucleus

In the ipsilateral thalamus of the control group, the ventrolateral part of LD (LDVL) had labelled cells. These cells were located in the ventromedial part of anterior sections, in the ventrolateral part of posterior sections of the LDVL. Contralateral LDVL had no labelled cells in control and study groups. In the ipsilateral thalamus of the control group, dorsomedial part of LD (LDDM) had labelled cells in the medial part. Contralateral LDDM of control group had few labelled cells medially.

Comparing the number and topography of labelled cells in the ipsilateral LDVL and LDVM, there was no significant difference between the study and control groups, with no labelled cell in the contralateral LDDM.

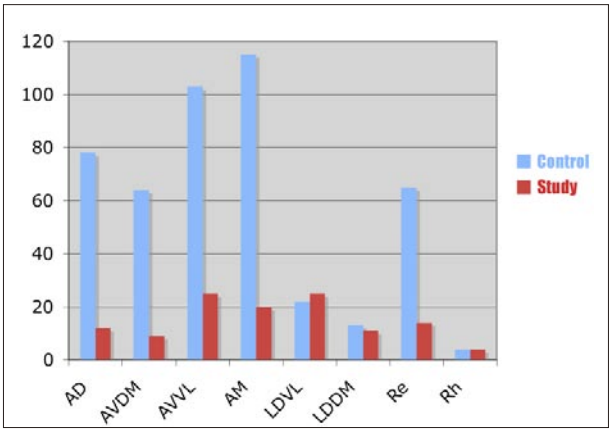

Chart 1. Comparison of the total number of labeled cells in ipsilateral thalamus of the study (red) and the control groups (blue).

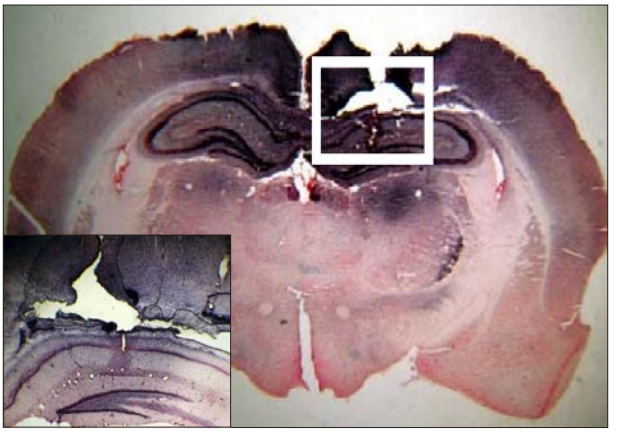

Figure 1. Photograph of the injection site of HRP. *(**Bregma:** -3.60; **Lateral:** 1.80; **Depth:** 3.20)*

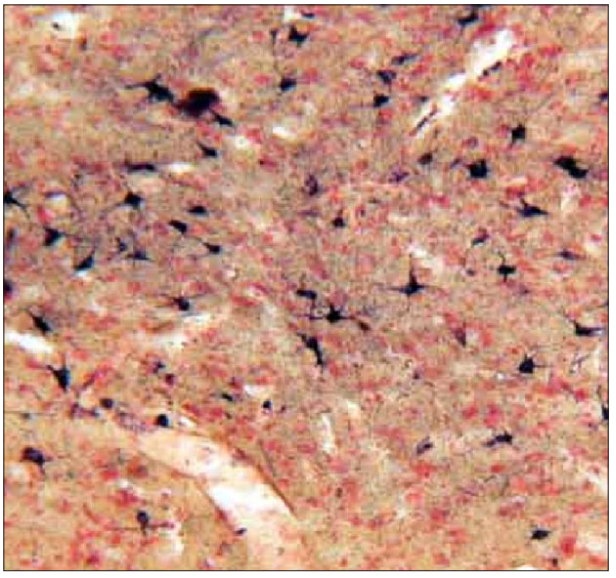

Figure 2. Labeled cells in ipsilateral AD of control group.

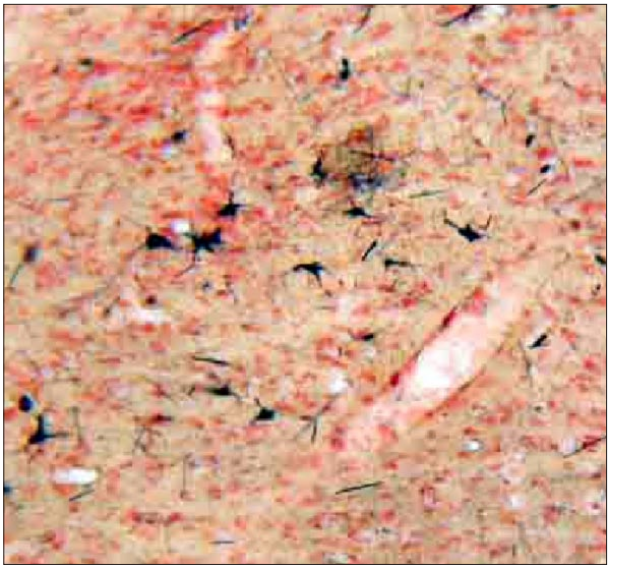

Figure 3. Labeled cells in ipsilateral AD of study group.

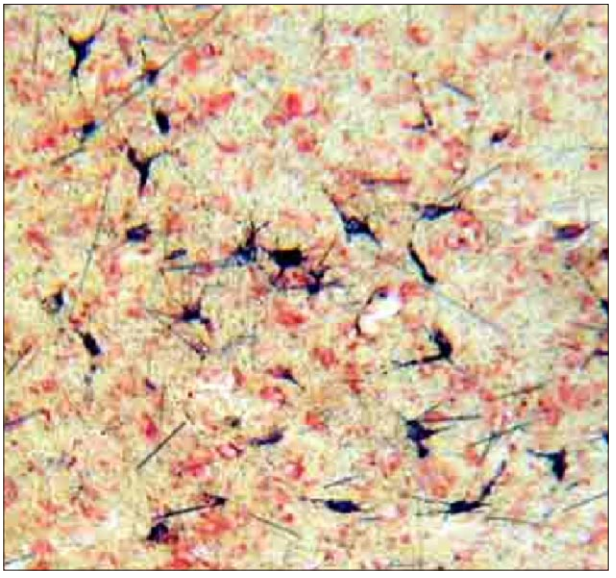

Figure 4. Labeled cells in anterior part of Re in control group.

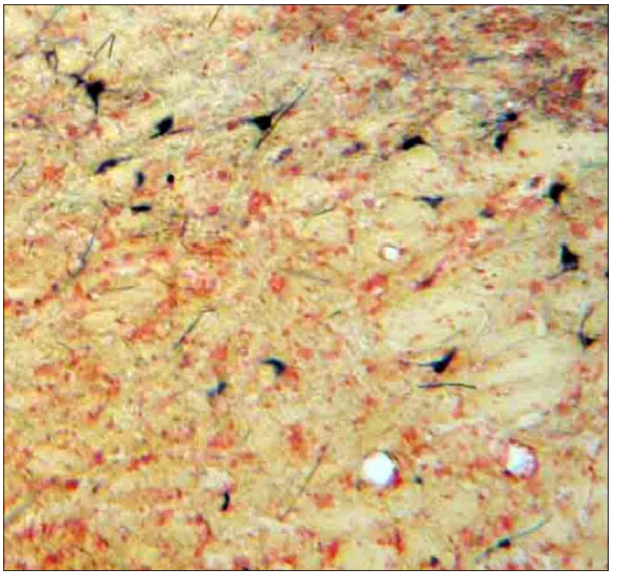

Figure 5. Labeled cells in anterior part of Re in study group.

Midline thalamic nuclei

In this group we found that the reuniens (Re) and rhomboid (Rh) nuclei send projections to CA1 hippocampal area.

Nucleus Reuniens (Re)

This nucleus was divided into right and left halves in rostral slices. After injection of tracer into CA1, labelled cells were observed in the rostral and caudal sections of Re (Fig 4). In this area, labelled cells were scattered between other cells. Rostral part of nucleus Re had no labelled cells.

The number of labelled cells in the Re of the study group was also lesser than the control group ($p<0.05$).

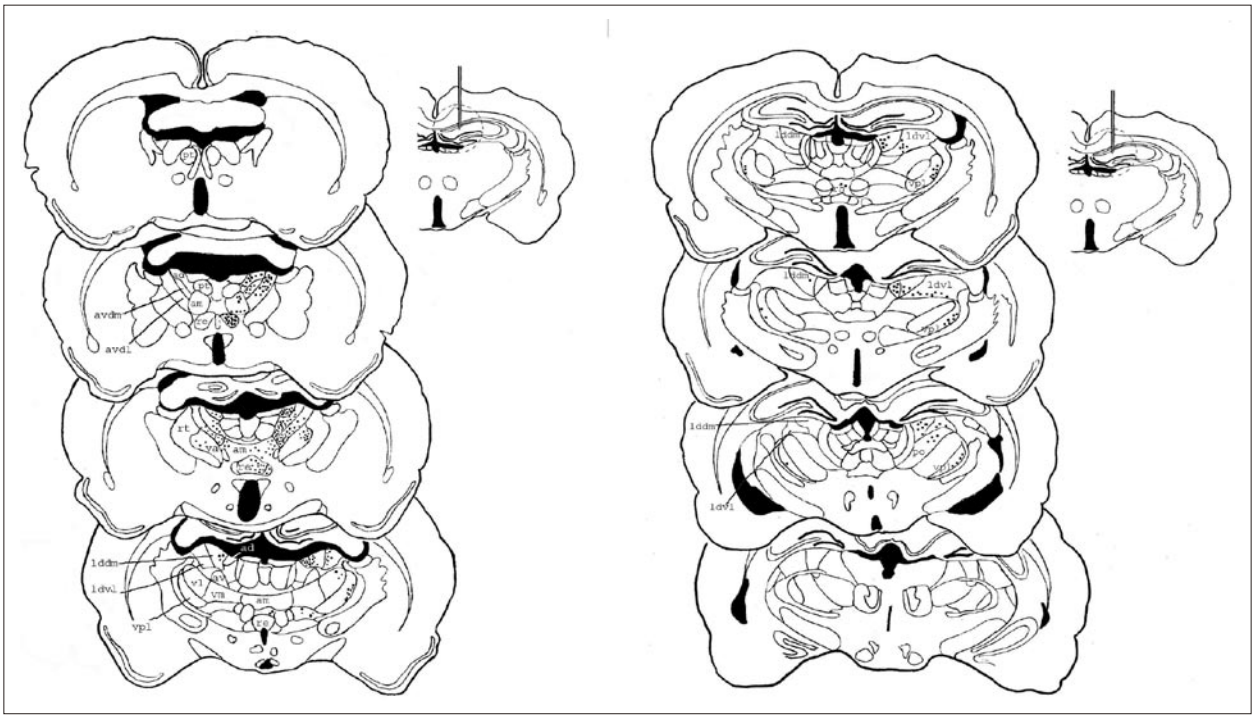

Figure 6. Drawing of dispersions of labeled cells in control group.

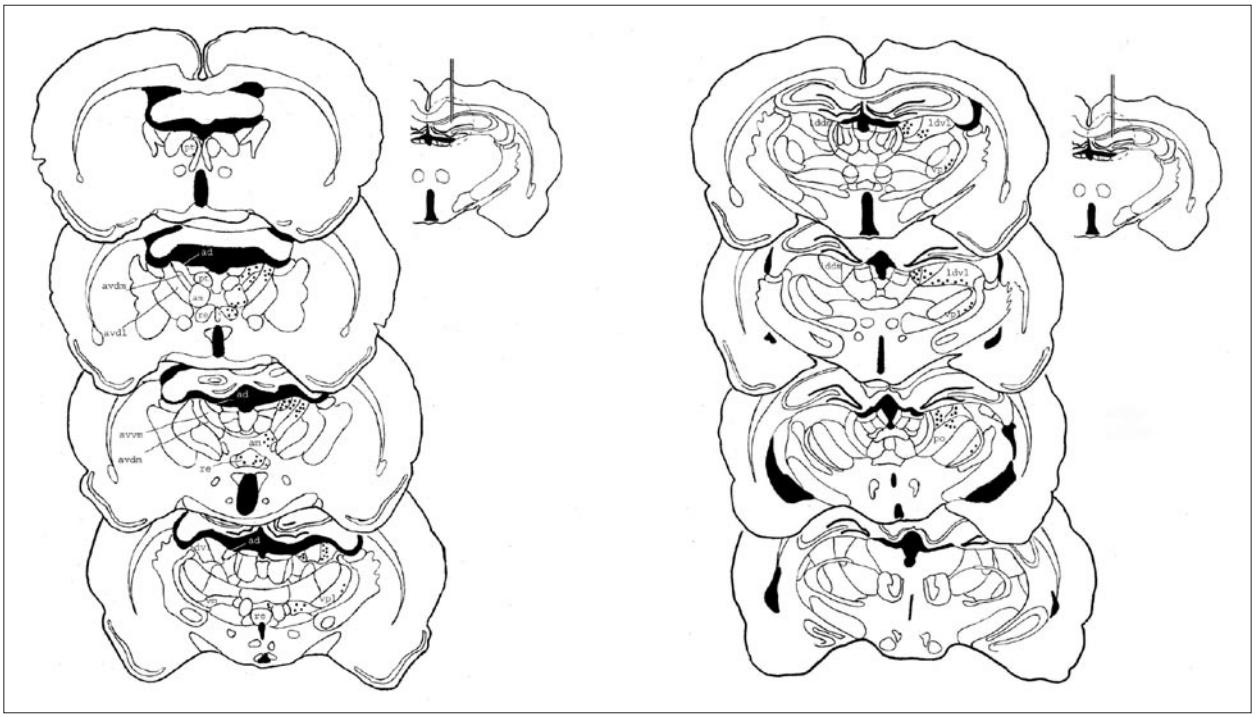

Figure 7. Drawing of dispersions of labeled cells in study group.

Topography of dispersion of the labelled cells was similar in both groups (Fig. 5).

Rhomboid nucleus

In the study and control groups there was no difference in the number and topography of dispersion of labelled cells. Labelled cells in this nucleus were located in the lateral part of the caudal slices.

Ventrolateral (VL) and ventromedial (VM) thalamic nuclei

On the side of injection of HRP, nucleus VL projected to CA1. Cells that sent fibers to hippocampus were in the anterodorsal part of VL. In the VL nucleus of study group we found no labelled cells.

More rostral parts of the VM in the control and study groups was found to send few projections to CA1. These were dispersed in the anterior sections of this nucleus.

Lateral posterior (LP) nucleus

We found that in the study and control groups on the side of injection there were few dispersed labelled cells througout the nucleus.

Posterior (Po) and ventral posterolateral (VPL) thalamic nuclei

In the dorsal part of the middle sections of the ipsilateral Po of the control group a few labelled cells were seen. This shows that this part of the Po sends projections to the hippocampus. In the study group, labelled cells in the Po were similar those of the control group.

Labelled cells were seen in the control group in the lateral part of the VPL adjacent to reticular thalamic nucleus. Labelled cells were in the ipsilateral and contralateral VPL. The number of labelled cells in the contralateral VPL were fewer than the ipsilateral side. We compared number and topography of connections between VPL and CA1 in the two groups and found that the number of labelled cells were reduced in the study group with no change in the topography of their connections.

Parataenial (Pt) thalamic nucleus

In the dorsal part of Pt, we found a few labelled cells on both sides in the control group. Controlateral Pt sent fewer projections to CA1 compared to the ipsilateral. Examinations of Pt in the study group revealed no labelled cells on both sides.

The topographical organization of the labeled cells for the control and study groups are given in Figure 6–7.

Discussion

In this study it was found that all anterior thalamic nuclei (AM, AV and AD) sent their projections to the hippocampus. Among these nuclei, the AV was found to have the most efferent projections to CA1. Anterior thalamic nuclei of both sides sent efferents to CA1, but the connection of ipsilateral anterior thalamic nuclei with the hippocampus was more profuse.

It was also found that Re and Rh nuclei project to CA1 hippocampal area. Connection of Re to hippocampus was dominant to that of Rh to hippocampus, and labelled cells were scattered in the anterior and middle sections of the nucleus showing that the anterior and middle sections of nucleus send projections to CA1. It was seen that LD thalamic nucleus on the side of the injection site sends projection to CA1. We also found that ipsilateral VPL, Po, LPMR, VM, VL and Pt send a few fibers to CA1 area.

Paxinos reports that all anterior thalamic nuclei and nucleus Reuniens send projections to the hippocampus [2]. Swanson and Wyss in their study on the subcortical afferents of the hippocampus using HRP as a tracer and tetramethylbebenzidin histochemical reaction found that all anterior thalamic nuclei, Re, PV and Pt send projection to the hippocampus. They concluded that AD sends more efferents to the ipsilateral hippocampus and found labelled cells of AM on both sides with ipsilateral predominancy [6].

Van Groen et al stated that AM sends projections to the entorhinal area and subiculum. There was no information about the hippocampus as a target of efferents [11].

Bokor et al found that the neurons of nucleus Re thalami send projection to the CA1 hippocampal subfield. It is also reported that these cells are located in the dorsolateral part of the nucleus Re [12].

Su and Bentivoglio described efferents of Re and stated that Re sends projections to hippocampus, amygdala and nucleus accumbens [13]. Herkenham found in his study with autoradiography and HRP tracing that there is a direct connection between Re and hippocampus in which those efferents pass through the genu of internal capsule and terminate in the stratum lacunosom moleculare of CA1 [14].

In the examination of the number and dispersion of labelled cells in animals fed with low protein diet (8%), we found that the amount of efferent projections from all anterior thalamic nuclei, Re, Rh, VPL, Po, VL, VA, and Pt was lower than the control group.

According to Mokler et al [15] protein malnutrition causes changes in the hippocampal formation.

Viana et al [16] reported that protein-energy malnutrition tends to cause significant decreases in muscarinic receptors in the hippocampus and basal ganglia. However, no significant differences in acethylcholinesterase activity or protein content were observed between control and undernutritioned animals in any of the brain areas studied.

Granados-Rojas et al [17] stated that prenatal protein malnutrition induces long-lasting deleterious effects on the progression of developmental programs controlling synaptogenesis and/or synaptic consolidation, likely by affecting a myriad of cellular progresses.

Andrade et al [18] found that protein deprivation experienced in adult rats causes reduction in the volume of the subiculum and the total number of its neurons. They reported that protein malnutrition causes a marked regressive change in the basal dendritic trees of the pyramidal subicular neurons. However, the spine density was increased in malnourished rats. They concluded that the effects of long-term protein deprivation are region specific and that the resulting structural alternations are confined to the three-layered components of the

hippocampal region. Andrade et al [8] also showed that among hippocampal neurons, dentate granule cells are selectively vulnerable to food restriction. Nonetheless the reorganization which takes place in their dendrites and synapses is capable of minimizing the functional importance that were expected to occur following changes in the hippocampal neuronal circuitry induced by this type of dietary restriction.

Results of these experiments show that the hippocampal formation is vulnerable to protein malnutrition. Due to this type of restriction, significant functional and morphological changes have been reported. Our findings show that under the influence of protein malnutrition efferent projections from anterior nuclear group and midline thalamic nuclei to the CA1 hippocampal area had decreased. According to our results, it was assessed that in protein malnourished rats after injection of a retrograde tracer in CA1 hippocampal area, the number of labelled cells in the anterior and midline thalamic nuclei is reduced compared to normal animals.

We conclude that neuronal activity of the thalamus and hippocampal formation may be affected by the influence of protein restriction or the progression of developmental programs controlling synaptogenesis, or axonal transport may be reduced.

Acknowledgement

This study was supported by Qazvin University of Medical Science Research Unit.

References

[1] **Amaral DG, Witter MP.** The three dimensional organization of the hippocampal formation. A review of anatomical data. **Neuroscience. 1989; 31: 571–591.**

[2] **Paxinos G.** The rat nervous system. **2nd Ed., San Diego, Academic Press. 1995; 829–648.**

[3] **Guyton AC.** Textbook of medical physiology. **9th Ed., Philadelphia, Saunders. 1998; 841–843.**

[4] **Carpenter MB.** Core text of neuroanatomy. **4th Ed., Baltimore, Williams and Wilkins. 1991; 370–373.**

[5] **Kiernan JA, Barr ML.** The human nervous system. **7th Ed., Philadelphia, Lippincott-Raven. 1998; 324–350.**

[6] **Wyss JM, Swanson LW, Cowan WM.** A study of subcortical afferents to the hippocampal formation in the rat. **Neuroscience. 1979; 4: 463–476.**

[7] **Pitkanen A, Maria Pikkarainen M, Nurminen N, Ylinen A.** Reciprocal connections between the amygdala and hippocampal formation, perirhinal cortex and postrhinal cortex in rat. A review. **Ann. N. Y. Acad. Sci. 2000; 911: 369–391.**

[8] **Andrade JP, Lukoyanov NV, Paula-Barbosa MM.** Chronic food restriction is associated with subtle dendretic alternations in granule cells of the rat hippocampal formation. **Hippocampus. 2002; 12: 149–164.**

[9] **Krause MV, Mahan LK, Escott-Stump S.** Krause's food, nutrition and diet therapy. **10th Ed., Philadelphia, Saunders. 2000; 242.**

[10] **Mesulam MM, Van Hoesen GW, Pandya DN, Geschwind N.** Limbic and sensory connections of the inferior parietal lobule (area PG) in the rhesus monkey: a study with a new method for horseradish peroxidase histochemistry. **Brain Res. 1977; 136: 393–414.**

[11] **van Groen T, Kadish I, Wyss JM.** Efferent connections of anteromedial nucleus of the thalamus of the rat. **Brain Res. Brain Res. Rev. 1999; 30: 1–26.**

[12] **Bokor H, Csaki A, Kocsis K, Kiss J.** Cellular architecture of the nucleus reuniens thalami and its putative aspartatergic/glutamatergic projection to the hippocampus and medial septum in the rat. **Eur. J. Neurosci. 2002; 16: 1227–1239.**

[13] **Su HS, Bentivoglio M.** Thalamic midline cell populations projecting to the nucleus accumbens, amygdala, and hippocampus in the rat. **J. Comp. Neurol. 1990; 297: 582–593.**

[14] **Herkenham M.** The connections of nucleus Reuniens thalami: evidence for a direct thalamo-hippocampal pathway in the rat. **J. Comp. Neurol. 1978; 177: 589–610.**

[15] **Mokler DJ, Bronzino JD, Galler JR, Morgane PJ.** The effects of median raphe electrical stimulation on serotonin release in the hippocampal formation of the prenatally protein malnourished rats. **Brain Res. 1999; 838: 95–103.**

[16] **Viana GS, Figueiredo RM, Bruno JA.** Effect of protein-energy malnutrition on muscarinic receptor density and acetylcholinesterase activity in rat brain. **Ann. Nutr. Metab. 1997; 41: 52–59.**

[17] **Granados-Rojas L, Larriva-Sahd J, Cintra L, Gutierrez-Ospina G, Rondan A, Diaz-Cintra S.** Prenatal protein malnutrition decreases mossy fibers-CA3 thorny excrescences asymmetrical synapses in adult rats. **Brain Res. 2002; 933: 164–171.**

[18] **Andrade JP, Madeira MD, Paula-Barbosa MM.** Differential vulnerability of the subiculum and entorhinal cortex of the adult rat to prolonged protein deprivation. **Hippocampus. 1998; 8: 33–47.**