Neurobiology of Learning and Memory 119 (2015) 1–9

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

β_2-Adrenoceptor stimulation restores frontal cortex plasticity and improves visuospatial performance in hidden-prenatally-malnourished young-adult rats

Patricio Sáez-Briones [a], Rubén Soto-Moyano [b], Héctor Burgos [c,d], Amparo Castillo [e,f], Luis Valladares [b], Carlos Morgan [b,*], Hernán Pérez [b], Rafael Barra [g], Luis Constandil [g], Claudio Laurido [g], Alejandro Hernández [g,*]

[a] Faculty of Medicine, University of Santiago de Chile, Santiago, Chile
[b] Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile
[c] School of Psychology, Central University of Chile, Santiago, Chile
[d] School of Psychology, University of Santiago de Chile, Santiago, Chile
[e] School of Psychology, Autonomous University of Chile, Santiago, Chile
[f] Doctoral Program in Psychology, Faculty of Social Sciences, University of Chile, Santiago, Chile
[g] Faculty of Chemistry and Biology, University of Santiago de Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 26 May 2014
Revised 11 October 2014
Accepted 4 November 2014
Available online 20 November 2014

Keywords:
β_2-Adrenoceptor
Clenbuterol
Long-term potentiation
Prefrontal cortex
Prenatal protein malnutrition
Visuospatial performance

ABSTRACT

Moderate reduction in dietary protein composition of pregnant rats from 25% to 8% casein, calorically compensated by carbohydrates, has been described as a "hidden malnutrition" because it does not alter body and brain weights of pups at birth. However, this dietary treatment leads to altered central noradrenergic systems, impaired cortical long-term potentiation (LTP) and worsened visuo-spatial memory performance. Given the increasing interest on the role played by β_2-adrenoceptors (β_2-ARs) on brain plasticity, the present study aimed to address the following in hidden-malnourished and eutrophic control rats: (i) the expression levels of β_2-ARs in the frontal cortex determined by immunohistochemistry, and (ii) the effect of the β_2 selective agonist clenbuterol on both LTP elicited *in vivo* in the prefrontal cortex and visuospatial performance measured in an eight-arm radial maze. Our results showed that, prenatally malnourished rats exhibited a significant reduction of neocortical β_2-AR expression in adulthood. Concomitantly, they were unable to elicit and maintain prefrontal cortex LTP and exhibited lower visuospatial learning performance. Administration of clenbuterol (0.019, 0.038 and 0.075 mg/kg i.p.) enhanced LTP in malnourished and control animals and restored visuospatial learning performance in malnourished but not in normal rats, in a dose-dependent manner. The results suggest that decreased density of neocortical β_2-ARs during postnatal life, subsequent to hidden prenatal malnutrition might affect some synaptic networks required to elicit neocortical LTP and form visuospatial memory, since those neuroplastic deficits were counteracted by β_2-AR stimulation.

1. Introduction

Moderate reduction of the protein content in the diet of pregnant rats, calorically compensated by carbohydrates, results in apparently normal development in utero of fetuses as assessed by normal maternal weight gain during pregnancy and normal body and brain weights of pups at birth. However, this insidious form of protein maternal malnutrition in rat, the so-called "hidden" prenatal malnutrition (Resnick, Morgane, Hasson, & Miller, 1982), leads to a variety of morpho-functional abnormalities in the brain of the adult progeny, including decreased cortical release of noradrenaline (Soto-Moyano et al., 1998),

Abbreviations: β_2-AR, β_2-adrenoceptor; CC, corpus callosum; LTP, long-term potentiation.

* Corresponding authors at: Human Nutrition Unit, Institute of Nutrition and Food Technology (INTA), University of Chile, Av. El Líbano 5524, Macul, Santiago 138-11, RM 7830490, Chile (C. Morgan). Laboratory of Neurobiology, Department of Biology, Faculty of Chemistry and Biology, University of Santiago of Chile, Ave. Libertador B. O'Higgins 3363, Casilla 40 Correo 33, Santiago, Chile (A. Hernández).

E-mail addresses: cmorgan@inta.uchile.cl (C. Morgan), alejandro.hernandez@usach.cl (A. Hernández).

http://dx.doi.org/10.1016/j.nlm.2014.11.003
1074-7427/

increased neocortical expression of the α_{2C}-adrenoceptor subtype (Sierralta et al., 2006; Soto-Moyano et al., 2005), decreased β_1-adrenoceptor density and reduction of both cyclic-AMP dependent protein kinase α and calcium/calmodulin-dependent protein kinase α expression in the prefrontal cortex (Flores et al., 2011), as well as weakened electrophysiological indices, such as a decreased ability of callosal–cortical synapses to perform temporal summation (Soto-Moyano et al., 1998) and maintenance of long-term synaptic potentiation (Barra et al., 2012; Flores et al. 2011; Hernández et al., 2008; Soto-Moyano et al., 2005).

It has been hypothesized that many of the deficits in functional plasticity observed in prenatally malnourished animals are due, at least in part, to the above mentioned modifications in brain noradrenergic systems (Barra et al., 2012; Flores et al., 2011; Soto-Moyano et al., 2005). In fact, central noradrenaline critically influences long-term potentiation (LTP) in cerebral cortex (Kobayashi, 2007; Marzo, Bai, & Otani, 2009; Nowicky, Christofi, & Bindman, 1992; Radisavljevic, Cepeda, Peacock, Buchwald, & Levine, 1994) and hippocampus (Bramham, Bacher-Svendsen, & Sarvey, 1997; Hopkins & Johnston, 1988; Marzo et al., 2009; O'Dell et al., 2010; Schimanski, Ali, Baker, & Nguyen, 2007), being involved in memory formation (Crowe, Ng, & Gibbs, 1990; Gibbs, 1991; Sternberg, Korol, Novack, & McGaugh, 1986) through balanced activation of specific receptors. For instance, animal studies have revealed that α_1-adrenoceptor activation is associated to decreased hippocampal LTP (Tachibana et al., 2004) and impaired memory performance (Arnsten & Jentsch, 1997; Birnbaum, Gobeske, Auerbach, Taylor, & Arnsten, 1999; Mao, Arnsten, & Li, 1999). In turn, activation of α_{2A}-adrenoceptors has been related to improve working memory performance (Arnsten & Jin, 2012; Franowicz et al., 2002; Li, Mao, Wang, & Mei, 1999; Wang et al., 2007), while activation of the α_{2C}-adrenoceptor subtype seems to be associated to decreased memory formation (Björklund et al., 1998; Björklund et al., 1999; Björklund et al., 2000). In addition, activation of β-adrenoceptors leads to enhanced LTP in the hippocampus (Bramham et al., 1997; Hopkins & Johnston, 1988; Schimanski et al., 2007) and to memory facilitation (Crowe et al., 1990; Gibbs, 1991; Gibbs & Summers, 2000).

In recent years, there has been a renewed interest on the role that β_2-adrenoceptors (β_2-ARs) play in neuroplasticity. It has been reported that the enhancement of pyramidal cell action potential frequency induced in the hippocampus by the non-specific β-adrenoceptor agonist isoproterenol, can be blocked by the administration of selective β_2-AR antagonists (Hillman, Doze, & Porter, 2005). This finding suggests that β_2-ARs are mainly responsible for the strengthening effect of the stimulation of nonspecific β-adrenoceptors on LTP, at least in the hippocampal formation. β_2-ARs are G protein-coupled cell membrane receptors that activate PKA via adenylyl cyclase, thereby producing important effects on brain plasticity (for reviews see Kandel, 2012; Nguyen & Woo, 2003). In this regard, it has been reported that activation of β_2-ARs supports prolonged hippocampal θ-tetanus-LTP (Qian et al. 2012), which is a β_2-AR-PKA-dependent long-lasting form of hippocampal synaptic plasticity (Havekes et al., 2012), which is essential for β_2-AR-mediated novelty-induced enhancement of LTP in the hippocampus (Li et al., 2013). More recent data showed that activation of β_2-ARs enhances prefrontal cortex LTP and behavioral memory in the rat (Zhou et al., 2013), thus extending the role of β_2-adrenoceptor signaling to neuroplastic capabilities of the cortical mantle. Consistent with the foregoing electrophysiological data, activation of β_2-ARs at the rat prefrontal cortex improves performance on delayed alternation tasks in T-maze (Ramos, Colgan, Nou, & Arnsten, 2008) and enhances trace fear memory (Zhou et al., 2013).

Considering the current evidence supporting the role that β_2-ARs play on brain plasticity, and the fact that the impaired neuroplastic indices found in neocortex of prenatally malnourished adult offspring are linked to altered brain noradrenergic function, the present study aimed to address two relevant questions: first, whether hidden prenatal malnutrition in the rat does modify β_2-AR expression in the frontal cortex of the young adult offspring; and second, whether pharmacological activation of β_2-ARs in these animals could improve prefrontal cortex LTP and visuospatial learning performance.

2. Materials and methods

2.1. Animals and diets

The experimental protocols and animal management were in accordance with the NIH Guide for the Care and Use of Laboratory Animals (National Research Council., 1985) and were approved by the Committee for the Ethical Use of Experimental Animals at INTA, University of Chile, and the Institutional Ethical Committee of the University of Santiago de Chile. The experiments were carried out on 136 male Sprague–Dawley rats born from mothers submitted to rearing procedures, already described (Barra et al., 2012; Flores et al., 2011; Resnick et al., 1982; Soto-Moyano et al., 2005). Briefly, virgin female rats fed isocaloric purified diets containing either normal (25% casein, providing 22.5% protein) or low (8% casein, providing 7.2% protein) protein amounts. The other components of the purified diets were: (i) Normal diet: carbohydrate, 50.2%; fat, 15.0%; vitamin mix, 1.0%; salt mix, 4.7%; water, 1.7%; cellulose, 4.2%; L-methionine, 0.4%. (ii) Low protein diet: carbohydrate, 66.5%; fat, 15.0%; vitamin mix, 1.0%; salt mix, 4.7%; water, 1.0%; cellulose, 4.2%; L-methionine, 0.4%. Both diets provide about 4.3 kcal/g. The dietary paradigm was started one day prior to mating and continued throughout pregnancy. The body weight gain of the pregnant mothers was controlled daily. At birth, all pups were weighed and litters were culled to eight male pups. Afterwards, pups born from malnourished mothers were fostered by well-nourished dams. Pups born from eutrophic mothers were also fostered to well-nourished dams to equalize other factors that may depend on the rearing conditions in both groups (i.e. stress due to cross-fostering). After weaning, at 22 days of age, all pups were fed a standard laboratory diet, providing 22.5% protein.

2.2. β_2-Adrenoceptor immunohistochemistry

Normal and malnourished rats of 60 days of age ($N = 4$ each group, taken from two different litters) were deeply anesthetized with urethane (1.5 g/kg i.p.) and perfused transcardially with freshly made fixative (4% paraformaldehyde, 0.2% picric acid in 100 mM phosphate buffer saline (PBS), pH 6.9). The brains were removed from the skulls and postfixed for 14 h by immersion in the same fixative. After rinsing in 100 mM PBS, the brains were dehydrated with graded alcohol solutions and xylenes, and embedded in Histowax. Sagital sections of 5 μm thickness were cut with a Reichert–Jung rotary microtome, collected on slides coated with chromalaun–gelatine and stored until immunostaining.

The immunostaining of β_2-ARs was done in humid chambers following antigen retrieval by treatment with a microwave oven (model H2500, Energy Beam Sciences Inc., MA, USA) and using the Tyramide Signal Amplification system (TSA) kit NEL 700 (Perkin-Elmer Life Sciences Inc., MA, USA) to detect antibody attachment. For antigen retrieval, dewaxed sections were microwave-irradiated exactly as described before (Sierralta & Thole, 1996). To quench any remaining free aldehyde group, sections were incubated for 30 min in 0.1 M glycine dissolved in buffer

TNT (0.1% Triton X100/150 mM NaCl/50 mM Tris/HCl, pH 7.4). After blocking for 30 min with freshly prepared blocking solution from the TSA-kit, sections were incubated (4 h RT and overnight at 4 °C) with a rabbit antibody against the β_2-AR (Catalog number sc-569, Santa Cruz Biotechnology, CA, USA). The antibody was diluted (1:100) with TNT containing 1% BSA and 1% normal rabbit serum. After washing with TNT containing BSA and normal rabbit serum, sections were incubated for 1 h at RT with a 1:1000 dilution of a secondary antibody conjugated with peroxidase (rabbit anti-guinea pig, Zymed Laboratories, CA, USA). After washing with TNT, sections were incubated at RT with biotinylated-tyramine (TSAkit, NEN, USA) for exactly 8 min, washed with TNT and incubated for 30 min with streptavidine–peroxidase from the TSA-kit. After washings, the peroxidase reaction was developed using buffered 3,3′-diaminobenzidine tablets dissolved in 0.03% H_2O_2 (Rockland, PA, USA).

Throughout the immunostaining procedure, sections were washed three times for 15 min between each step. One set of sections was used for control staining, substituting the primary antibodies with normal guinea pig serum, or with the β_2-AR antibody preincubated with a 10-fold excess of the antigen, provided by Santa Crux Biotechnology (control peptide). Counterstain was done with Meyer's hematoxylin and Eukitt was used for mounting. Images were acquired with an Olympus CX31 microscope fitted with a Cool Snap-Pro Color Digital camera controlled by Image Pro (Media Cybernetics, MD, USA). The same software was used to calculate the expression level scores (ELS) of the receptor using measurement tools provided in the software, including automatic detection of similarly colored objects to measure and measuring of intensity parameters of all tracked objects.

For each animal, the selected slides corresponded to sagittal sections located at 1, 3, 4 and 5 mm from the mid sagittal plane. 200 × 200 μm regions situated at 1000, 1400, 1800, 2200 and 2600 μm rostrally from bregma, 300–500 μm depth from the cortical surface, were used for quantitative analysis, totalizing 80 studied regions per animal. In these regions, we assessed both the percent of stained tissue, as number of cells expressing β_2-ARs in percentage, and the intensity of staining, as the number of β_2-ARs/cell. Combining these two parameters allowed to obtain the expression levels scores as the number of β_2-ARs/area.

2.3. Cortical LTP determinations

Experiments were carried out in 32 normal and 32 malnourished rats of 55–60 days of age. Rats were weighed, anesthetized with 1.5 g/kg i.p. urethane and placed in a stereotaxic apparatus. A single dose of 1.5 mg/kg of d-tubocurarine was injected i.m. and adequate ventilation was maintained by means of a respirator pump. LTP was induced in the rat frontal cortex according to the method reported elsewhere (Flores et al., 2011). For this purpose, field cortical responses evoked by electrical stimulation of the corpus callosum (CC) were recorded by means of an electrode placed on the cortical surface (active electrode) in reference to another electrode located on the excised muscles over the frontal bone (reference electrode). Since the responses were led into a differential amplifier, d-tubocurarine was used to avoid electrical responses from muscles that may introduce electrical noise to the reference electrode. d-Tubocurarine does not penetrate the normal intact brain-blood barrier and is therefore devoid of effects in the central nervous system when administered systemically. Reinforcement of anesthesia during the experiments was not necessary since surgical procedures and recordings lasted no longer than 3 h and, in our experience with non paralyzed rats, 1.5 g/kg i.p. urethane induces profound anesthesia lasting more than 5 h. Animals never regained consciousness and no changes in heart rate in response to stimulation were detected throughout the experiments.

After partial exposure of the frontal lobe of both cerebral hemispheres (two symmetrical holes of 2-mm diameter each bilaterally drilled in the frontal bone), electrical stimulation of the CC was carried out by means of a bipolar electrode that penetrated through the right frontal cortex at the de Groot coordinates $A = 6.8$ mm, $L = 2.0$ mm, according to the atlas of Pellegrino and Cushman (1967). The stimulating electrode consisted of two side-by-side glued 100-μm-diameter insulated tungsten wires with a 0.5-mm tip separation; one tip of the electrode was located over the CC and the other tip penetrated the CC until the de Groot coordinate $V = 2.5$ mm respect to the cortical surface. Cortical evoked responses were recorded from the left frontal cortex with a surface monopolar silver ball electrode of 0.5 mm diameter located on the contralateral cortex at similar surface de Groot coordinates to those utilized for transcortical stimulation of the CC. Test stimuli consisted of 100 μs duration square-wave pulses generated by means of a Grass S11 stimulator in conjunction with a Grass SIU-5 stimulus isolation unit and a Grass CCU 1A constant current unit. Before beginning each experiment, a full input–output series was performed at a stimulus intensity of 300–1100 μA, and test stimuli with a stimulation intensity yielding responses with peak-to-peak amplitude of 50% of the maximum were used for the remainder of the experiment. After a 30-min stabilization period, a 5-min control period of 30 averaged basal responses was recorded; then, the rats received a single dose of the β_2-AR clenbuterol (0.019, 0.038 and 0.075 mg/kg i.p.; $N = 12$ for each group). Twelve rats receiving saline served as controls. Clenbuterol (Sigma–Aldrich, St. Louis, MO) or saline were administered as a 100 μl/100 g body weight volume. Twenty minutes after drug or saline administration, a new control period of 5-min was recorded. Thereafter, a tetanizing stimulus consisting of a single train of 100 μs duration square-wave pulses at 312 Hz and 500 ms duration, with intensity 50% higher than the test stimuli, was applied. Recordings were amplified by a Grass P-511 preamplifier (0.8–1000 Hz bandwidth), displayed on a Philips PM 3365A digital oscilloscope, digitized at a rate of 10,000/s by an A/D converter interfaced to an Acer PC, and stored for retrieval and off-line analysis. In all experiments body temperature and expired CO_2 were monitored and remained within normal limits. Basal responses evoked in the rat cerebral cortex by contralateral stimulation of the CC begin with an early downward surface positive deflection (*P*) followed by a late upward surface negative wave (*N*). *P–N* latency and *P–N* peak-to-peak amplitude were measured using time and voltage cursors provided in the digital oscilloscope. Slope was determined as the amplitude/time ratio on the nearest sample to the 10% and the 90% level between cursors set on peaks *P* and *N*. The efficacy of the tetanizing train to potentiate cortical evoked responses was evaluated by measuring both the peak-to-peak amplitude and the maximal slope increase. The amplitudes were used for analyses of the experiments, according to a procedure reported elsewhere (Mondaca et al., 2004; Soto-Moyano et al., 2005). Changes (in percentage) of the peak-to-peak amplitude increase were plotted as time-course curves. To appreciate the global effect of clenbuterol over the total period of LTP testing (1 h), the area under the curves (AUC) was determined as the integral from 0 to 60 min after tetanization using the Origin 6.0 software (Microcal Software, Inc., Northampton, MA, USA) and plotted as bar graph. At the end of the electrophysiological experiments, the animals were sacrificed with an overdose of urethane.

2.4. Visuo-spatial memory evaluation

Visuospatial learning performance was evaluated in 64 rats (32 normal, 32 malnourished) by employing an 8-arm radial Olton maze (from Laffayette Instrument Co., Laffayette, IN, USA), according to Soto-Moyano et al. (2005). The maze consisted of eight

equally spaced plexiglas arms (70-cm-long, 8-cm-wide), extending from a central octagonal hub (34-cm-across). It was placed 92 cm from the floor in a room with white walls. During the adaptation and testing sessions, all the maze arms were baited with rice puffs. Spatial cues in the extra-maze environment were provided by the experimenter itself, together with different articles for dressing placed on different hangers fixed on the walls of the room; the position of these articles and the position of the experimenter never changed during the 15-day of maze testing in each group of rats. To test animals in this maze, a strong motivation for food is required; this was induced by keeping them on a restricted diet (8 g/day/rat) starting on day 45 of age and until a 15% body weight deficit was obtained. Thereafter, at 53, 54 and 55 days of age, each animal was submitted to an adaptation period which consisted in placing the rat into the center of the maze to explore and run to the end of the arms and consume the bait. Between 56 and 70 days of age, animals were submitted daily to the visuospatial memory test (one assay daily, fifteen days of testing). In each daily assay, rats received a single dose of the β_2-AR agonist clenbuterol (0.019, 0.038 or 0.075 mg/kg clenbuterol i.p. dissolved in saline solution; $N = 8$ for each group), and 30 min after the injection they were placed in the central platform where they could freely run the maze until obtaining the eight baits of food with a cut-off time of 10 min. Eight rats receiving a similar volume of saline (100 µl/100 g body weight) served as controls. The number of errors (entry to already visited arms) was measured as a significant parameter for memory evaluation and plotted as time-course curves. In the full-baited eight-arm radial arm maze, learning is usually inferred from the day-to-day performance improvement. However, in order to appreciate the global effect of clenbuterol on visuospatial performance, the total number of errors accumulated over the whole 15-day testing period was also computed.

2.5. Statistical analyses

All statistical analyses were performed with GraphPad Prism 3.0 software (GraphPad Software, Inc., San Diego, CA, USA). For the analysis of the immunohistochemical data, intergroup comparisons were made using two-tailed unpaired Student's *t*-test. For analysing intergroup differences in time-course after each dose of clenbuterol both in LTP studies and in visuospatial memory evaluation, a two-factor ANOVA of repeated measures was performed followed by the Bonferroni multiple comparisons *post hoc* test. To analyze the global effect of increasing doses of clenbuterol over the complete testing period (AUC, in the electrophysiological experiments; total number of errors, in visuospatial memory evaluation), a two-way ANOVA was used followed by the Bonferroni multiple comparisons test.

3. Results

Body weight measurements revealed that there were no significant differences in body weight gain of pregnant mothers receiving 7.2% or 22.5% protein diet (data not shown). No significant differences in body and brain weights were observed between rats born from mothers receiving either 7.2% or 22.5% dietary protein (data not shown). Full data on the effects of diets on maternal weight gain and on body and brain weights of pups have been published previously for our laboratory (Soto-Moyano et al., 1998; Soto-Moyano et al., 2005).

3.1. β_2-Adrenoceptor expression in cerebral cortex

The β_2-AR subtype was visualized immunohistochemically with a commercially available antibody previously characterized by Western blot (Zhao et al., 2011) and immunocytochemistry (Pérez Piñero, Bruzzone, Sarappa, Castillo, & Lüthy, 2012). Preabsorption of the antibody to the control peptide supplied did not result in attachment of the secondary, peroxidase-labeled immunoglobulins, demonstrating the specificity of the staining approach. In the frontal cortex of normal rats, β_2-AR-immunoreactive neurons were observed in all regions, without significant variation in the distribution of immunoreactivity in both the dorsoventral and mediolateral aspects of individual sections. Larger and smaller neocortical neurons exhibited moderate β_2-AR-immunoreactivity localized in extranuclear regions (Fig. 1A, left panel).

Immunostaining showed a lower expression of β_2-ARs in the frontal cortex of malnourished animals compared to eutrophic controls. The prefrontal cortex of malnourished animals exhibited a lower number of cells expressing the β_2 receptor (Fig. 1B, left panel; $P < 0.05$, two-tailed unpaired Student's *t*-test) while the endowment of β_2-ARs/cell did not change significantly (Fig. 1B, middle panel), thereby resulting in a significantly lower number of β_2ARs/area (Fig. 1B, right panel; $P < 0.01$, two-tailed unpaired Student's *t*-test) when the two factors (percent of cells expressing β_2-ARs and number of β_2-ARs/cell) were combined.

3.2. Cortical LTP in vivo

Transcallosal responses evoked in the rat prefrontal cortex begin with an early downward surface positive deflection followed by a late prominent upward surface negative wave. Detailed characterization of early and late components of these responses upon tetanization during *in vivo* recording, giving insight into what synapses in what layer are being activated during each component, have been described elsewhere (Barra et al., 2012; Burgos et al., 2010; Chapman et al. 1998; Trepel & Racine, 1998). As previously reported (Barra et al., 2012; Flores et al., 2011; Soto-Moyano et al., 2005), shape, latencies, and wave amplitudes of non-potentiated, basal field responses evoked in the prefrontal cortex of prenatally malnourished rats did not significantly differ from those of normal eutrophic rats.

Evaluation of cortical LTP revealed that, in normal animals, tetanic stimulation of callosal fibers produced a significant increase in peak-to-peak amplitude of transcallosal cortical responses evoked in the prefrontal cortex, which remained unchanged throughout the 60-min recording period (Fig. 2A, saline series). In contrast, in the neocortex of malnourished animals, a lower (not significant) increase in the peak-to-peak amplitude of the evoked responses was observed after applying the same tetanic stimulation protocol, which rapidly declined and disappeared (Fig. 2B, saline series).

In normal rats, pretreatment with clenbuterol did not change the peak-to-peak amplitude of transcallosally-evoked cortical responses prior to the application of the tetanizing train. However, in these animals clenbuterol dose-dependently enhanced the magnitude of the LTP induced in the prefrontal cortex, as revealed by the increasing potentiating effect of the tetanizing stimulus as the dose of clenbuterol heightens (Fig. 2A). Fig. 2A reveals that normal eutrophic rats receiving the higher dose of clenbuterol exhibited a significantly bigger LTP than those receiving saline, while increased LTP in the group of rats injected with the lower dose of clenbuterol could only be detected up to 30 min after tetanization ($P < 0.05$ for intergroup comparisons, Bonferroni multiple comparisons test).

Fig. 2C illustrates the global effect over the total testing period of the three doses of clenbuterol administered to normal eutrophic and malnourished rats. Two-way ANOVA followed by the Bonferroni *post hoc* test revealed a clear dose-dependent, enhancing effect of clenbuterol on LTP elicited in the prefrontal cortex of normal

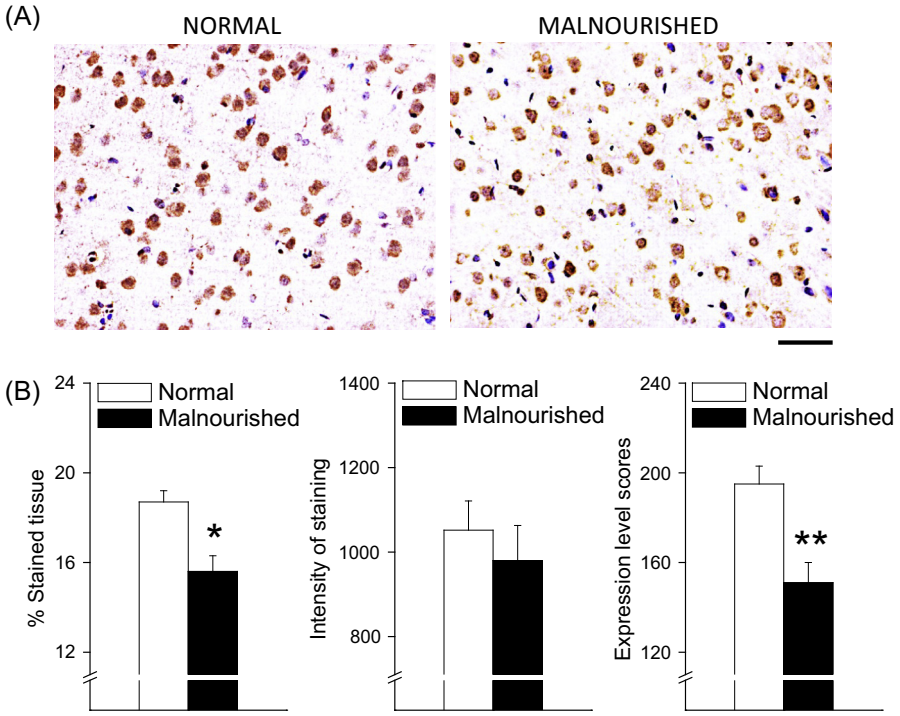

Fig. 1. (A) Expression pattern (25×) of β_2-ARs by immunohistochemistry, in a representative section of the prefrontal cortex of a normal (left panel) and a malnourished (right panel) rats of 60 days of age. The β_2-AR immunoreactivity was homogeneously distributed throughout the perikarya of small and larger neurons, allocated in dark, diffusely stained neurons intermingled with lighter labeled cells. Scale bar, 100 μm. (B) Expression of β_2-ARs in the frontal cortex of normal and malnourished rats of 60 days of age. Left panel shows% stained tissue (percent of cells expressing β_2-ARs); middle panel shows the intensity of staining (number of β_2-ARs/cell); right panel shows the expression levels scores (number of β_2-ARs/area). Values are means ± SEM. For each group $N = 4$ rats. Comparisons between normal and malnourished rats were carried out using two-tailed unpaired Student's *t*-test: * $P < 0.05$, ** $P < 0.01$.

rats. As in normal animals, malnourished rats treated with clenbuterol did not change the peak-to-peak amplitude of cortically evoked responses prior to tetanization, but the higher dose of the drug (0.075 mg/kg) fully restored in these animals the ability of the prefrontal cortex to develop and maintain LTP after tetanization (Fig. 2C, Bonferroni multiple comparisons test). Fig. 2C also illustrates the global effect over the total testing period of the three doses of clenbuterol administered to malnourished rats. Two-way ANOVA followed by the Bonferroni *post hoc* test revealed a dose-dependent, enhancing effect of clenbuterol on LTP evoked in the prefrontal cortex of prenatally malnourished rats.

3.3. Visuospatial memory

Evaluation of visuospatial performance in a full-baited eight-arm radial maze revealed that normal rats committed a number of errors that significantly declined from a mean of 7.2 for the first 3-day block of assays to a mean of 0.7 for the last 3-day block of assays (Fig. 3A, left panel, saline series), which is in agreement with the day-to-day improvement in performance described for that maze. A similar trend was observed in malnourished rats, but the number of errors committed by these animals was clearly higher than those found in control animals, declining from a mean value of 16.5 for the first 3-day assay block to a mean value of 6.2 for the last 3-day assay block (Fig. 3B, left panel, saline series). Consistently, malnourished rats committed almost a 4-fold greater number of errors than normal rats during the whole 15 days of testing (Fig. 3, right panels, saline series).

The two higher doses of clenbuterol significantly increased the number of errors committed by normal rats over animals receiving saline solution, notably during the first 6 days of visuospatial performance evaluation (Fig. 3A, left panel, $P < 0.05$, Bonferroni multiple comparisons test). Analysis of the total number of errors accumulated during the fifteen days of testing revealed consistent results, as clenbuterol treatment significantly ($P < 0.05$, Student–Newman–Keuls multiple comparisons test) increased the total number of errors (Fig. 3A, right panel). In contrast, clenbuterol significantly decreased ($P < 0.05$, Bonferroni multiple comparisons test) the number of errors in the malnourished group (Fig. 3B, left panel). This reinforcing effect of clenbuterol in visuospatial learning performance was dose-dependent when considering the total number of errors committed by the malnourished rats during the complete 15 days of maze testing (Fig. 3B, right panel).

4. Discussion

Mild reduction in the protein content of maternal diet did not significantly alter body and brain weights of pups at birth, indicating that the mild protein deficiency resulted in apparently normal fetal development as assessed by body and brain weights at birth, as reported previously (Resnick et al., 1982; Soto-Moyano et al., 1998; Soto-Moyano et al., 2005). According to Resnick et al. (1982) and Morgane et al. (1993), fetal growth retardation and reduction in brain weight after prenatal malnutrition occur only after protein restriction with maternal diets providing less than 6% protein. However, in spite of the apparently normal body and brain fetal development, the present study showed that hidden prenatally-malnourished rats had impaired prefrontal cortex LTP and presented lower visuospatial memory performance in adult life, together with exhibiting lower expression level scores of β_2-ARs in the prefrontal cortex. Additionally, it was found that i.p. administration of the selective β_2-AR agonist clenbuterol fully restored the ability of prefrontal cortex to develop and maintain

LTP and improved visuospatial memory performance in the undernourished rats.

The foregoing immunohistochemical results revealed that β_2-ARs were moderately present in the frontal cortex of normal rats at postnatal day 60. In this regard, binding assay studies have shown that 63% of total β-adrenoceptors found in frontal cortex during the intrauterine life of the rat corresponded to the β_2 subtype, while postnatal β_2 binding sites represent only 28% of the total population of adrenoceptors (Erdtsieck-Ernste, Feenstra, & Boer, 1991). However, more recent data have revealed that both β_1- and β_2-AR-positive cells were densely distributed throughout the medial prefrontal cortex of adult mice, and that more than 60% of GABA immunoreactive interneurons expressed β_2-ARs (Liu, Liang, Ren, & Li, 2014).

The current results also showed that by postnatal day 60, prenatally malnourished rats exhibited about 25% decrease in β_2-AR expression in the frontal cortex, as compared to eutrophic controls. The mechanisms by which malnutrition during fetal life decreased the β_2-AR population in the frontal cortex are unknown, but they probably include prenatally programmed changes in cell expression of proteins that interact with these receptors during trafficking to the cell membrane, stabilization in the sorting location and endocytosis. Indeed, protein restriction during gestation in rats results in reduced expression of the microtubule-associated protein MAP1B while increasing the expression of MAP1A (Gressens et al., 1997), which are proteins known to play key roles in anchoring ionotropic receptors to microtubules (Sheng & Pak, 2000). For instance, MAP1B plays a role in AMPA receptor endocytosis (Davidkova & Carroll, 2007), which might be related to the changes in density of hippocampal kainate receptors observed in rats subjected to prenatal undernutrition (Fiacco, Rosene, Galler, & Blatt, 2003). However, there are still no data regarding the effects of early undernutrition over components of the trafficking machinery for G protein-coupled receptors, such as β2-ARs. Further studies investigating the expression level of proteins involved in β_2-AR trafficking and endocytosis in the brain of prenatally-malnourished rats would be helpful in elucidating these aspects. In this regard, one may speculate about the fact that maternal low-protein diet during gestation and lactation of rats results in increased β-arrestin levels in heart tissue of the progeny when adults (Fernandez-Twinn, Ekizoglou, Wayman, Petry, & Ozanne, 2006), which is known to promote clathrin-dependent internalization of β_2-ARs (Han, Kommaddi, & Shenoy, 2013), but comparable data from brain tissue are yet lacking.

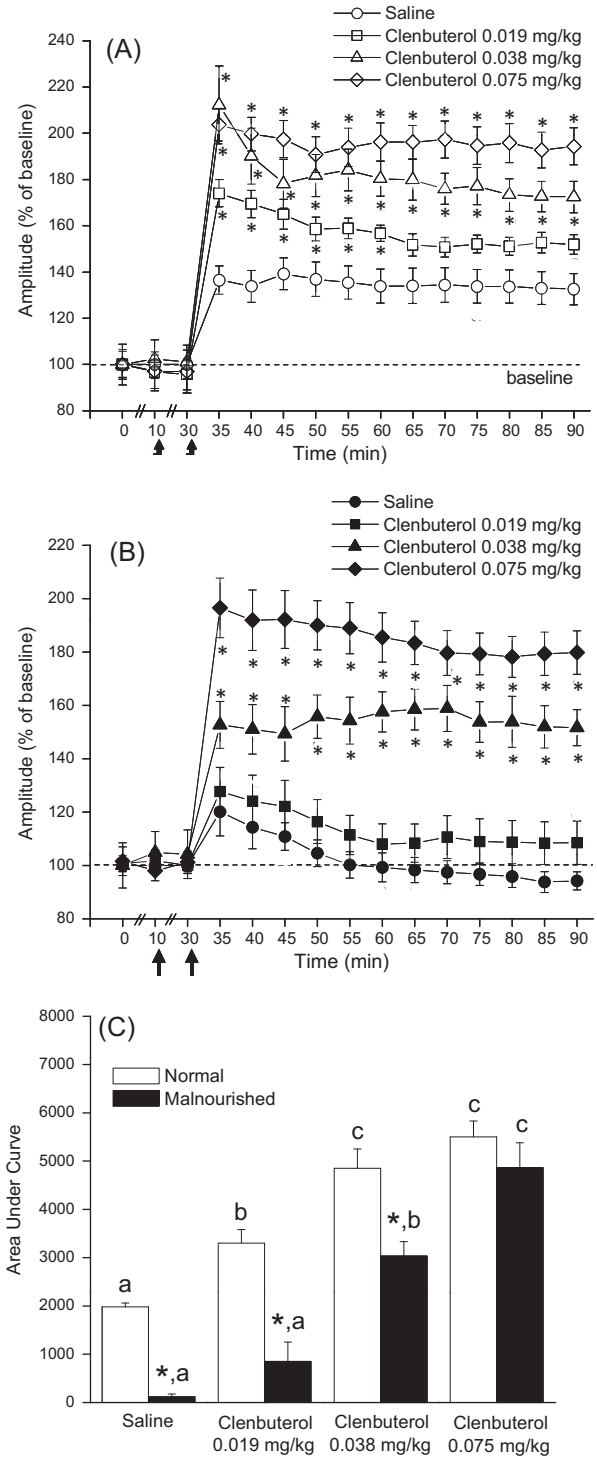

Fig. 2. Effect of i.p. clenbuterol (0.19, 0.38 and 0.75 mg/kg) or saline on LTP induced *in vivo* in the prefrontal cerebral cortex of either normal eutrophic or hidden malnourished rats of 55–60 day of age. (A): Time-course of LTP in normal rats. (B): Time-course of LTP in malnourished rats. Drug injection and time of application of the tetanizing train are indicated by the left and right arrows, respectively. $N = 8$ rats in each group. Values are means ± S.E.M of peak-to-peak amplitude change (in percentage) of 30 cortical evoked responses per rat with respect to the baseline, which was determined as the average of the peak-to-peak amplitude of 30 basal evoked responses per rat recorded at time zero, after a 30-min stabilization period. The potentiating effect of the tetanizing train over the time-course (intragroup variable: time effect) as well as the effect of the different doses of clenbuterol (intergroup variable: treatment effect) on LTP were analyzed using two-factor ANOVA of repeated measures followed by the Bonferroni *post hoc* test. For eutrophic control rats (A), the time effect showed a P ANOVA = 0.0001, $F = 127.8$, and the intergroup variable showed a P ANOVA < 0.0001, $F = 60.71$; the interaction time × treatment showed a P ANOVA = 0.001, $F = 2.72$. For hidden malnourished rats (B), the time effect showed a P ANOVA = 0.0001, $F = 248.4$, and the treatment effect showed a P ANOVA < 0.0001, $F = 18.72$; the interaction time × treatment showed a P ANOVA = 0.001, $F = 4.67$. Asterisks indicate significant intergroup differences for each corresponding time ($P < 0.05$, according to the Bonferroni multiple comparisons test). (C): Area under the curves (AUC) representing the global potentiating effect after tetanization (prefrontal cortex LTP) in normal and malnourished rats under clenbuterol or saline, over the complete testing period. Comparison of the effect of the different doses of clenbuterol or saline (treatment factor) on AUCs from normal and malnourished rats (nutritional status factor) was assessed using two-way ANOVA followed by the Bonferroni *post hoc* test. For the treatment (clenbuterol) factor, P ANOVA < 0.0001, $F = 52.25$, and for the nutritional status factor P ANOVA < 0.0001, $F = 62.50$; the interaction treatment × nutritional status, P ANOVA = 0.0594, $F = 2.62$. For the treatment (clenbuterol) factor comparisons were labeled with letters, and within each group columns with different letters are significantly different ($P < 0.05$, according to the Bonferroni multiple comparisons test), while similar letters means no significant difference between columns. For the nutritional status factor comparisons were labeled with an asterisk, and columns from malnourished rats with an asterisk are significantly different to the corresponding one from normal animals ($P < 0.05$, according to the Bonferroni multiple comparisons test).

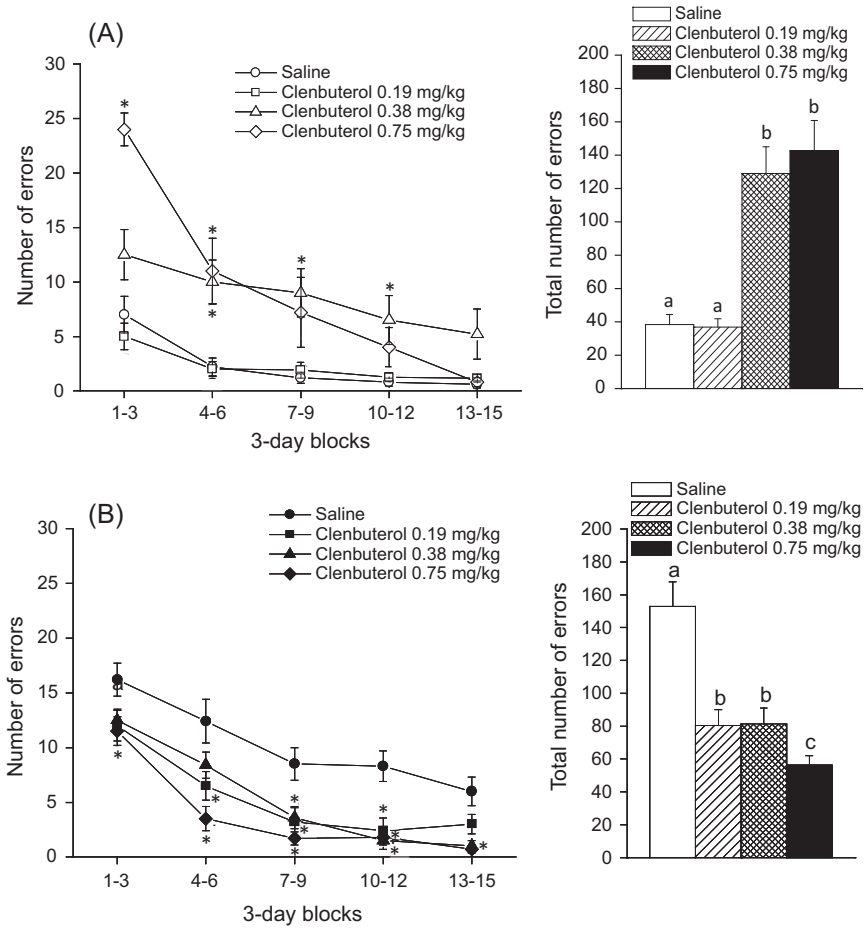

Fig. 3. Effect of i.p. clenbuterol (0.19, 0.38 and 0.75 mg/kg) or saline on visuospatial performance of either normal eutrophic (A) or hidden malnourished (B) rats of 56–70 day of age. *Left panels*: time-course of the number of errors committed by the animals in solving the task in the radial maze. $N = 8$ rats in each group. Values are the means ± S.E.M. of scores recorded during 15 days of testing (one assay daily) grouped in 3-day blocks. The decrease in the number of errors in solving the task over the time-course (intragroup variable: time effect) as well as the effect of the different doses of clenbuterol (intergroup variable: treatment effect) on learning performance were analysed using two-factor ANOVA of repeated measures followed by the Bonferroni *post hoc* test. For eutrophic control rats (A), the time effect showed a P ANOVA < 0.0001, $F = 44.56$, and the treatment effect showed a P ANOVA < 0.0002, $F = 6.494$; the interaction time × treatment showed a P ANOVA = 0.1333, $F = 0.54$. For hidden malnourished rats (B), the time effect showed a P ANOVA < 0.0001, $F = 31.25$, and the treatment effect showed a P ANOVA < 0.0001, $F = 23.13$; the interaction time × treatment showed a P ANOVA = 0.527, $F = 0.2424$. Asterisks indicate significant intergroup differences for each corresponding 3-day block ($P < 0.05$, according to the Bonferroni multiple comparisons test). *Right panels*: total number of errors over the complete testing period of visuospatial performance in rat groups under clenbuterol or saline. Comparison of the effect of the different doses of clenbuterol or saline on the total number of errors was assessed using one-way ANOVA followed by the Student–Newman–Keuls *post hoc* test. For eutrophic control rats (A), P ANOVA < 0.0001, $F = 35.81$; for hidden malnourished rats (B), P ANOVA < 0.0001, $F = 16.07$. Columns with different superscripts are significantly different ($P < 0.05$, according to the Student–Newman–Keuls multiple comparisons test).

Consistent with previous studies (Barra et al., 2012; Flores et al., 2011; Soto-Moyano et al., 2005), the present results showed that hidden prenatal malnutrition in rats did impair neocortical LTP and decreased visuospatial memory performance in adult animals. Interestingly, the administration of the selective β_2-AR agonist clenbuterol to malnourished animals restored the ability of prefrontal cortical neurons to elicit and maintain LTP and improved task execution in the radial eight-arm maze. These data are in agreement with recent findings showing that activation of β_2-ARs plays a prominent role in the elicitation of LTP in hippocampus (Havekes et al., 2012; Li et al., 2013; Qian et al. 2012) and in prefrontal cortex (Zhou et al., 2013), as well as with those showing that stimulation of β_2-ARs in rat prefrontal cortex improves performance on alternation tasks (Ramos et al., 2008) and enhanced trace fear memory (Zhou et al., 2013). As already known, β_2-ARs are G protein-coupled receptors present in the cell membrane, known to be coupled to PKA activity via adenylyl cyclase; in turn, PKA is known to be critically involved in the regulation of calcium permeability of glutamatergic NMDA receptors, thereby controlling the induction and long-term expression of LTP (Abel & Nguyen, 2008; Skeberdis et al. 2006), at least in the hippocampus. Moreover, it has been recently reported that hidden prenatal malnutrition leads to reduced PKA expression in frontal cortex tissue from young adult rats (Flores et al., 2011), which is consistent with the reduced β_2-AR expression reported herein. In addition, β_2-AR-dependent signals in the brain are also transduced through the phosphoinositide-3 kinase PI3-K (Schmidt, Holsboer, & Spengler, 2001). In its turn, PI3-K activates several downstream targets, including PKB, PKC, MAPK, S6K, and p70S6K (Chan, Rittenhouse, & Tsichlis, 1999), which are protein kinases known to be involved in neuroplasticity and memory consolidation (for review see Costa-Mattioli, Sossin, Klann, & Sonenberg, 2009; Dineley et al., 2001; Micheau & Riedel, 1999). Thus, both PKA and PI3-K β_2-AR-dependent effector pathways help to explain observations showing that the β_2-AR agonist salbutamol ameliorates neurotoxin-induced amnesia and enhanced memory consolidation (Crowe & Shaw, 1997), and that β_2-AR antagonists can block the memory-enhancing effect of noradrenaline (Sternberg et al., 1986) while inducing amnesia, when given systemically (Davies & Payne, 1989). Taken together, all these observations agree with the clenbuterol-induced

improvement of synaptic plasticity and behavioral memory that we observed in malnourished rats. Nevertheless, in contrast to the strengthening effect of clenbuterol in learning performance of prenatally malnourished rats, the present results also showed that similar doses of clenbuterol resulted in lower visuospatial learning, when administered to eutrophic control rats. Likely, this result can be explained, at least in part from the differential expression levels of β_2-AR found in cerebral cortex between groups, and/or from a possible differential desensitization of β_2-ARs upon chronic clenbuterol treatment during the fifteen days of maze testing. For instance, unlike adult rats, β_2 signaling in the brain of eutrophic neonates is resistant to homologous desensitization by β_2 agonists (Slotkin, Tate, Cousins, & Seidler, 2001), but this question has not been addressed in malnourished animals. Nevertheless, there has been reported that early undernutrition may selectively affect some mechanisms involved in the control of down-regulation of β adrenoceptors (Keller, Cuadra, Molina, & Orsingher, 1990), which may be understood as an inability of the general β adrenoceptor population of malnourished rats to become desensitized. How this peculiar behavior may be related to the strengthening effect of clenbuterol in the visuospatial learning of malnourished rats remains unknown.

Our results show that the β_2-AR agonist clenbuterol restored neocortical LTP and improved visuospatial learning in prenatally-malnourished young adult rats. However, at the present time it is unclear whether the deleterious effects of prenatal malnutrition on both prefrontal cortex LTP and visuo-spatial memory were due to postnatal decreases in β_2-AR expression, as observed by us. As recently reported, β_2-AR knockout mice show defective hippocampal LTP (Qian et al., 2012), but the effect of lacking β_2-AR expression on visuospatial performance has not yet been studied. Furthermore, the fact that clenbuterol administered to normal control rats resulted in increased neocortical LTP but impaired learning performance in the radial maze is indicative that neocortical LTP is a form of neural plasticity that underlies some learning capabilities, though by no means fully underlying all types of visuo-spatial learning. Indeed, learning involved in visuospatial tasks has rather been associated to hippocampal LTP (Buzsáki & Moser, 2013), although it is also clear that the prefrontal cortex plays critical roles in the representation of space and its function (Funahashi, 2013). Indeed, abnormalities in the electrophysiological properties of hippocampal neurons in malnourished animals seem unlikely to contribute to the deficits in visuospatial performance reported herein, on the basis that those early alterations disappear when animals reached adulthood (Rushmore, Luebke, & Galler, 1998). In contrast, many structural changes and functional disturbances remain in neocortex long after postnatal nutrition has been restored, reflecting the limited structural/functional recovery capacity of the neocortex compared to hippocampus, due to lack of postnatal neurogenesis in the former (Levitsky & Strupp, 1995; Morgane et al., 1993).

In summary, the present data show that mild prenatal protein malnutrition results in decreased β_2-AR expression in the rat cerebral cortex during postnatal life, together with decreased LTP and visuospatial performance in adulthood, and that administration of the β_2-AR agonist clenbuterol improves neocortical LTP and visuospatial memory performance in these animals. These results may offer some hints to further investigate possible changes in the role of β_2-ARs in the development of plasticity in the adult brain as a result of alterations in maternal protein nutrition during pregnancy. Brain plasticity is regulated by numerous individual molecular mechanisms, and all of these are regulated by genes which are under epigenetic regulation by a *complex* interplay of dietary and environmental influences (Langley-Evans, 2009), thus highlighting the role of adequate maternal protein nutrition during pregnancy for later brain plasticity.

Acknowledgments

This work was partially supported by Grants 021243HK and 021001SB from DICYT, University of Santiago de Chile, and Grant 3130573 from FONDECYT. The authors declare that they have no competing financial interests.

References

Abel, T., & Nguyen, P. V. (2008). Regulation of hippocampus-dependent memory by cyclic AMP-dependent protein kinase. *Progress in Brain Research, 169*, 97–115.
Arnsten, A. F., & Jentsch, J. D. (1997). The α_1-adrenergic agonist, cirazoline, impairs spatial working memory performance in aged monkeys. *Pharmacology, Biochemistry and Behavior, 58*, 55–59.
Arnsten, A. F., & Jin, L. E. (2012). Guanfacine for the treatment of cognitive disorders: A century of discoveries at Yale. *The Yale Journal of Biology and Medicine, 85*, 45–58.
Barra, R., Soto-Moyano, R., Valladares, L., Morgan, C., Pérez, H., Burgos, H., et al. (2012). Knockdown of α_{2C}-adrenoceptors in the occipital cortex rescued long-term potentiation in hidden prenatally malnourished rats. *Neurobiology of Learning and Memory, 98*, 228–234.
Birnbaum, S., Gobeske, K. T., Auerbach, J., Taylor, J. R., & Arnsten, A. F. (1999). A role for norepinephrine in stress-induced cognitive deficits: A-1-adrenoceptor mediation in the prefrontal cortex. *Biological Psychiatry, 46*, 1266–1274.
Björklund, M., Sirviö, J., Puoliväli, J., Sallinen, J., Jäkälä, P., Scheinin, M., et al. (1998). A_{2C}-Adrenoceptor-overexpressing mice are impaired in executing nonspatial and spatial escape strategies. *Molecular Pharmacology, 54*, 569–576.
Björklund, M., Sirviö, J., Riekkinen, M., Sallinen, J., Scheinin, M., & Riekkinen, P. Jr., (2000). Overexpression of α_{2C}-adrenoceptors impairs water maze navigation. *Neuroscience, 95*, 481–487.
Björklund, M., Sirviö, J., Sallinen, J., Scheinin, M., Kobilka, B. K., & Riekkinen, P. Jr., (1999). A_{2C}-Adrenoceptor overexpression disrupts execution of spatial and non-spatial search patterns. *Neuroscience, 88*, 1187–1198.
Bramham, C. R., Bacher-Svendsen, K., & Sarvey, J. M. (1997). LTP in the lateral perforant path is β-adrenergic receptor-dependent. *Neuroreport, 8*, 719–724.
Burgos, H., Castillo, A., Flores, O., Puentes, G., Morgan, C., Gatica, A., et al. (2010). Effect of modafinil on learning performance and neocortical long-term potentiation in rats. *Brain Research Bulletin, 83*, 238–244.
Buzsáki, G., & Moser, E. I. (2013). Memory, navigation and theta rhythm in the hippocampal–entorhinal system. *Nature Neuroscience, 16*, 130–138.
Chan, T. O., Rittenhouse, S. E., & Tsichlis, P. N. (1999). AKT/PKB and other D3 phosphoinositide-regulated kinases: Kinase activation by phosphoinositide-dependent phosphorylation. *Annual Review of Biochemistry, 68*, 965–1014.
Chapman, C. A., Trepel, C., Ivanco, T. L., Froc, D. J., Wilson, K., & Racine, R. J. (1998). Changes in field potentials and membrane currents in rat sensorimotor cortex following repeated tetanization of the corpus callosum in vivo. *Cerebral Cortex, 8*, 730–742.
Costa-Mattioli, M., Sossin, W. S., Klann, E., & Sonenberg, N. (2009). Translational control of long-lasting synaptic plasticity and memory. *Neuron, 61*, 10–26.
Crowe, S. F., Ng, K. T., & Gibbs, M. E. (1990). Memory consolidation of weak training experiences by hormonal treatments. *Pharmacology, Biochemistry and Behavior, 37*, 729–734.
Crowe, S. F., & Shaw, S. (1997). Salbutamol overcomes the effect of the noradrenergic neurotoxin DSP-4 on memory function in the day-old chick. *Behavioral Pharmacology, 8*, 216–222.
Davidkova, G., & Carroll, R. C. (2007). Characterization of the role of microtubule-associated protein 1B in metabotropic glutamate receptor-mediated endocytosis of AMPA receptors in hippocampus. *Journal of Neuroscience, 27*, 13273–13278.
Davies, D. C., & Payne, J. M. (1989). Amnesia of a passive avoidance task due to the beta 2-adrenoceptor antagonist ICI 118,551. *Pharmacology, Biochemistry and Behavior, 32*, 187–190.
Dineley, K. T., Weeber, E. J., Atkins, C., Adams, J. P., Anderson, A. E., & Sweatt, J. D. (2001). Leitmotifs in the biochemistry of LTP induction: Amplification, integration and coordination. *Journal of Neurochemistry, 77*, 961–971.
Erdtsieck-Ernste, B. H., Feenstra, M. G., & Boer, G. J. (1991). Pre- and postnatal developmental changes of adrenoceptor subtypes in rat brain. *Journal of Neurochemistry, 57*, 897–903.
Fernandez-Twinn, D. S., Ekizoglou, S., Wayman, A., Petry, C. J., & Ozanne, S. E. (2006). Maternal low-protein diet programs cardiac β-adrenergic response and signaling in 3-mo-old male offspring. *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology, 291*, R429–R436.
Fiacco, T. A., Rosene, D. L., Galler, J. R., & Blatt, G. J. (2003). Increased density of hippocampal kainate receptors but normal density of NMDA and AMPA receptors in a rat model of prenatal protein malnutrition. *Journal of Comparative Neurology, 456*, 350–360.
Flores, O., Pérez, H., Valladares, L., Morgan, C., Gatica, A., Burgos, H., et al. (2011). Hidden prenatal malnutrition in the rat: Role of β_1-adrenoceptors on synaptic plasticity in the frontal cortex. *Journal of Neurochemistry, 119*, 314–323.
Franowicz, J. S., Kessler, L. E., Borja, C. M., Kobilka, B. K., Limbird, L. E., & Arnsten, A. F. (2002). Mutation of the α_{2A}-adrenoceptor impairs working memory performance and annuls cognitive enhancement by guanfacine. *Journal of Neuroscience, 22*, 8771–8777.

Funahashi, S. (2013). Space representation in the prefrontal cortex. *Progress in Neurobiology, 103*, 131–155.
Gibbs, M. E. (1991). Behavioral and pharmacological unravelling of memory formation. *Neurochemical Research, 16*, 715–726.
Gibbs, M. E., & Summers, R. J. (2000). Separate roles for β_2- and β_3-adrenoceptors in memory consolidation. *Neuroscience, 95*, 913–922.
Gressens, P., Muaku, S. M., Besse, L., Nsegbe, E., Gallego, J., Delpech, B., et al. (1997). Maternal protein restriction early in rat pregnancy alters brain development in the progeny. *Developmental Brain Research, 103*, 21–35.
Han, S. O., Kommaddi, R. P., & Shenoy, S. K. (2013). Distinct roles for β-arrestin2 and arrestin-domain-containing proteins in β_2 adrenergic receptor trafficking. *EMBO Reports, 14*, 164–171.
Havekes, R., Canton, D. A., Park, A. J., Huang, T., Nie, T., Day, J. P., et al. (2012). Gravin orchestrates protein kinase A and β_2-adrenergic receptor signaling critical for synaptic plasticity and memory. *Journal of Neuroscience, 32*, 18137–18149.
Hernández, A., Burgos, H., Mondaca, M., Barra, R., Núñez, H., Pérez, H., et al. (2008). Effect of prenatal protein malnutrition on long-term potentiation and BDNF protein expression in the rat entorhinal cortex after neocortical and hippocampal tetanization. *Neural Plasticity, 2008*, 646919. http://dx.doi.org/10.1155/2008/646919.
Hillman, K. L., Doze, V. A., & Porter, J. E. (2005). Functional characterization of the beta-adrenergic receptor subtypes expressed by CA1 pyramidal cells in the rat hippocampus. *Journal of Pharmacology and Experimental Therapeutics, 314*, 561–567.
Hopkins, W. F., & Johnston, D. (1988). Noradrenergic enhancement of long-term potentiation at mossy fiber synapses in the hippocampus. *Journal of Neurophysiology, 59*, 667–687.
Kandel, E. R. (2012). The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. *Molecular Brain, 5*, 14. http://dx.doi.org/10.1186/1756-6606-5-14.
Keller, E. A., Cuadra, G. R., Molina, V. A., & Orsingher, O. A. (1990). Perinatal undernutrition affects brain modulatory capacity of beta-adrenergic receptors in adult rats. *Journal of Nutrition, 120*, 305–308.
Kobayashi, M. (2007). Differential regulation of synaptic transmission by adrenergic agonists via protein kinase A and protein kinase C in layer V pyramidal neurons of rat cerebral cortex. *Neuroscience, 146*, 1772–1784.
Langley-Evans, S. C. (2009). Nutritional programming of disease: Unravelling the mechanism. *Journal of Anatomy, 215*, 36–51.
Levitsky, D. A., & Strupp, B. J. (1995). Malnutrition and the brain: Changing concepts, changing concerns. *Journal of Nutrition, 125*, 2212S–2220S.
Li, S., Jin, M., Zhang, D., Yang, T., Koeglsperger, T., Fu, H., et al. (2013). Environmental novelty activates β2-adrenergic signaling to prevent the impairment of hippocampal LTP by Aβ oligomers. *Neuron, 77*, 929–941.
Li, B. M., Mao, Z. M., Wang, M., & Mei, Z. T. (1999). A-2 Adrenergic modulation of prefrontal cortical neuronal activity related to spatial working memory in monkeys. *Neuropsychopharmacology, 21*, 601–610.
Liu, Y., Liang, X., Ren, W. W., & Li, B. M. (2014). Expression of β1- and β2-adrenoceptors in different subtypes of interneurons in the medial prefrontal cortex of mice. *Neuroscience, 257*, 149–157.
Mao, Z. M., Arnsten, A. F., & Li, B. M. (1999). Local infusion of an α-1 adrenergic agonist into the prefrontal cortex impairs spatial working memory performance in monkeys. *Biological Psychiatry, 46*, 1259–1265.
Marzo, A., Bai, J., & Otani, S. (2009). Neuroplasticity regulation by noradrenaline in mammalian brain. *Current Neuropharmacology, 7*, 286–295.
Micheau, J., & Riedel, G. (1999). Protein kinases: Which one is the memory molecule? *Cellular and Molecular Life Sciences, 55*, 534–548.
Mondaca, M., Hernández, A., Pérez, H., Valladares, L., Sierralta, W., Fernández, V., et al. (2004). A_2-Adrenoceptor modulation of long-term potentiation elicited in vivo in rat occipital cortex. *Brain Research, 1021*, 292–296.
Morgane, P. J., Austin-LaFrance, R., Bronzino, J., Tonkiss, J., Díaz-Cintra, S., Cintra, L., et al. (1993). Prenatal malnutrition and development of the brain. *Neuroscience and Biobehavioral Reviews, 17*, 91–128.
National Research Council (1985). Guide for the care and use of laboratory animals. Publication no. 85–23 (rev.). National Institutes of Health, Bethesda, MD.
Nguyen, P. V., & Woo, N. H. (2003). Regulation of hippocampal synaptic plasticity by cyclic AMP-dependent protein kinases. *Progress in Neurobiology, 71*, 401–437.
Nowicky, A. V., Christofi, G., & Bindman, L. J. (1992). Investigation of β-adrenergic modulation of synaptic transmission and postsynaptic induction of associative LTP in layer V neurones in slices of rat sensorimotor cortex. *Neuroscience Letters, 137*, 270–273.
O'Dell, T. J., Connor, S. A., Gelinas, J. N., & Nguyen, P. V. (2010). Viagra for your synapses: Enhancement of hippocampal long-term potentiation by activation of beta-adrenergic receptors. *Cellular Signalling, 5*, 728–736.
Pellegrino, L. J., & Cushman, A. J. (1967). A stereotaxic atlas of the rat brain. In R.M. Elliott, G. Lindzey, K. MacCorquodale (Eds.), New York: Appleton-Century-Crofts.
Pérez Piñero, C., Bruzzone, A., Sarappa, M. G., Castillo, L. F., & Lüthy, I. A. (2012). Involvement of α2- and β2-adrenoceptors on breast cancer cell proliferation and tumour growth regulation. *British Journal of Pharmacology, 166*, 721–736.
Qian, H., Matt, L., Zhang, M., Nguyen, M., Patriarchi, T., Koval, O. M., et al. (2012). β2-Adrenergicreceptor supports prolonged theta tetanus-induced LTP. *Journal of Neurophysiology, 107*, 2703–2712.
Radisavljevic, Z., Cepeda, C., Peacock, W., Buchwald, N. A., & Levine, M. S. (1994). Norepinephrine modulates excitatory amino acid-induced responses in developing human and adult rat cerebral cortex. *International Journal of Developmental Neuroscience, 12*, 353–361.
Ramos, B. P., Colgan, L. A., Nou, E., & Arnsten, A. F. (2008). β2 adrenergic agonist, clenbuterol, enhances working memory performance in aging animals. *Neurobiology of Aging, 29*, 1060–1069.
Resnick, O., Morgane, P. J., Hasson, R., & Miller, M. (1982). Overt and hidden forms of chronic malnutrition in the rat and their relevance to man. *Neuroscience and Biobehavioral Reviews, 6*, 55–75.
Rushmore, J., Luebke, J., & Galler, J. R. (1998). Electrophysiological properties of rat hippocampal principal cells are unaltered by prenatal protein malnutrition. *Hippocampus, 8*, 380–389.
Schimanski, L. A., Ali, D. W., Baker, G. B., & Nguyen, P. V. (2007). Impaired hippocampal LTP in inbred mouse strains can be rescued by β-adrenergic receptor activation. *European Journal Neuroscience, 25*, 1589–1598.
Schmidt, P., Holsboer, F., & Spengler, D. (2001). β2-Adrenergic receptors potentiate glucocorticoid receptor transactivation via G protein βγ-subunits and the phosphoinositide 3-kinase pathway. *Molecular Endocrinology, 15*, 553–564.
Sheng, M., & Pak, D. T. (2000). Ligand-gated ion channel interactions with cytoskeletal and signaling proteins. *Annual Review of Physiology, 62*, 755–778.
Sierralta, W., Hernández, A., Valladares, L., Pérez, H., Mondaca, M., & Soto-Moyano, R. (2006). Mild prenatal protein malnutrition increases alpha 2C-adrenoceptor expression in the rat cerebral cortex during postnatal life. *Brain Research Bulletin, 69*, 580–586.
Sierralta, W. D., & Thole, H. H. (1996). Retrieval of estradiol receptor in paraffin sections of resting porcine uteri by microwave treatment. Immunostaining patterns obtained with different primary antibodies. *Histochemistry and Cell Biology, 105*, 357–363.
Skeberdis, V. A., Chevaleyre, V., Lau, C. G., Goldberg, J. H., Pettit, D. L., Suadicani, S. O., et al. (2006). Protein kinase A regulates calcium permeability of NMDA receptors. *Nature Neuroscience, 9*, 501–510.
Slotkin, T. A., Tate, C. A., Cousins, M. M., & Seidler, F. J. (2001). Beta-adrenoceptor signaling in the developing brain: sensitization or desensitization in response to terbutaline. *Developmental Brain Research, 131*, 113–125.
Soto-Moyano, R., Alarcón, S., Belmar, J., Kusch, C., Pérez, H., Ruiz, S., et al. (1998). Prenatal protein restriction alters synaptic mechanisms of callosal connections in the rat visual cortex. *International Journal of Developmental Neuroscience, 16*, 75–84.
Soto-Moyano, R., Valladares, L., Sierralta, W., Pérez, H., Mondaca, M., Fernández, V., et al. (2005). Mild prenatal protein malnutrition increases α_{2C}-adrenoceptor density in the cerebral cortex during postnatal life and impairs neocortical long-term potentiation and visuo-spatial performance in rats. *Journal of Neurochemistry, 93*, 1099–1109.
Sternberg, D. B., Korol, D., Novack, G. D., & McGaugh, J. L. (1986). Epinephrine-induced memory facilitation: Attenuation by adrenoceptor antagonists. *European Journal of Pharmacology, 129*, 189–193.
Tachibana, K., Matsumoto, M., Togashi, H., Kojima, T., Morimoto, Y., Kemmotsu, O., et al. (2004). Milnacipran, a serotonin and noradrenaline reuptake inhibitor, suppresses long-term potentiation in the rat hippocampal CA1 field via 5-HT1A receptors and alpha 1-adrenoceptors. *Neuroscience Letters, 357*, 91–94.
Trepel, C., & Racine, R. J. (1998). Long-term potentiation in the neocortex of the adult, freely moving rat. *Cerebral Cortex, 8*, 719–729.
Wang, M., Ramos, B. P., Paspalas, C. D., Shu, Y., Simen, A., Duque, A., et al. (2007). A_{2A}-Adrenoceptors strengthen working memory networks by inhibiting cAMP-HCN channel signaling in prefrontal cortex. *Cell, 129*, 397–410.
Zhao, W., Tong, T., Wang, L., Li, P. P., Chang, Y., Zhang, L. L., et al. (2011). Chicken type II collagen induced immune tolerance of mesenteric lymph node lymphocytes by enhancing beta2-adrenergic receptor desensitization in rats with collagen-induced arthritis. *International Immunopharmacology, 11*, 12–18.
Zhou, H. C., Sun, Y. Y., Cai, W., He, X. T., Yi, F., Li, B. M., et al. (2013). Activation of β2-adrenoceptor enhances synaptic potentiation and behavioral memory via cAMP-PKA signaling in the medial prefrontal cortex of rats. *Learning and Memory, 20*, 274–284.