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João Paulo Ferreira de Castro

Effects of Malnutrition in the Number of Hippocampal Formation Dentate Neurons of Adult Rats

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Effects of Malnutrition in the Number of Hippocampal Formation Dentate Neurons of Adult Rats

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Prof. Doutor José Paulo Alves Vieira de Andrade

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Effects of Malnutrition in the Number of Hippocampal Formation Dentate Neurons of Adult Rats

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ABBREVIATIONS

PV-ir – Parvalbumin-immunoreactive

Groups of animals:

FD – Food-deprived

LPD – Low-protein diet

RecLPD – Recovery low-protein diet

ABSTRACT

Several studies have demonstrated the vulnerability of the adult hippocampal formation to malnutrition. In this study, we have compared the effects of two models of dietary deprivation, food restriction and protein malnutrition, in the number of neurons of dentate gyrus and parvalbumin-immunoreactive (PV-ir) interneuronal subpopulation, related to the control of calcium homeostasis and fine tuning of the hippocampal circuits. We also tested if nutritional rehabilitation of protein-deprived rats could reverse the alterations found.

Two month-old rats were randomly assigned to control, food-deprived (40% caloric restriction) or low-protein diet groups (8% casein). After 6 months of treatment, 10 rats from the low-protein diet group were selected at random and fed *ad libitum* for 2 months. Using stereological methodology, the numbers of granule and hilar cells as well as the PV-ir interneurons were quantified.

It was found that the total number of granule and hilar neurons was reduced in protein-malnourished rats and the exposure to a normoproteic diet does not lead to a normalization of neuronal numbers. PV-ir interneurons located in the granule layer and hilus were increased in protein-deprived rats when compared to the other groups, but their number returned to values similar to controls after protein rehabilitation. Our data also shows that food deprivation does not affect significantly the number of neurons or PV-ir interneurons of the adult dentate gyrus.

These results support the view that protein deprivation may provoke a disturbance in calcium homeostasis leading to neuronal death. The up-regulation of PV-ir cells could be due to a protective mechanism to counteract the calcium overload and protect the surviving neurons of the dentate gyrus. This imbalance in cell-ratio favoring GABAergic interneurons may justify learning and memory impairments described in

protein-deprived animals. The contrast between the results of food restriction and protein deprivation should be further analyzed.

Keywords: malnutrition; dentate gyrus; granule cells; hilar neurons; GABAergic interneurons; parvalbumin.

In Kafka's romance "A Hunger Artist", the protagonist is presented as a man who found peace and pleasure in fasting for extended periods of time. Despite the romantic view of hunger set forth in some literature and ascetic doctrines, starvation was always a painful presence in the world (WHO, 2012). There are several causes of hunger, such as natural disasters, war, poverty, poor agricultural infrastructures and over-exploitation of the environment. Today, according to the most recent estimates, there are 925 million undernourished people in the world (WFP, 2012), 98% of which live in developing countries and the remaining in the developed world (WFP, 2012).

Among several forms of malnutrition one of the most important is the incapacity to ingest a sufficient amount of food to supply our daily needs. This subset of malnutrition is more common in developing countries (FAO, 2012, WFP, 2012). Although extreme food restriction leads to an inevitably painful death, it is well established that moderate levels of food restriction are associated to beneficial effects (Andrade et al., 2002, Andrade et al., 2006). Several studies showed that rodents and monkeys submitted to a reduced, but moderate, percentage of food intake in comparison to controls, extend their mean and maximum lifespans by up to 50% (Lee et al., 2002a, Lee et al., 2002b, Michael Anson et al., 2003, Dirks and Leeuwenburgh, 2006). It has also been shown that the restriction of calories reduces or delays the incidence of several diseases typical of adulthood and senescence (Walford et al., 1987, Bronson and Lipman, 1991, Masoro, 1992, Weindruch, 1996). Indeed, food restriction was demonstrated to reduce susceptibility to the toxic effects of several chemicals (Hart et al., 1992), increase the resistance to epileptic seizures (Bough et al., 1999) and protect neurons from metabolic and ischemic insults (Bruce-Keller et al., 1999, Duan and Mattson, 1999). The mechanisms underlying the beneficial effects of food restriction in the central nervous system (CNS) are not completely understood, although some

advance that reduction of oxidative damage and increased expression of genes that encode cytoprotective proteins such as heat shock protein 70 and neurotrophins may be related (Duan et al., 2001, Lee et al., 2002a, Lee et al., 2002b, Michael Anson et al., 2003). On the other hand, food restriction seldom produces adverse effects. Some studies demonstrated that chronic food restriction causes slight physiological and behavioral alterations, including diminished ability to cope with stress provoked by cold (Campbell and Richardson, 1988), peripheral neuropathy (Faundez et al., 1990), slower wound healing (Harrison e and Archer, 1987) and reduced reproduction status (Gill and Rissman, 1997, Leonhardt et al., 1999). With respect to the CNS, studies have shown that food-deprived animals display lower performance in the operant conditioning and radial maze tasks (Roberts et al., 1983, Hao et al., 2000), enhanced behavioral responsiveness to hypothalamic stimulation (Carr, 1996, Abrahamsen et al., 1997) and to many drugs of abuse (De Vaca and Carr, 1998), as well as abnormally high activity (Morse et al., 1995, Altemus et al., 1996, Heiderstadt et al., 2000).

However, malnutrition is not just an empty stomach. Daily undernourishment is a less visible form of hunger, which affects many more people (Morgane et al., 1978, Waterlow et al., 1992). In fact, one of the most serious subsets of malnutrition results from a deficiency in the protein content of the diet (Lukoyanov and Andrade, 2000). For many years it was believed that the adult brain was resistant to malnutrition in general and protein deprivation in particular (Morgane et al., 1978, Lukoyanov and Andrade, 2000). Nevertheless, several studies have unveiled the enormous vulnerability of the adult hippocampal formation to protein deprivation (Paula-Barbosa et al., 1989, Andrade et al., 1996, Andrade and Paula-Barbosa, 1996, Lukoyanov and Andrade, 2000, Mesquita et al., 2002). For example, it has been found that exposure of adult rats to a low-protein diet for 6 months results in neuronal loss in the dentate gyrus, CA3,

CA1 (Paula-Barbosa et al., 1989, Andrade et al., 1995a) and subiculum of the hippocampal formation (Andrade et al., 1998). Moreover, protein-deprived adult rats present degenerative alterations in dendritic arborization (Andrade et al., 1996), loss of synaptic contacts (Andrade et al., 1995b) and changes in the cholinergic and GABAergic systems mainly in the hilar region (Andrade and Paula-Barbosa, 1996). In agreement with this, behavioral studies indicate that some aspects of learning and memory are altered in these malnourished rats (Lukoyanov and Andrade, 2000).

Adult rats submitted to protein malnutrition have an irreversible reduction in the total number of hippocampal inhibitory GABAergic neurons (Andrade and Paula-Barbosa, 1996). However, it would be important to know if the several largely non-overlapping subpopulations with different structural and functional characteristics are equally affected by protein deprivation (Celio, 1990, Brady and Mufson, 1997, Lister et al., 2011). Quantifying the entire GABAergic inhibitory population probably obscures differential effects on individual interneurons subpopulations and a change in a particular subgroup might not be noticed if there was compensation in another subpopulation (Lister et al., 2011). Thus, we have utilized a specific antibody to quantify a particular type of GABAergic interneuron, the parvalbumin-immunoreactive (PV-ir) interneurons of the dentate gyrus. These neurons compose approximately half of the population of hilar GABAergic neurons (Celio, 1990, Lister et al., 2011) and express parvalbumin, a member of the calcium-binding proteins group that also includes calbindin D-28K and calretinin (Celio, 1990, De Jong et al., 1996, Lawrence et al., 2010). Although the functions of these proteins are not yet well defined, they contribute to the organization of the cytoskeleton, axonal transport, membrane excitability and the synthesis and release of neurotransmitters providing a common mechanism of resistance to neurodegeneration in some disorders (Celio, 1990, De Jong et al., 1996, Sisó et al.,

2003, Vreugdenhil et al., 2003). PV-ir cells are predominantly chandelier cells and basket cells (75-85%) (Baude et al., 2007) that provide inhibition to the principal neurons at the axon initial segment and cell body, respectively, regulating the precise timing of principal cells activation (Freund, 2003, Vreugdenhil et al., 2003, Lister et al., 2011, Szilágyi et al., 2011). These types of interneurons are thus an important population in the regulation of the output from the granule cells and a change in this population would affect synaptic transmission through the hippocampal formation, with possible implications in cognitive functions including learning and memory (Freund, 2003, Lister et al., 2011).

Therefore, we sought to evaluate and compare, in this study, the effects of two models of malnutrition in the structure of the dentate gyrus of the hippocampal formation of the adult rat. In order to do that, we quantified the total number of granule and hilar neurons and also evaluated the effects in the neuronal density of PV-ir interneurons. Furthermore, and bearing in mind that some of the protein malnutrition-induced alterations described in the hippocampal formation of adult rats are reversible, we also found it of interest to test whether animals previously exposed to protein deprivation followed by nutritional rehabilitation would recover from the anatomical impairments.

2. EXPERIMENTAL PROCEDURES

2.1 Animals and Diets

Forty male Wistar rats, obtained from the colony of the Charles River Laboratories (Barcelona, Spain), were used in the present study. Animals were housed individually and maintained under standard laboratory conditions (20-22ºC and a 12:12-h light-dark cycle). At 2 months of age, rats were randomly assigned to a control group, a low-protein diet group or a food-deprived group. Control rats (n=10) were fed *ad libitum* throughout the entire experimental period with standard laboratory chow (Letica, Spain) containing: proteins (17%), supplemented with lysine (0.7%), methionine (0.3%) and cysteine (0.5%), carbohydrates (57%), fat (4%) and salts (7%). Low-protein diet group (n=20) were fed for 6 months with a low-protein diet (MP Biomedicals, USA) containing: casein (8%), supplemented with methionine (0.3%), carbohydrates (78%), fat (10%) and ICN Salt Mixture U.S.P. XIV (4%). Both diets were supplemented with ICN Diet Vitamin Fortification Mixture. All rats had free access to food and water throughout the experimental period. Food-deprived group (n=10) were fed 40% of the amount of food consumed by control animals every day for 6 months. The latter were fed once a day at 08:00 a.m.

At 6 months of treatment, 10 rats from the low-protein diet group were selected at random and switched to the *ad libitum* feeding regimen (Letica, Spain) for 2 months (recovery low-protein diet group). All the animals were killed at 10 months of age.

The handling and care of the animals were conducted according to the UE guiding principles in animal research (86/609/UE) and Portuguese Act no. 129/92, whose directives are strictly followed in Faculty of Medicine of Porto.

2.2 Tissue Preparation

2.2.1 General Procedures

Five control, five food-deprived, five low-protein diet and five nutritionally rehabilitated rats were randomly selected, deeply anesthetized with chloral hydrate (i.p., 1ml/100g body weight, 6% solution) and transcardially perfused with a fixative solution containing 1% paraformaldehyde and 1% glutaraldehyde in 0.12 M phosphate buffer at pH 7.4. The brains were removed from the skulls, weighed, codified to allow blind estimations, placed in fresh fixative and processed for glycolmethacrilate embedding.

Another set of 5 rats per experimental group, were killed by transcardiac perfusion of a fixative solution containing 4% paraformaldehyde in phosphate buffer at pH 7.6. Subsequently, the brains were rapidly removed from the skulls, coded for blind processing and analysis, stored in the same fixative solution for 1h and maintained overnight in a 10% sucrose solution at 4ºC. Blocks containing the right and left hippocampal formations were mounted on a vibratome, serially sectioned in the coronal plane at a nominal thickness of 40 μm, collected in phosphate buffered saline and used for immunocytochemistry.

2.2.2 Glycolmethacrilate Embedding

After 30 days of postfixation, blocks of tissue containing the entire hippocampal formation and the adjacent neocortical shell, from 5 animals per group, were dehydrated through a graded series of ethanol solutions and embedded in glycolmethacrylate, as described in detail elsewhere (West et al., 1991). These blocks were then serially sectioned in the horizontal plane at a nominal thickness of 40 μm using a Jung Multicut microtome. Every tenth section was collected using a systematic random sampling

procedure (Gundersen and Jensen, 1987), mounted serially, and stained with a modified Giemsa solution (West et al., 1991).

2.2.3 Immunocytochemistry

One set of vibratome sections containing the hippocampal formations were selected, using a systematic random sampling procedure, from 5 rats of each experimental group. These sections were used for PV immunostaining using the rabbit polyclonal antibody against PV (PV-28, SWant, Switzerland). For immunocytochemical staining, all sections were processed simultaneously, using the avidin-biotin technique with diaminobenzidine as the chromogen as previously described (Mesquita et al., 2002, Silva et al., 2002). The same procedure was followed for control sections in parallel, which were incubated without primary antiserum. No immunostaining was observed in these sections.

2.3 Morphometric Analysis:

2.3.1 Estimation of Total Neuron Numbers

The total number of neurons was estimated on glycolmethacrylate-embedded sections by applying the optical fractionator method (West et al., 1991). The boundaries of the granular layer of the dentate gyrus and its hilus were consistently defined at all levels along the septo-temporal axis of the hippocampal formation on the basis of cell morphology and cytoarchitectonic criteria (Amaral DG, 1995). Estimations were carried out using the C.A.S.T.-Grid System (Olympus, Denmark) and a mean of 14 sections were used per animal. Beginning at a random starting position, visual fields were systematically sampled along the x and y axes, using a raster pattern procedure. Neurons were counted in every frame using the optical dissector at a final magnification

of x2000. The coefficient of error (CE) of the individual estimates was calculated according to Gundersen et al. (Gundersen et al., 1999) and ranged between 0.07 for hilar region and 0.09 for granular layer.

2.3.2 Estimation of the Density of PV-ir Neurons

Brain sections immunostained for PV were analyzed using a light microscope equipped with a camera lucida at final magnification of ×130. From the PV-stained sections obtained for each brain, every third section was systematically sampled to yield a set of 10 to 12 sections to be included in the analysis. Level-matched sections were used for all groups. Camera lucida diagrams of PV-ir perikarya were drawn, unilaterally, from the hippocampal fissure to the pial surface and extending towards the initial segment of CA3. Dentate gyrus and hilus PV-ir neurons were identified as darkly stained perikarya. The number of neurons that fell on each layer of dentate gyrus was then counted from the drawings. The same camera lucida diagrams were used for measuring the areas of the layers. For this purpose, a transparent sheet bearing a test system composed of a set of regularly spaced points (Gundersen et al., 1988) was overlaid on the drawings and the number of points that fell within the limits of each layer was counted. The area of each layer was then estimated by multiplying the number of points that fell on that layer by the area per point of the test system (0.00869 mm^2). Cell profile counts were performed in molecular layer, granular layer and hilus of dentate gyrus. The cell counts obtained were divided by the values of the corresponding laminar areas to yield the values of the areal densities (N/mm^2).

2.4 Statistical Analysis

The data were analyzed for statistical significance using one-way Anova test. Post-hoc analyses were performed whenever appropriate, using the Newman-Keuls test. Differences were considered to be significant if $P<0.05$. Data were shown as means ± 1 standard deviations (S.D.).

3. RESULTS

3.1 Animals and Diets

Daily food intake, measured at 08:00h every day was 31.6 ± 1.25g in control rats, 29.4 ± 4.1g in low-protein diet animals and 19.2 ± 0.9g in the food-deprived group. Nutritionally rehabilitated rats consumed 32.0 ± 1.5g per day.

The mean body weights of control, food-deprived, low protein-diet and nutritionally rehabilitated rats at the end of experiment are shown in Table 1. By the end of the experiment, the mean body weight of control rats was similar to low-protein and nutritionally rehabilitated rats. On average, the body weight of food-deprived animals was 35% lower than that of control rats ($P<0.0005$).

No significant difference was detected between the mean brain weights of control (1.54 ± 0.025 g), food-deprived (1.54 ± 0.04 g), low protein-diet and nutritionally rehabilitated (1.54 ± 0.05 g) animals at the end of the experiment.

3.2 Morphometric Analysis

3.2.1 Total Neuronal Numbers

The total number of dentate gyrus granule cells and hilar cells estimated in the hippocampal formations from control, food-deprived, low-protein diet and nutritionally rehabilitated groups are shown in Table 2.

ANOVA revealed a significant effect of feeding regimen on the total number of hippocampal neurons. In the low-protein diet group, the total number of granule cells was reduced by approximately 4% and 6% relatively to control and food-deprived groups, respectively ($P<0.05$). In nutritionally rehabilitated group, a reduction of approximately 4.4% and 7% was observed when compared to control and food-deprived groups, respectively ($P<0.05$).

A similar reduction was observed in the number of hilar cells. In the low-protein diet group, a reduction of approximately 6% and 5% was detected comparatively to control and food-deprived groups, respectively ($P<0.05$). In the recovery low-protein diet group of animals, we found a decrease of approximately 5% and 4% comparatively to control and food-deprived groups, respectively ($P<0.05$).

3.2.2 Density of PV-immunoreactive Neurons

The density of PV-ir neurons by layer of the dentate gyrus and hilus of the hippocampal formation is presented in Figure 1. PV immunoreactivity was observed in the soma, dendritic tree and in the axons of stained interneurons. PV-ir cells were heterogeneous in shape and in dentate gyrus, most of the cells were larger than the unstained granule cells and were located within or adjacent to the granular layer.

In the molecular layer of dentate gyrus, ANOVA did not detect any significant effect of feeding regimen in the density of PV-ir cells.

In the granular layer, it was detected a significant effect of feeding regimen both by ANOVA and Newman-Keuls test. In the low-protein diet group, the density of PV-ir cells was increased almost four times comparatively to controls ($P<0.05$).

A significant effect of feeding regimen was also revealed in the hilus of the hippocampal formation. In the protein-deprived group, the density of PV-ir cells was increased approximately three times relatively to control, food-deprived and nutritionally rehabilitated animals ($P<0.05$).

FD groups presented no differences when compared to controls.

4. DISCUSSION

In accordance to previous studies, chronic protein malnutrition produces significant loss of granule cells and hilar neurons (Paula-Barbosa et al., 1989, Andrade et al., 1995a, Lukoyanov and Andrade, 2000) and the exposure to a normoproteic diet after a lengthy period of protein deprivation does not lead to a normalization of the neuronal numbers (Andrade et al., 1995a, Lukoyanov and Andrade, 2000).

In rat, the neurogenesis of the granule cells continues during adulthood but it is higher during the first year of life (Masiulis et al., 2011). Granule neurons are generated by neuroblasts located deep to the granular layer, in the sub-granular region of the dentate fascia (Masiulis et al., 2011). Thus, the reduction of granule cells after protein malnutrition probably depends both on a deficient neurogenesis and neuronal death. In fact, some studies have demonstrated a reduction in the number of these cells in conditions such as perinatal malnutrition (Cintra et al., 1990) and hypothyroidism (Madeira et al., 1991) denoting the susceptibility of granule cells to the metabolic alterations after the reduction of protein intake. Since nutritional rehabilitation does not lead to a recovery on the number of granule cells, we can advance that most of the neuronal stem cells have degenerated or lost the potential to generate new neurons. On the other hand, as the proliferation of most of the hilar cells occurs in the prenatal period (Bayer, 1980, Altman and Bayer, 1990), we can conclude that the reduction of their number depends mainly on neuronal degeneration.

The mechanisms by which protein deprivation induces neuronal death are multiple. Protein deprivation leads to a set of hormonal alterations, such as a rise in the levels of cortisol that can trigger neuronal death (Smith et al., 1975, Garcia-Belenguer et al., 1993). Moreover, an increase of the number of glutamate receptors after protein deprivation was reported (Blatt et al., 1994). Since this neurotransmitter is involved in

processes of excitotoxicity, this is a very likely mechanism by which low-protein treatment may induce neuronal degeneration in the hippocampal formation (Lopes da Silva et al., 1990, Farooqui and Horrocks, 1991). In addition, the uncontrolled elevation of the intracellular calcium concentration associated to this condition is toxic to neurons, leading to excessive cell activation, injury and, ultimately, to the neuronal death found in the protein-deprived group (Celio, 1990, De Jong et al., 1996).

The disturbance in calcium homeostasis in the protein-deprived group can be partially corroborated by the finding of a significant increase of almost four times on the density of PV-ir neurons in the granular layer and of three times in the hilar region of these animals when compared to controls. The majority of PV-ir interneurons in the hippocampal formation belong to the perisomatic inhibitory neuronal group. It is recruited by converging excitatory glutamatergic afferents, which are deeply interconnected and positioned for the fine-tuning and control of granule cells efferents (Sauer and Bartos, 2010). They were electrophysiologically characterized as fast-firing cells, found in high numbers in metabolic active brain regions and reported to buffer free intracellular calcium by transiently binding calcium ions with high affinity (Sauer and Bartos, 2010).

We may hypothesize that protein malnutrition induces metabolic events resulting in the loss of calcium buffering in the hippocampal formation. Since the intracellular calcium ions are critical to neuronal survival and signal transduction pathways, the loss of control becomes toxic to neurons and may lead to cell death (Celio, 1990, De Jong et al., 1996, Lawrence et al., 2010). The increase of labeling denotes a change in the control of PV synthesis and expression probably due to the plastic reorganization of local circuits that are altered by the damage inflicted to neurons by the prolonged protein malnutrition (Andrade et al., 1995b). In other words, there is

an attempt to protect the remaining granule cells and hilar neurons of the dentate gyrus and the observed up-regulation of PV-ir neurons may be interpreted as a survival strategy reflecting a protective mechanism to counteract the calcium overload (Schmidt-Kastner et al., 1992, Idrizbegovic et al., 2004, Gomes da Silva et al., 2010). Similarly, after a physiological situation such as aerobic physical exercise in young rats, an increase of the number of PV-ir neurons in the hippocampal formation was reported and it was equally advanced that the augment of the number of these neurons could protect against calcium overload rendering the other neurons more resistant to neurotoxicity (Gomes da Silva et al., 2010). Also, in rats submitted to gastric restrictive procedure in a model simulating bariatric surgery, there was an increase of the expression of PV-ir interneurons in CA1 and CA3 hippocampal fields (Sonoda et al., 2011). Similar increase of PV-ir neurons in rodents was described in other brain regions such as the cochlear nucleus neurons and several visual system structures (Schmidt-Kastner et al., 1992, Idrizbegovic et al., 2004, Gomes da Silva et al., 2010). It appears that neurons containing PV are resistant to ischemia (Nitsch et al., 1989, Leifer and Kowall, 1993), epilepsy (Sloviter et al., 1991) and N-methyl-D-aspartate receptor agonists (Waldvogel et al., 1991) although they were demonstrated to be vulnerable in the dentate gyrus and CA1 region of the hippocampal formation of Alzheimer's disease patients (Brady and Mufson, 1997). Moreover, in humans, the number of PV-ir neurons in the hippocampal formation, as well as the PV mRNA, was decreased in schizophrenia (Konradi et al., 2011).

The alteration of the density of PV-ir neurons probably alters the control of excitability and neuronal synchrony by modifying GABA-mediated responses in the hippocampal formation. In normal conditions, the ratio of PV-ir interneurons to granule cells within the dentate gyrus is about 1:200 (Celio, 1990). Therefore, the nutritional

insult results in an altered ratio favoring GABAergic interneurons. Functionally, there could be an increase of the inhibitory drive due to the changes in the relation of inhibitory to granule cells affecting the axonal processing of feed-forward information (Seidel et al., 2008) and justify partially the detrimental changes observed in learning and memory described in these protein-deprived animals (Lukoyanov and Andrade, 2000). This finding is consistent with the earlier observations of increased inhibition in the hippocampal formation of protein malnourished animals (Morgane et al., 2002) and supports the hypothesis that imbalances in the cell ratios is one factor underlying cognitive changes observed in this model of protein malnutrition (Andrade and Paula-Barbosa, 1996). However, further studies using other techniques are required to establish whether the population of PV-ir interneurons in this subset of protein malnourished rats maintains the same number of synapses, but on a reduced number of targets, compared to controls. After 2 months of normoproteic diet the recovered rats presented a return to the number observed in the age-matched controls. This structural recovery, probably related to the decrease of excitotoxicity and calcium overload, was previously demonstrated to be accompanied by a total functional recovery (Lukoyanov and Andrade, 2000) as well as by a normal number of mossy fiber-CA3 synapses (Andrade et al., 1995b).

The present results also show that prolonged food restriction does not lead to a significant reduction in the number of granule and hilar cells in the hippocampal formation of the adult rat. This is possibly due to the fact that they receive in total more proteins per gram body weight than rats exposed to protein deprivation. It is thus plausible that the decrease in protein intake underwent by food-deprived rats might be insufficient to trigger widespread neuronal degeneration as observed after low-protein feeding (Andrade et al., 1998, Lukoyanov and Andrade, 2000). We have also found that

the number of PV-ir neurons in food-deprived animals is similar to controls showing that calcium homeostasis in the hippocampal formation of food-restricted animals is apparently maintained. The present data support the view that food restriction has no major effects in the structure of the hippocampal formation of the adult rat as well as the absence of significant changes in learning and memory, although might present other behavioral alterations (Andrade et al., 2002, Lukoyanov et al., 2002). It would be of interest to investigate whether feeding rats with a more severely restricted diet than that used in the present experiment and, consequently, with a more profound reduction in protein intake, would produce degenerative changes in the hippocampal formation.

In conclusion, chronic food restriction has no effect neither on the number of principal cells nor on the number of PV-ir interneurons in the dentate fascia of the hippocampal formation of adult rat. In contrast, we found that long-term protein deprivation induces a significant loss of granule and hilar neurons in the dentate gyrus and that nutritional rehabilitation does not lead to normalization of those values. On the other hand, protein deprivation results in an increase in the density of PV-ir cells both in granular layer and hilus when compared to controls. Hence, our evidence suggests that the PV-ir interneurons are not only spared by the deprivational nutritional insult but there is also an increase of their relative number. The consumption of a normoproteic diet leads to restoration of the number observed in controls.

Further studies are necessary to compare these two types of nutritional deprivation, in order to understand the molecular mechanisms leading to the changes in calcium homeostasis in the hippocampal formation related to each one of these deprivational nutritional conditions.

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The author confirms that he has read the Journal's position on issues involved in ethical publication and affirms that this report is consistent with those guidelines. The author has no conflict of interest to disclose.

REFERENCES

Abrahamsen GC, Kandawire MJ, Carr KD (1997) Aminoglutethimide, a corticosteroid synthesis inhibitor, facilitates brain stimulation reward in food-restricted rats: An investigation of underlying mechanisms. Psychopharmacology 133:405-412.

Altemus M, Glowa JR, Galliven E, Leong YM, Murphy DL (1996) Effects of serotonergic agents on food-restriction - Induced hyperactivity. Pharmacology Biochemistry and Behavior 53:123-131.

Altman J, Bayer SA (1990) Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. Journal of Comparative Neurology 301:365-381.

Amaral DG WM (1995) The hippocampal formation. In: Paxinos G, editor. The rat nervous system 2nd ed.:p 443-493.

Andrade JP, Castanheira-Vale AJ, Paz-Dias PG, Madeira MD, Paula-Barbosa MM (1996) The dendritic trees of neurons from the hippocampal formation of protein-deprived adult rats. A quantitative Golgi study. Experimental Brain Research 109:419-433.

Andrade JP, Lukoyanov NV, Paula-Barbosa MM (2002) Chronic food restriction is associated with subtle dendritic alterations in granule cells of the rat hippocampal formation. Hippocampus 12:149-164.

Andrade JP, Madeira MD, Paula-Barbosa MM (1995a) Effects of long-term malnutrition and rehabilitation on the hippocampal formation of the adult rat. A morphometric study. Journal of anatomy 187 (Pt 2):379-393.

Andrade JP, Madeira MD, Paula-Barbosa MM (1995b) Evidence of reorganisation in the hippocampal mossy fiber synapses of adult rats rehabilitated after prolonged undernutrition. Experimental Brain Research 104:249-261.

Andrade JP, Madeira MD, Paula-Barbosa MM (1998) Differential vulnerability of the subiculum and entorhinal cortex of the adult rat to prolonged protein deprivation. Hippocampus 8:33-47.

Andrade JP, Mesquita R, Assuncao M, Pereira PA (2006) Effects of food restriction on synthesis and expression of brain-derived neurotrophic factor and tyrosine kinase B in dentate gyrus granule cells of adult rats. Neuroscience letters 399:135-140.

Andrade JP, Paula-Barbosa MM (1996) Protein malnutrition alters the cholinergic and GABAergic systems of the hippocampal formation of the adult rat: An immunocytochemical study. Neuroscience letters 211:211-215.

Baude A, Bleasdale C, Dalezios Y, Somogyi P, Klausberger T (2007) Immunoreactivity for the GABAA receptor α1 subunit, somatostatin and connexin36 distinguishes axoaxonic, basket, and bistratified interneurons of the rat hippocampus. Cerebral Cortex 17:2094-2107.

Bayer SA (1980) Development of the hippocampal region in the rat I. Neurogenesis examined with 3H-thymidine autoradiography. Journal of Comparative Neurology 190:87-114.

Blatt GJ, Chen JC, Rosene DL, Volicer L, Galler JR (1994) Prenatal protein malnutrition effects on the serotonergic system in the hippocampal formation: An immunocytochemical, ligand binding, and neurochemical study. Brain Research Bulletin 34:507-518.

Bough KJ, Valiyil R, Han FT, Eagles DA (1999) Seizure resistance is dependent upon age and calorie restriction in rats fed a ketogenic diet. Epilepsy Research 35:21-28.

Brady DR, Mufson EJ (1997) Parvalbumin-immunoreactive neurons in the hippocampal formation of Alzheimer's diseased brain. Neuroscience 80:1113-1125.

Bronson RT, Lipman RD (1991) Reduction in rate of occurrence of age related lesions in dietary restricted laboratory mice. Growth, Development and Aging 55:169-184.

Bruce-Keller AJ, Umberger G, McFall R, Mattson MP (1999) Food restriction reduces brain damage and improves behavioral outcome following excitotoxic and metabolic insults. Annals of Neurology 45:8-15.

Campbell BA, Richardson R (1988) Effects of chronic dietary restriction on sensory-motor function and susceptibility to stressor stimuli in the laboratory rat. Experimental Gerontology 23:417-427.

Carr KD (1996) Feeding, drug abuse, and the sensitization of reward by metabolic need. Neurochemical Research 21:1455-1467.

Celio MR (1990) Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience 35:375-475.

Cintra L, Diaz-Cintra S, Galvan A, Kemper T, Morgane PJ (1990) Effects of protein undernutrition on the dentate gyrus in rats of three age groups. Brain Research 532:271-277.

De Jong GI, Naber PA, Van Der Zee EA, Thompson LT, Disterhoft JF, Luiten PGM (1996) Age-related loss of calcium binding proteins in rabbit hippocampus. Neurobiology of Aging 17:459-465.

De Vaca SC, Carr KD (1998) Food restriction enhances the central rewarding effect of abused drugs. Journal of Neuroscience 18:7502-7510.

Dirks AJ, Leeuwenburgh C (2006) Caloric restriction in humans: Potential pitfalls and health concerns. Mechanisms of Ageing and Development 127:1-7.

Duan W, Lee J, Guo Z, Mattson MP (2001) Dietary restriction stimulates BDNF production in the brain and thereby protects neurons against excitotoxic injury. Journal of Molecular Neuroscience 16:1-12.

Duan W, Mattson MP (1999) Dietary restriction and 2-deoxyglucose administration improve behavioral outcome and reduce degeneration of dopaminergic neurons in models of Parkinson's disease. Journal of neuroscience research 57:195-206.

FAO (2012) Hunger. vol. 2012.

Farooqui AA, Horrocks LA (1991) Excitatory amino acid receptors, neural membrane phospholipid metabolism and neurological disorders. Brain Research Reviews 16:171-191.

Faundez V, Cordero ME, Rosso P, Alvarez J (1990) Calibers and microtubules of nerve fibers: Differential effect of undernutrition in developing and adult rats. Brain Research 509:198-204.

Freund TF (2003) Interneuron Diversity series: Rhythm and mood in perisomatic inhibition. Trends in Neurosciences 26:489-495.

Garcia-Belenguer S, Oliver C, Mormede P (1993) Facilitation and feedback in the hypothalamo-pituitary-adrenal axis during food restriction in rats. Journal of Neuroendocrinology 5:663-668.

Gill CJ, Rissman EF (1997) Female sexual behavior is inhibited by short- and long-term food restriction. Physiology and Behavior 61:387-394.

Gomes da Silva S, Doná F, da Silva Fernandes MJ, Scorza FA, Cavalheiro EA, Arida RM (2010) Physical exercise during the adolescent period of life increases hippocampal parvalbumin expression. Brain and Development 32:137-142.

Gundersen HJ, Jensen EB (1987) The efficiency of systematic sampling in stereology and its prediction. Journal of Microscopy 147:Pt 3/.

Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, West MJ (1988) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96:379-394.

Gundersen HJG, Jensen EBV, Kiêu K, Nielsen J (1999) The efficiency of systematic sampling in stereology - Reconsidered. Journal of Microscopy 193:199-211.

Hao S, Avraham Y, Mechoulam R, Berry EM (2000) Low dose anandamide affects food intake, cognitive function, neurotransmitter and corticosterone levels in diet-restricted mice. European Journal of Pharmacology 392:147-156.

Harrison e D, Archer JR (1987) Genetic differences in effects of food restriction on aging in mice. Journal of Nutrition 117:376-382.

Hart RW, Leakey JEA, Chou M, Duffy PH, Allaben WT, Feuers RJ (1992) Modulation of chemical toxicity by modification of caloric intake. Advances in Experimental Medicine and Biology 322:73-81.

Heiderstadt KM, McLaughlin RM, Wright DC, Walker SE, Gomez-Sanchez CE (2000) The effect of chronic food and water restriction on open-field behaviour and serum corticosterone levels in rats. Laboratory Animals 34:20-28.

Idrizbegovic E, Bogdanovic N, Willott JF, Canlon B (2004) Age-related increases in calcium-binding protein immunoreactivity in the cochlear nucleus of hearing impaired C57BL/6J mice. Neurobiology of Aging 25:1085-1093.

Konradi C, Yang CK, Zimmerman EI, Lohmann KM, Gresch P, Pantazopoulos H, Berretta S, Heckers S (2011) Hippocampal interneurons are abnormal in schizophrenia. Schizophrenia Research 131:165-173.

Lawrence YA, Kemper TL, Bauman ML, Blatt GJ (2010) Parvalbumin-, calbindin-, and calretinin-immunoreactive hippocampal interneuron density in autism. Acta Neurologica Scandinavica 121:99-108.

Lee J, Duan W, Mattson MP (2002a) Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. Journal of Neurochemistry 82:1367-1375.

Lee J, Seroogy KB, Mattson MP (2002b) Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice. Journal of Neurochemistry 80:539-547.

Leifer D, Kowall NW (1993) Immunohistochemical patterns of selective cellular vulnerability in human cerebral ischemia. Journal of the neurological sciences 119:217-228.

Leonhardt S, Shahab M, Luft H, Wuttke W, Jarry H (1999) Reduction of luteinzing hormone secretion induced by long-term feed restriction in male rats is associated with increased expression of GABA-synthesizing enzymes without alterations of GnRH gene expression. Journal of Neuroendocrinology 11:613-619.

Lister JP, Blatt GJ, Kemper TL, Tonkiss J, Debassio WA, Galler JR, Rosene DL (2011) Prenatal protein malnutrition alters the proportion but not numbers of parvalbuminimmunoreactive interneurons in the hippocampus of the adult sprague-Dawley rat. Nutritional Neuroscience 14:165-178.

Lopes da Silva FH, Witter MP, Boeijinga PH, Lohman AHM (1990) Anatomic organization and physiology of the limbic cortex. Physiological Reviews 70:453-511.

Lukoyanov NV, Andrade JP (2000) Behavioral effects of protein deprivation and rehabilitation in adult rats: Relevance to morphological alterations in the hippocampal formation. Behavioural Brain Research 112:85-97.

Lukoyanov NV, Pereira PA, Mesquita RM, Andrade JP (2002) Restricted feeding facilitates time-place learning in adult rats. Behavioural Brain Research 134:283-290.

Madeira MD, Cadete-Leite A, Andrade JP, Paula-Barbosa MM (1991) Effects of hypothyroidism upon the granular layer of the dentate gyrus in male and female adult rats: a morphometric study. The Journal of comparative neurology 314:171-186.

Masiulis I, Yun S, Eisch AJ (2011) The Interesting Interplay Between Interneurons and Adult Hippocampal Neurogenesis. Molecular Neurobiology 1-16.

Masoro EJ (1992) Retardation of aging processes by food restriction: An experimental tool. American Journal of Clinical Nutrition 55:1250S-1252S.

Mesquita RM, Pereira PA, Andrade JP (2002) Low levels of brain-derived neurotrophic factor and tyrosine kinase receptor B are related to loss of dentate granule cells after prolonged low-protein feeding in the rat. Neuroscience letters 330:155-158.

Michael Anson R, Guo Z, de Cabo R, Iyun T, Rios M, Hagepanos A, Ingram DK, Lane MA, Mattson MP (2003) Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. Proceedings of the National Academy of Sciences of the United States of America 100:6216-6220.

Morgane PJ, Miller M, Kemper T (1978) The effects of protein malnutrition on the developing central nervous system in the rat. Neuroscience and Biobehavioral Reviews 2:137-230.

Morgane PJ, Mokler DJ, Galler JR (2002) Effects of prenatal protein malnutrition on the hippocampal formation. Neuroscience and Biobehavioral Reviews 26:471-483.

Morse AD, Russell JC, Hunt TWM, Wood GO, Epling WF, Pierce WD (1995) Diurnal variation of intensive running in food-deprived rats. Canadian Journal of Physiology and Pharmacology 73:1519-1523.

Nitsch C, Scotti A, Sommacal A, Kalt G (1989) GABAergic hippocampal neurons resistant to ischemia-induced neuronal death contain the Ca2(+)-binding protein parvalbumin. Neuroscience letters 105:263-268.

Paula-Barbosa MM, Andrade JP, Castedo JL, Azevedo FP, Camoes I, Volk B, Tavares MA (1989) Cell loss in the cerebellum and hippocampal formation of adult rats after long-term low-protein diet. Exp Neurol 103:186-193.

Roberts HJ, Wearden JH, Smart JL (1983) Undernutrition of weanling and adult rats: Effects on operant responding. Behavioural Brain Research 10:287-296.

Sauer JF, Bartos M (2010) Recruitment of early postnatal parvalbumin-positive hippocampal interneurons by GABAergic excitation. Journal of Neuroscience 30:110-115.

Schmidt-Kastner R, Meller D, Eysel UT (1992) Immunohistochemical changes of neuronal calcium-binding proteins parvalbumin and calbindin-D-28k following unilateral deafferentation in the rat visual system. Experimental Neurology 117:230-246.

Seidel K, Helmeke C, Poeggel G, Braun K (2008) Repeated neonatal separation stress alters the composition of neurochemically characterized interneuron

subpopulations in the rodent dentate gyrus and basolateral amygdala. Developmental Neurobiology 68:1137-1152.

Silva SM, Madeira MD, Ruela C, Paula-Barbosa MM (2002) Prolonged alcohol intake leads to irreversible loss of vasopressin and oxytocin neurons in the paraventricular nucleus of the hypothalamus. Brain Research 925:76-88.

Sisó S, Tort S, Aparici C, Pérez L, Vidal E, Pumarola M (2003) Abnormal neuronal expression of the calcium-binding proteins, parvalbumin and calbindin D-28k, in aged dogs. Journal of Comparative Pathology 128:9-14.

Sloviter RS, Sollas AL, Barbaro NM, Laxer KD (1991) Calcium-binding protein (calbindin-D28K) and parvalbumin immunocytochemistry in the normal and epileptic human hippocampus. The Journal of comparative neurology 308:381-396.

Smith SR, Bledsoe T, Chhetri MK (1975) Cortisol metabolism and the pituitary adrenal axis in adults with protein calorie malnutrition. Journal of Clinical Endocrinology and Metabolism 40:43-52.

Sonoda EY, Gomes da Silva S, Arida RM, Giglio PN, Margarido NF, Martinez CA, Pansani AP, Maciel RS, Cavalheiro EA, Scorza FA (2011) Hippocampal plasticity in rats submitted to a gastric restrictive procedure. Nutritional Neuroscience 14:181-185.

Szilágyi T, Orbán-Kis K, Horváth E, Metz J, Pap Z, Pávai Z (2011) Morphological identification of neuron types in the rat hippocampus. Romanian Journal of Morphology and Embryology 52:15-20.

Vreugdenhil M, Jefferys JG, Celio MR, Schwaller B (2003) Parvalbumin-deficiency facilitates repetitive IPSCs and gamma oscillations in the hippocampus. Journal of neurophysiology 89:1414-1422.

Waldvogel HJ, Faull RLM, Williams MN, Dragunow M (1991) Differential sensitivity of calbindin and parvalbumin immunoreactive cells in the striatum to excitotoxins. Brain Research 546:329-335.

Walford RL, Harris SB, Weindruch R (1987) Dietary restriction and aging: Historical phases, mechanisms and current directions. Journal of Nutrition 117:1650-1654.

Waterlow J, Kusuma C, Somnasang P (1992) The development of stunting in children in north-east Thailand. Pediatric Reviews and Communications 6:186.

Weindruch R (1996) Caloric restriction and aging. Scientific American 274:46-52.

West MJ, Slomianka L, Gundersen HJG (1991) Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anatomical Record 231:482-497.

WFP (2012) Hunger: Who are the hungry? , vol. 2012.

WHO (2012) 10 facts on nutrition. vol. 2012.

FIGURE LEGENDS

Figure 1 – Graphic representation of estimates of density of PV-ir interneurons of dentate gyrus molecular layer (A), granular layer (B) and hilus (C). A: No significant effect of food regimen was found. B: The density of PV-ir interneurons is significantly increased in low-protein diet group relatively to controls ($P<0.05$).
C: The density of PV-ir interneurons of low-protein diet animals is significantly increased comparatively to all the other groups ($P<0.05$). Control; FD – food-deprived; LPD – low-protein diet; RecLPD – recovery low-protein diet. Columns represent means and vertical bars correspond to 1 S.D. * $P<0.05$.

Figure 2 – A: Photomicrograph of a methacrylate-stained section of the hippocampal formation of a protein-deprived rat. Sections like this were used to estimate total number of neurons in the molecular layer (ML), granular layer (GL) and hilus (H) of the dentate gyrus of the hippocampal formation. Scale bar = 400 μm.
B: Photomicrograph of a parvalbumin-stained section taken from the midseptotemporal region of the hippocampal formation of a protein-deprived rat. Sections like this were used to estimate the density of PV-ir interneurons in the molecular layer (ML), granular layer (GL) and hilus (H) of the dentate gyrus of the hippocampal formation. Scale bar = 400 μm.

Figure 3 – Representative photomicrograph of level-matched PV-immunostained brain sections of each group of this experiment: control (A), food-deprived (B), low-protein diet (C), and recovery low-protein diet animals (D). Note that PV-ir interneurons are more abundant in low-protein diet group than in the other groups. Scale bar = 200 μm.

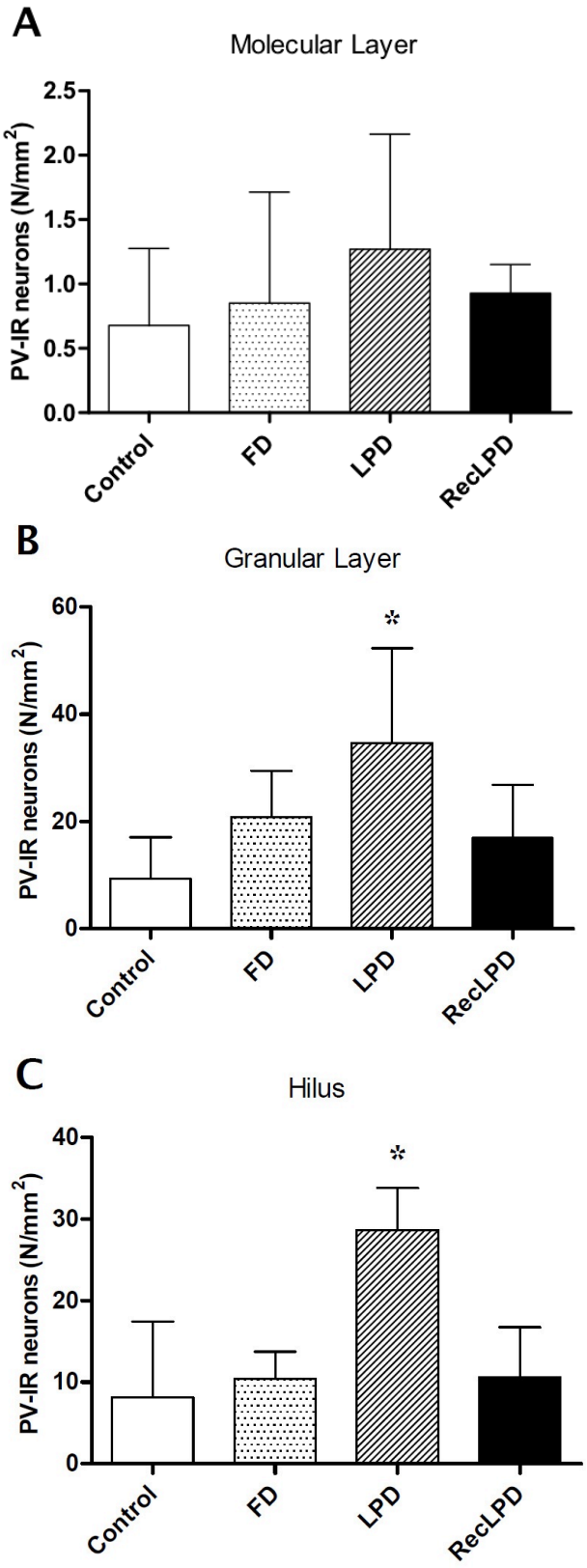
A
Molecular Layer
PV-IR neurons (N/mm2)
2.5
2.0
1.5
1.0
0.5
0.0
Control
FD
LPD
RecLPD
B
Granular Layer
PV-IR neurons (N/mm2)
60
40
20
0
*
Control
FD
LPD
RecLPD
C
Hilus
PV-IR neurons (N/mm2)
40
30
20
10
0
*
Control
FD
LPD
RecLPD

Figure 1

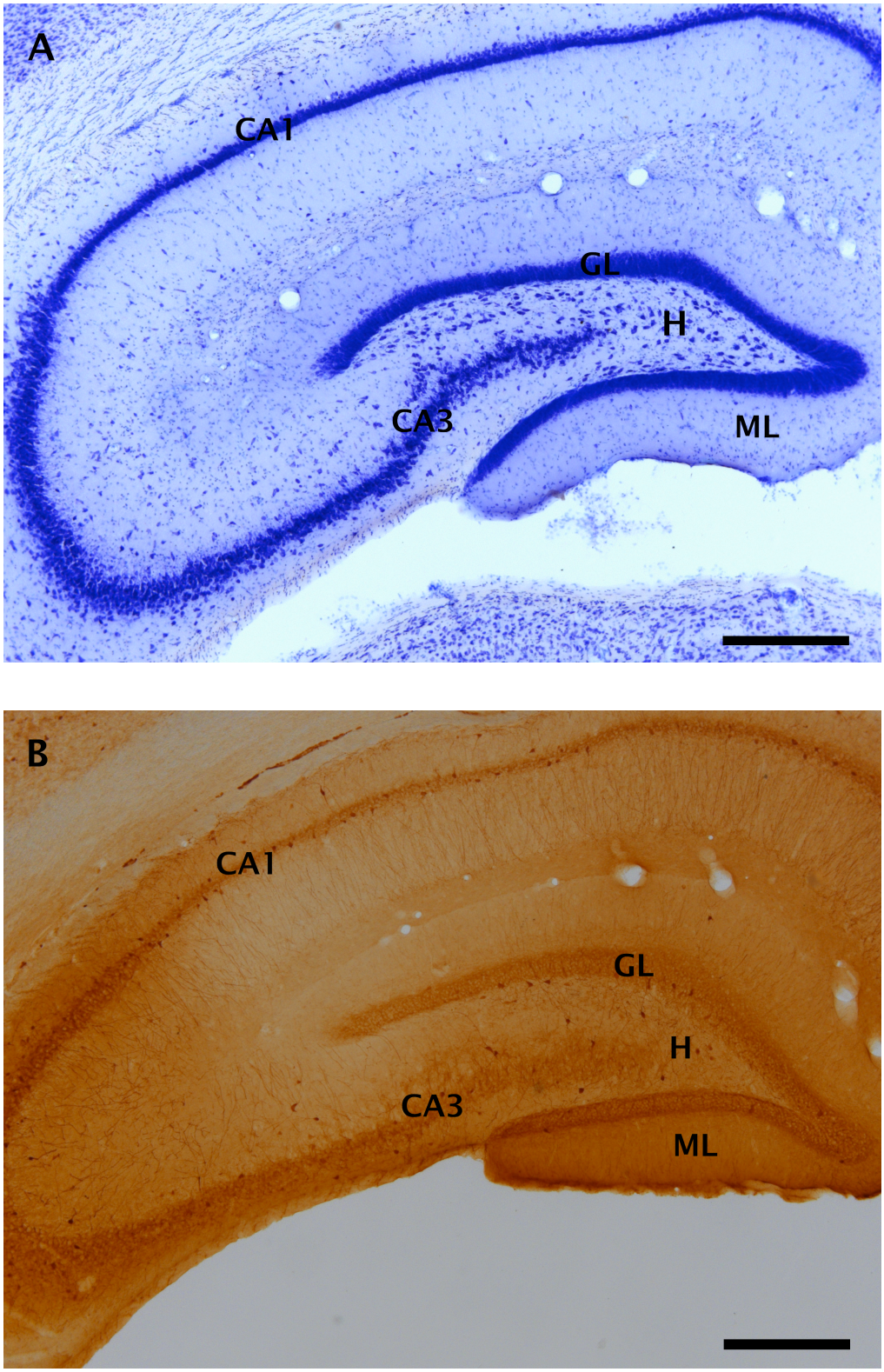

Figure 2

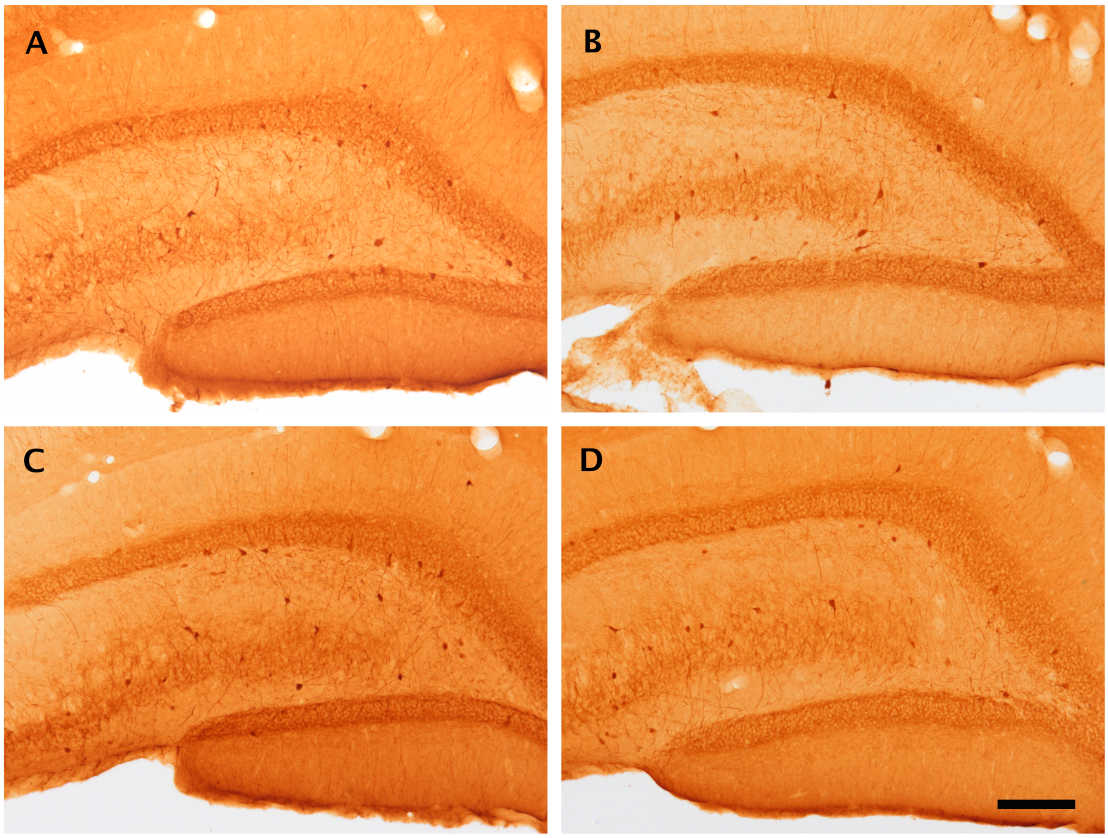

Figure 3

Table 1 – Mean body weights (g) of control, food-deprived, low-protein diet and recovery low-protein diet group after 6 and 8 months of treatment †

	6 months	8 months
Control	770 ± 24.1	831 ± 25.2
Food-deprived	531 ± 10.8	550 ± 17.7
Low-protein diet	741 ± 12.3	791 ± 14.1
Recovery LPD	-	852 ± 31.1

† Values are presented as mean ± SD.

Table 2 – Total number of granule and hilar cells in the dentate gyrus of control, food-deprived, low-protein diet and nutritionally rehabilitated rats †

Groups	Granule cells	Hilar cells
Control	1114824 ± 44487	53112 ± 2538
Food-deprived	1142042 ± 37162	52528 ± 1160
Low-protein diet	1071822 ± 42596 *	49739 ± 1110 *
Recovery LPD	1065745 ± 44336 *	50119 ± 1558 *

† Values are presented as mean ± SD.

* $P<0.05$ vs. control and food-deprived groups.

GUIDE FOR AUTHORS

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