

# Brain Glutathione Content and Glutamate Uptake Are Reduced in Rats Exposed to Pre- and Postnatal Protein Malnutrition<sup>1</sup>

Ana Maria Feoli,<sup>2,4</sup> Ionara Siqueira,<sup>3</sup> Lucia Maria V. Almeida,<sup>4</sup> Ana Carolina Tramontina,<sup>4</sup> Cíntia Battu,<sup>4</sup> Susana T. Wofchuk,<sup>4</sup> Carmem Gottfried,<sup>4</sup> Marcos Luiz Perry,<sup>4</sup> and Carlos-Alberto Gonçalves<sup>4\*</sup>

<sup>2</sup>Faculdade de Enfermagem, Nutrição e Fisioterapia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil and <sup>3</sup>Departamento de Farmacologia, and <sup>4</sup>Departamento de Bioquímica, ICBS, Universidade Federal do Rio Grande de do Sul, Porto Alegre, Brazil

## Abstract

The brain is particularly susceptible to oxidative insults and its antioxidant defense is dependent on its glutathione content. Protein malnutrition (PMN) is an important and very common insult during development and compromises antioxidant defenses in the body, particularly glutathione levels. We investigated whether brain glutathione content and related metabolic pathways, predominantly regulated by astrocytes (particularly glutamate uptake and glutamine synthesis), are altered by pre- and postnatal PMN in rats. Thus, we measured the glutathione content, glutamine synthetase (GS) activity, and glutamate uptake activity in the cerebral cortex (Cx) and hippocampus of rats subjected to pre- and postnatal PMN and in nourished controls. Although malnourished rats exhibited an ontogenetic profile of glutathione levels in both brain regions similar to that of controls, they had lower levels on postnatal d 2 (P2); in Cx this decrease persisted until postnatal d 15. In addition, we found other changes, such as reduced total antioxidant reactivity and glutathione peroxidase activity on P2, and these were not accompanied by alterations in free radical levels or lipoperoxidation in either brain region. Moreover, malnourished rats had elevated GS and reduced glutamate uptake. Taken together, these alterations indicate specific changes in astrocyte metabolism, possibly responsible for the higher vulnerability to excitotoxic/oxidative damage in malnourished rats. The lower antioxidant defense appears to be the main alteration that causes oxidative imbalance, rather than an increase in reactive oxygen species. Moreover, a recovery of altered metabolic variables may occur during adulthood, despite persistent PMN. *J. Nutr.* 136: 2357–2361, 2006.

## Introduction

Cellular glutathione is the major antioxidant compound that acts directly both in removing reactive oxygen species (ROS)<sup>5</sup> and as a substrate for several peroxidases (1). The brain is particularly susceptible to oxidative insults and is, therefore, very dependent on its glutathione content, especially during development, when brain metabolism and growth are maximal. In addition, various pathologies affecting the nervous system involve oxidative stress, possibly associated with a diminution in glutathione levels. The rate-limiting enzyme for glutathione is the L- $\gamma$ -glutamyl-cysteine synthase, although glutamate levels, particularly in astrocytes, are equally important for the production of new glutathione (1,2).

Malnutrition is an important and very common insult to human development and compromises antioxidant defenses in the body, particularly glutathione levels (3). There are several studies in animal models showing the biochemical changes in the brain caused by protein malnutrition (PMN) that involve specific neurotransmitters (4), and, more recently, we have shown changes in the neurotransmission mediated by glutamate, the main excitatory neurotransmitter (5).

Astrocytes are intimately related to glutamatergic transmission and antioxidant defense (6,7). These cells play essential roles during development and adulthood in migration and synaptogenesis, release of neurotrophic factors, control of energetic metabolism, regulation of extracellular ionic composition, and uptake/recycling of neurotransmitters.

Our previous results indicate important alterations in astrocyte markers (S100B protein and glial fibrillary acidic protein) induced by PMN in rats, as well as alterations in the oxidative status of these rats (8). Together, these alterations suggest that the brain content of glutathione and related metabolic pathways, predominantly regulated by astrocytes (particularly glutamate uptake and glutamine synthesis), may be altered by PMN and this, therefore, could contribute to a higher vulnerability of oxidative damage in malnourished rats (9).

<sup>1</sup> Supported by Brazilian funds from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

<sup>5</sup> Abbreviations used: CAT, catalase; Cx, cerebral cortex; DCF, 2'-7'-dichlorofluorescein; GS, glutamine synthetase; GPx, glutathione peroxidase; Hc, hippocampus; PMN, protein malnutrition; P2, postnatal d 2; P15, postnatal d 15; P60, postnatal d 60; SOD, superoxide dismutase; ROS, reactive oxygen species; TAR, total antioxidant reactivity.

\* To whom correspondence should be addressed. E-mail: casg@ufrgs.br.

In this study, we measured glutathione content, glutamine synthetase (GS) activity, and glutamate uptake activity, as well as other parameters of oxidative stress, namely free radical content, lipid peroxidation, total antioxidant reactivity (TAR), and antioxidant enzyme activities, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), in cerebral cortex (Cx) and hippocampus (Hc) from rats subjected to pre- and postnatal PMN.

## Materials and Methods

**Chemicals.** Thiobarbituric acid and Trolox were obtained from Merck, 2,2'-azobis (2-amidinopropane), dihydrochloride was obtained from Wako Chemicals USA, and 2'-7'-dichlorofluorescein diacetate, 2'-7'-dichlorofluorescein (DCF), phenylmethylsulfonyl fluoride, 5-amino-2,3-dihydro-1,4-phtalazinedione (luminol), H<sub>2</sub>O<sub>2</sub>, glutathione reductase (all reagents used for glutathione assay and glutamic acid stock solution) were purchased from Sigma Chemical. L-[2,3-<sup>3</sup>H] glutamic acid (<sup>3</sup>H] glutamate) (specific activity 49 Ci/mmol) was from Amersham International.

**Animals.** Albino Wistar rats were maintained under standard conditions (12-h light/12-h dark, 22 ± 2°C); they consumed food and water ad libitum. The experimental protocol was developed according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil.

**Diets.** The rats had free access to isocaloric diets (Table 1) containing 25% or 7% protein (casein), salts, and vitamins as recommended by the AOAC (10) and as previously described by our group (11).

**Malnutrition model.** Prenatal and lactational malnutrition was induced by restricting the protein content of the mothers' diet to 7% (control group: 25% protein) throughout the gestation and lactational periods, resulting in a decrease in the global amount of nutrients accessible to pups; whereas malnutrition for up to 21 and 60 d was induced by feeding pups the same 7% protein diet. Both diets were isocaloric, and they were consumed ad libitum. The litter size was adjusted to 8 pups/mother on d 1 postpartum and they were maintained at 22°C on a 12-h-light/12-h-dark cycle until experimental age. For biochemical assays, the rats were killed on postnatal d 2 (P2), postnatal d 15 (P15), or postnatal d 60 (P60) by decapitation and specific brain regions (Cx and Hc) were dissected out.

**TABLE 1** Nutritional composition of the diets

	25% protein diet	7% protein diet
	<i>g/kg</i>	
Casein (87% protein) <sup>1</sup>	287	80.5
L-Methionine	1.5	1.5
Fat (soybean oil)	150	150
Carbohydrate (corn starch)	501.5	708
Salt mix <sup>2,3</sup>	40	40
Vitamin mix <sup>2,4</sup>	10	10
Non-nutritive fiber	10	10

Energy for both diets: 18 kJ/g.

<sup>1</sup> Casein, purity 87% (from Farmaquímica) supplemented with 0.15% L-methionine (from Merck).

<sup>2</sup> Salt and vitamin compositions are according to Schweigert et al. (11).

<sup>3</sup> Mineral mixture (from Roche) mg/100 g of ration: NaCl, 557; KCl, 3.2; KH<sub>2</sub>PO<sub>4</sub>, 1556; MgSO<sub>4</sub>, 229; CaCO<sub>3</sub>, 1526; FeSO<sub>4</sub>·7H<sub>2</sub>O, 108; MnSO<sub>4</sub>·H<sub>2</sub>O, 16; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 2.2; CuSO<sub>4</sub>·5H<sub>2</sub>O, 1.9; CaCl<sub>2</sub>·6H<sub>2</sub>O, 0.09.

<sup>4</sup> Vitamin mixture (from Roche), mg/100 g of ration: vitamin A (retinyl acetate), 4; vitamin D (cholecalciferol), 0.5; vitamin E (α-tocopherol), 10; vitamin K (menadione), 0.5; choline, 200; PABA, 10; inositol, 10; niacin, 4; pantothenic acid, 4; riboflavin, 0.8; thiamin, 0.5; pyridoxine, 0.5; folic acid, 0.2; biotin, 0.04; vitamin B-12, 0.003.

**Total glutathione.** Total glutathione content was determined by a slightly modified assay, as described previously (12,13). Briefly, the tissue was homogenized in PBS (0.01 mol/L, pH 7.6), 6.3 mmol edetic acid (pH 7.5), and Triton-X (0.05%) and protein was precipitated with 1% sulfosalicylic acid. Supernatant was assayed with 462.6 μmol/L 5,5'-dithiobis-(2-nitrobenzoic acid), 0.5 kU/L glutathione reductase, and 0.3 mmol/L NADPH; reduced 5,5'-dithiobis-(2-nitrobenzoic acid) was measured at 412 nm.

**Free radicals.** Samples were incubated with 2'-7'-dichlorofluorescein diacetate (100 μmol/L) for 30 min. The formation of the oxidized fluorescent derivative (DCF) was monitored at excitation and emission wavelengths of 488 nm and 525 nm, respectively, using a fluorescence spectrophotometer (Hitachi F-2000) (14).

**Lipid peroxidation.** Samples were incubated with 46 mmol/L trichloroacetic acid and 0.60 mol/L thiobarbituric acid at 37°C for 30 min. An equal volume of n-butanol was added and, after centrifugation (1000 × g; 10 min), the organic phase was collected for fluorescence measurements at excitation and emission wavelengths of 515 and 553 nm, respectively (15). Results were expressed as pmol malondialdehyde/mg protein, using 1,1,3,3-tetramethoxypropane as standard.

**TAR.** TAR values were determined by assessing the decrease in the luminol-enhanced chemiluminescence induced by 2,2'-azobis (2-amidinopropane), dihydrochloride, in the presence Trolox or tissue samples (16,17). Values were expressed as equivalents of Trolox mg protein.

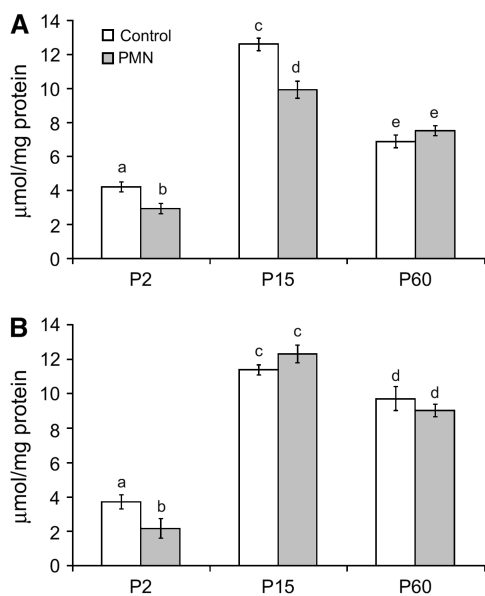
**Antioxidant enzymes.** SOD activity was determined using a RANSOD kit (Randox Labs). The CAT activity was assayed at 25°C by determining the rate of degradation of H<sub>2</sub>O<sub>2</sub> at 240 nm in 10 mmol/L potassium phosphate buffer (pH 7.0). Values were expressed as pmol H<sub>2</sub>O<sub>2</sub> consumed · min<sup>-1</sup> · mg protein<sup>-1</sup> (18). GPx activity was measured at 25°C in 600 μL of a solution containing 100 mmol/L potassium phosphate buffer, pH 7.7, 1 mmol/L EDTA, 0.4 mmol/L sodium azide, 2 mmol/L glutathione, 0.1 mmol/L NADPH, and 0.62 U glutathione reductase, using tert-butylhydroperoxide as a substrate at 340 nm. Values were expressed as nmol NADPH oxidized · min<sup>-1</sup> · mg protein<sup>-1</sup> (19).

**GS activity.** The enzymatic assay was performed according to Petito and co-workers (20). Briefly, samples were added to a reaction mixture containing 10 mmol/L MgCl<sub>2</sub>; 50 L-glutamate; 100 mmol/L imidazole-HCl buffer (pH 7.4); 10 mmol/L 2-mercaptoethanol; 50 mmol/L hydroxylamine-HCl; and 10 mmol/L ATP and incubated for 15 min at 37°C. The reaction was stopped by the addition of 0.4 mL of a solution containing 370 mmol/L ferric chloride, 670 mmol/L HCl, and 200 mmol/L trichloroacetic acid. After centrifugation (2000 × g; 10 min), the supernatant was measured at 530 nm and compared to the absorbance generated by standard quantities of γ-glutamylhydroxamate (from Sigma) treated with ferric chloride reagent.

**[<sup>3</sup>H]Glutamate uptake in slices.** Coronal slices (0.4 mm) from Cx and Hc, obtained in a McIlwain chopper, were incubated at 37°C in HBSS, pH 7.2 with 100 μmol/L sodium glutamate (containing 0.33 μCi/mL [<sup>3</sup>H] glutamate) (21). Incubation was stopped after 7 min for cortex and 5 min for Hc by 2 ice-cold washes with 1 mL HBSS, immediately followed by addition of 0.5 mol/L NaOH. Sodium-independent uptake was determined using N-methyl-D-glucamine instead of sodium chloride, which was subtracted from the total uptake to obtain the sodium-dependent uptake. The intracellular [<sup>3</sup>H] glutamate content was determined by scintillation counting.

**Protein content.** Protein concentration was determined using the Lowry's method (22), using bovine serum albumin as the standard.

**Statistical analysis.** Results are expressed as mean ± SEM. Data were analyzed statistically by Student's *t* test between malnourished and controls at each age, with the level of significance set at *P* < 0.05. Two-way ANOVA with Duncan's post hoc test was used to characterize the ontogenetic profile of glutathione content in control and malnourished rats.



**Figure 1** Glutathione content in the Cx (A) and Hc (B) of rats exposed to pre- and postnatal PMN and of nourished controls on P2, P15, and P60. Values are means  $\pm$  SEM,  $n = 6-8$ . Two-way ANOVA,  $F_{2,25} = 152.3$ ,  $P < 0.001$ , for age in Cx;  $F_{2,25} = 99.1$ ,  $P < 0.001$ , for age in Hc. An effect of diet was observed only in Cx;  $F_{1,25} = 11.15$ ,  $P = 0.003$ . A significant interaction was observed between age and diet for Cx ( $F_{2,25} = 8.69$ ,  $P = 0.001$ ) but not for Hc ( $F_{2,25} = 1.76$ ,  $P > 0.05$ ). Means without a common letter differ,  $P < 0.001$ .

## Results

The severity of the PMN of the rats was confirmed by their brain weights, which were 18% and 30% less than in controls on P2 and P60, respectively ( $P < 0.05$ ).

An ontogenetic variation in the glutathione content in Cx (Fig. 1A) and Hc (Fig. 1B) occurred in both groups. There was an increase in both brain regions between P2 and P15 ( $P < 0.001$ ). On P60, the glutathione content in Cx fell to 50% of the P15 control values but was still higher than on P2. In Hc, we found a smaller but significant difference between P15 and P60 rats. On P2, the glutathione content in both brain regions was significantly lower in PMN rats than in controls. This difference was maintained at P15 in the Cx but not in the Hc. At P60, the groups did not differ in either brain region.

On P2, TAR was lower in PMN rats than in controls (Table 2), as were the levels of free radicals in the Cx and Hc. Lipid

peroxidation, as indicated by TBARS, did not differ between the groups. The activity of GPx in Cx and Hc was lower in PMN rats than in controls (Table 2). The activities of SOD and CAT were not altered in PMN rats on P2. Interestingly, malnourished rats had a 25% greater activity of GS than controls in both regions, but only on P2 (Table 2). Controls and PMN rats did not differ at later times (data not shown). Also on P2, PMN rats had 15% lower glutamate uptake in the Hc and Cx than controls (Table 2). However, the groups did not differ on P60 (data not shown).

## Discussion

Glutathione is the most abundant low molecular weight thiol involved in antioxidant defense in animal cells. Virtually all cells are able to synthesize glutathione from glutamate, cysteine, and glycine, although hepatocytes are the major producers and exporters of glutathione (1). In the central nervous system, astrocytes play a central role in the metabolism of glutathione (7). Glutathione deficiency contributes to oxidative stress in many brain disorders, including seizure and stroke, as well as in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. PMN, therefore, could affect astrocyte activity and increase the brain vulnerability to oxidative stress.

With regard to the ontogeny of the brain glutathione content in control rats, an intense increase may be observed next to the synaptogenesis peak, which occurs in the postnatal wk 2 (23). Similarly, Nanda and co-workers (24) described an increment in the glutathione in Cx and cerebellum at the beginning of the postnatal wk 2. Although malnourished rats had a similar ontogenetic profile of glutathione content, we found a decrease in glutathione in the first 2 wk in both analyzed brain regions. In Hc, this decrease was observed only in the first wk. This glutathione deficit was accompanied by a reduced TAR and GPx activity, suggesting a lower antioxidant defense in malnourished rats. Moreover, glutathione plays other intracellular roles, including formation of S-nitroso-L-glutathione, cytokine production, cell proliferation, and apoptosis (1).

It is important to emphasize that this glutathione deficit is likely to be due to a prenatal nutritional insult. In fact, a full recovery in glutathione content occurred in these rats, despite the persistent nutritional postnatal insult. The mechanism involved in the glutathione deficit at P2 is unclear at present, but it could involve a down-regulation of the GCS expression

**TABLE 2** Effect of PMN on oxidative markers and glutamate metabolism on P2 in the brain of rats subjected to pre- and postnatal PMN and in controls<sup>1</sup>

	Cx		Hc	
	Control	PMN	Control	PMN
<b>Oxidative markers</b>				
TAR, <i>pmol eq. Trolox/mg protein</i>	121.80 $\pm$ 8.51	88.34 $\pm$ 5.20**	86.79 $\pm$ 8.47	62.40 $\pm$ 5.98*
Free radical content, <i>nmol DCF formed/mg protein</i>	5.33 $\pm$ 0.18	4.01 $\pm$ 0.19**	4.14 $\pm$ 0.09	3.31 $\pm$ 0.18**
Lipid peroxidation, <i>pmol malondialdehyde/mg protein</i>	0.59 $\pm$ 0.04	0.60 $\pm$ 0.04	0.28 $\pm$ 0.02	0.23 $\pm$ 0.01
<b>Antioxidant enzymes</b>				
GPx activity, <i>nmol NADPH/(min·mg protein)</i>	22.50 $\pm$ 2.35	10.21 $\pm$ 1.25**	6.94 $\pm$ 0.71	4.14 $\pm$ 0.64**
SOD activity, <i>U/mg protein</i>	1.41 $\pm$ 0.11	1.49 $\pm$ 0.21	3.36 $\pm$ 0.30	3.66 $\pm$ 0.58
CAT activity, <i>U/mg protein</i>	2.04 $\pm$ 0.22	1.65 $\pm$ 0.19	2.80 $\pm$ 0.42	2.47 $\pm$ 0.45
<b>Glutamate metabolism</b>				
GS activity, <i>µmol/(h·mg protein)</i>	0.13 $\pm$ 0.007	0.18 $\pm$ 0.007**	0.18 $\pm$ 0.120	0.30 $\pm$ 0.043*
Glutamate uptake, <i>nmol/(mg protein·min)</i>	0.66 $\pm$ 0.049	0.51 $\pm$ 0.046*	1.34 $\pm$ 0.057	1.07 $\pm$ 0.092*

<sup>1</sup> Values are means  $\pm$  SEM,  $n = 6-8$ . Asterisks indicate different from control: \* $P < 0.05$ , \*\* $P < 0.01$ .

( $\gamma$ -glutamyl-cysteine synthetase) by protein deficiency or a decrease in GCS activity by NO donors [see (25) for a review].

Our data indicate that PMN induced a deficit in the antioxidant defense but did not cause an increase in free radicals per se. In fact, we found a decrease in free radical levels in all brain structures. Interestingly, it has been reported that PMN is accompanied by a decrease in brain and liver mitochondrial metabolism in brain and liver (26,27), which could result in lower ROS production.

Glutamatergic neurotransmission and glutamate metabolism also undergo dramatic modifications during synaptogenesis due to changes in glutamate receptors (28) and glutamate transporters (29,30). Interestingly, we observed a higher GS activity in malnourished rats on P2. Glutamine is the main source of nitrogen for biosynthesis and has a strong relationship with the intracellular redox status (31). Glutamine, as a substrate, may promote amino acid, nucleotide, and protein synthesis, as well as that of glutathione, via glutamate delivery. For this, a glial phosphate-activated glutaminase is important (32); moreover, glutamine is an amino donor for glucosamine synthesis from fructose 6-P. Both glutamine and glucosamine are able to inhibit the pentose cycle and, therefore, NADPH production in endothelial cells (1). The elevated GS activity could be a compensatory mechanism for glutamate delivery (and, thus, glutathione), because glutamate uptake is reduced in malnourished rats. On the other hand, the resulting glutamine (and glucosamine) may inhibit NADPH production and contribute to the decrease in glutathione levels. However, we are not aware of any published report describing any effect of glutamine (or glucosamine) on NADPH production in astrocytes.

It has been proposed that brain GS works near its maximal capacity under physiological conditions (33). Moreover, GS is developmentally regulated and an increase in its activity is associated with astrocyte differentiation rather than proliferation (34). Thus, the increase observed in malnourished rats is possibly due to a greater amount of the enzyme. In agreement with this, we found that other glial markers of differentiation (glial fibrillary acidic protein and S100B protein) are also increased on P2 in malnourished rats (A. M. Feoli, unpublished results).

Another relevant finding is the reduced glutamate uptake during the postnatal wk 1 in the PMN group. Therefore, with regard to these parameters (GS and glutamate uptake), PMN appears to cause more damage/alteration during the prenatal phase of development. The lower glutamate entry, in turn, could affect cysteine uptake, which is dependent on glutamate efflux, (35) and, therefore, accentuate the decrease in glutathione levels. Note that oxidative stress impairs glutamate uptake and  $\gamma$ -glutamyltranspeptidase activity (2 alternatives to cysteine transport) (36). However, we did not find evidence of elevated free radicals or oxidative damage in samples from malnourished rats, regardless of the low levels of antioxidant defense (glutathione and TAR levels) in these rats.

In summary, the alterations in rat brain due to PMN that occurred during the postnatal wk 1, including the decreases in glutathione content, and in glutamate uptake and the increase in GS, indicate specific changes in astrocyte metabolism and, consequently, a higher vulnerability to excitotoxic/oxidative damage. The decreased antioxidant defense appears to be the main alteration that causes the oxidative imbalance, rather than an increase in ROS. Moreover, a full recovery of the alterations may occur during adulthood, in spite of persistent PMN. Other and sustained alterations cannot be ruled out, however (8).

## Literature Cited

1. Wu G, Haynes TE, Li H, Yan W, Meininger CJ. Glutamine metabolism to glucosamine is necessary for glutamine inhibition of endothelial nitric oxide synthesis. *Biochem J.* 2001;353:245–52.
2. Mates JM, Peres-Gomes C, Nunez de Castro I, Asenio M, Marquez J. Glutamine and its relationship with intracellular redox status, oxidative stress and cell proliferation/death. *Int J Biochem Cell Biol.* 2002;34:439–58.
3. Li J, Wang H, Stoner GD, Bray TM. Dietary supplementation with cysteine prodrugs selectively restores tissue glutathione levels and redox status in protein-malnourished mice (1). *J Nutr Biochem.* 2002;13:625–33.
4. Steiger JL, Alexander MJ, Galler JR, Farb DH, Russek SJ. Effects of prenatal malnutrition on GABAA receptor alpha1, alpha3 and beta2 mRNA levels. *Neuroreport.* 2003;14:1731–5.
5. Rotta LN, Schmidt AP, Mello e Souza T, Nogueira CW, Souza KB, Izquierdo IA, Perry ML, Souza DO. Effects of undernutrition on glutamatergic parameters in rat brain. *Neurochem Res.* 2003;28:1181–6.
6. Araque A, Perea G. Glial modulation of synaptic transmission in culture. *Glia.* 2004;47:241–8.
7. Takuma K, Baba A, Matsuda T. Astrocyte apoptosis: implications for neuroprotection. *Prog Neurobiol.* 2004;72:111–27.
8. Feoli AM, Siqueira IR, Almeida L, Tramontina AC, Vanzella C, Sbaraini S, Schweigert ID, Netto CA, Perry ML, et al. Effects of protein malnutrition on oxidative status in rat brain. *Nutrition.* 2006;22:160–5.
9. Bobyn PJ, Corbett D, Saucier DM, Noyan-Ashraf MH, Juurlink BH, Paterson PG. Protein-energy malnutrition impairs functional outcome in global ischemia. *Exp Neurol.* 2005;196:308–15.
10. Horwitz W. Official methods of analysis of the Association of Official Analytical Chemists (AOAC), Washington, DC, USA, 1980.
11. Schweigert I, de Oliveira DL, Scheibel F, da Costa F, Wofchuk ST, Souza D, Perry MLS. Gestational and postnatal malnutrition affects sensitivity of young rats to picrotoxin and quinolinic acid and uptake of GABA by cortical and hippocampal slices. *Brain Res Dev Brain Res.* 2005;154:177–85.
12. Allen S, Shea JM, Felmet T, Gadra J, Dehn P. A kinetic microassay for glutathione in cells plated on 96-well microtiter plates. *Methods Cell Sci.* 2000;22:305–12.
13. Tietze F. Enzymatic method for quantitative determination of nanogram amount of total oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem.* 1969;27:502–22.
14. Sriram K, Pai KS, Boyd MR, Ravindranath V. Evidence for generation of oxidative stress in brain by MPTP: in vitro and in vivo studies in mice. *Brain Res.* 1997;749:44–52.
15. Bromont C, Marie C, Bralet J. Increased lipid peroxidation in vulnerable brain regions after transient forebrain ischemia in rats. *Stroke.* 1989;20:918–24.
16. Lissi E, Salim-Hanna M, Pascual C, del Castillo MD. Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from luminol-enhanced chemiluminescence measurements. *Free Radic Biol Med.* 1995;18:153–8.
17. Evelson P, Travacio M, Repetto M, Escobar J, Llesuy S, Lissi EA. Evaluation of total reactive antioxidant potential (TRAP) of tissue homogenates and their cytosols. *Arch Biochem Biophys.* 2001;388:261–6.
18. Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121–6.
19. Wendel A. Glutathione peroxidase. *Methods Enzymol.* 1981;77:325–33.
20. Petito CK, Chung MC, Verkhovskiy LM, Cooper AJL. Brain glutamine synthetase increases following cerebral ischemia in the rat. *Brain Res.* 1992;569:275–80.
21. Thomazi AP, Godinho GF, Rodrigues JM, Schwalm FD, Frizzo ME, Moriguchi E, Souza DO, Wofchuk ST. Ontogenetic profile of glutamate uptake in brain structures slices from rats: sensitivity to guanosine. *Mech Ageing Dev.* 2004 Jul;125:475–81.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem.* 1951;193:265–75.
23. Morgane PJ, Mokler DJ, Galler JR. Effects of prenatal protein malnutrition on the hippocampal formation. *Neurosci Biobehav Rev.* 2002;26:471–83.

24. Nanda D, Tolputt J, Collard KJ. Changes in brain glutathione levels during postnatal development in the rat. *Brain Res Dev Brain Res.* 1996;94:238–41.
25. Lu SC. Regulation of glutathione synthesis. *Curr Top Cell Regul.* 2000;36:95–116.
26. Olorunsogo OO. Changes in brain mitochondrial bioenergetics in protein-deficient rats. *Br J Exp Pathol.* 1989;70:607–19.
27. Olorunsogo OO. Defective mitochondrial cation transport during dietary protein deficiency in rats. *Afr J Med Med Sci.* 1992;21:23–31.
28. Lujan R, Shigemoto R, Lopez-Bendito G. Glutamate and GABA receptor signalling in the developing brain. *Neuroscience.* 2005;130:567–80.
29. Furuta A, Rothstein JD, Martin LJ. Glutamate transporter protein subtypes are expressed differentially during rat CNS development. *J Neurosci.* 1997;17:8363–75.
30. Danbolt NC. Glutamate uptake. *Prog Neurobiol.* 2001;65:1–105.
31. Mates JM, Perez-Gomez C, Nunez de Castro I, Asenjo M, Marquez J. Glutamine and its relationship with intracellular redox status, oxidative stress and cell proliferation/death. *Int J Biochem Cell Biol.* 2002;34:439–58.
32. Olalla L, Aledo JC, Bannenberg G, Marquez J. The C-terminus of human glutaminase L mediates association with PDZ domain-containing proteins. *FEBS Lett.* 2001;488:116–22.
33. Felipe V, Butterworth RF. Neurobiology of ammonia. *Prog Neurobiol.* 2002;67:259–79.
34. Patel AJ, Hunt A, Gordon RD, Balazs R. The activities in different neural cell types of certain enzymes associated with the metabolic compartmentation glutamate. *Brain Res.* 1982;256:3–11.
35. McBean GJ, Flynn J. Molecular mechanisms of cystine transport. *Biochem Soc Trans.* 2001;29:717–22.
36. Trotti D, Rizzini BL, Rossi D, Haugeto O, Racagni G, Danbolt NC, Volterra A. Neuronal and glial glutamate transporters possess a SH-based redox regulatory mechanism. *Eur J Neurosci.* 1997;9:1236–43.