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ORIGINAL ARTICLE

Neonatal taurine and alanine modulate anxiety-like behavior and decelerate cortical spreading depression in rats previously suckled under different litter sizes

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Abstract The amino acids taurine and alanine play a role in several physiological processes, including behavior and the electrical activity of the brain. In this study, we investigated the effect of treatment with taurine or alanine on anxiety-like behavior and the excitability-dependent phenomenon known as cortical spreading depression (CSD), using rats suckled in litters with 9 and 15 pups (groups L_9 and L_{15}). From postnatal days 7 to 27, the animals received per gavage 300 mg/kg/day of taurine or alanine or both. At 28 days, we tested the animals in the elevated plus maze, and at 33–35 days, we recorded CSD and analyzed its velocity of propagation, amplitude, and duration. Compared with water-treated controls, the L_9 groups treated with taurine or alanine displayed anxiolytic behavior (higher number of entries in the open arms; $p < 0.05$), and reduced CSD velocity ($p < 0.001$). The effect of both amino acids on CSD was also found in the L_{15} groups and in five additional L_9 groups (naïve, water, taurine, alanine, or both) treated at adulthood (90–110 days). The L_{15} condition resulted in smaller durations and higher CSD velocities compared with the L_9 condition. Besides reinforcing previous evidence of behavioral modulation by taurine and alanine, our data are the first confirmation that treatment with these amino acids decelerates CSD regardless of lactation conditions (normal versus unfavorable lactation) or age at amino acid administration (young versus adult). The results suggest a modulating role for both amino acids on anxiety behavior and neuronal electrical activity.

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Introduction

The amino acids taurine and alanine are abundant in mammals, and experimental evidence points to a role for both amino acids in several physiological processes in the nervous system (Murakami and Furuse 2010; Wu et al. 2005; Rodríguez-Navarro et al. 2009; Wu and Prentice 2010; Santos-Silva et al. 2015; Schousboe et al. 2003). Taurine is involved in neuronal proliferation and differentiation (Chen et al. 1998), regulation of osmotic pressure and neuromodulation processes, and inhibitory neurotransmission (Wu et al. 2005; Rodríguez-Navarro et al. 2009; Wu and Prentice 2010). Supplementation with taurine is important because it operates as a scavenger of free radicals (Oliveira et al. 2010), is neuroprotective against glutamate-induced excitotoxicity (Pan et al. 2010) and ethanol-induced apoptosis (Taranukhin et al. 2010), and is cytoprotective (Wang et al. 2007). Taurine also improves glucose homeostasis in genetic obese animals (Santos-Silva et al. 2015).

Alanine is a simple amino acid that is involved in molecular biosynthesis in the central nervous system (CNS) (Westergaard et al. 1993). It can alleviate damage induced by oxidative stress (Estacion et al. 2003) and is cytoprotective (Dadsetan et al. 2013). In mammals, including humans, alanine participates in the metabolic pathways of glycolysis, gluconeogenesis, and the tricarboxylic acid cycle (Schousboe et al. 2003). It serves as the precursor of several substances, including glutamate, glutamine (Waagepetersen et al. 2000), β-alanine (Tiedje et al. 2010), and γ-aminobutyric acid (GABA) (Schousboe et al. 2003).

These substances share a close relationship in the CNS, participating in the fine and meticulous balance between excitatory and inhibitory mechanisms within the brain (Schousboe et al. 2003).

Cortical spreading depression (CSD) is a brain excitability-dependent, electrophysiological phenomenon, first described as a reduction in the spontaneous and evoked electrical activity of the cerebral cortex in response to a mechanical, electrical, or chemical stimulation of one point of the cortex (Leão 1944). It has been demonstrated in many animal species, including humans (Dohmen et al. 2008; Fabricius et al. 2008). CSD represents a transient neuronal depolarization accompanied by a negative shift in the DC (direct current) potential and a depression on electroencephalography. The phenomenon has been linked to brain excitability disorders and their diseases such as migraine with aura (Ferrari et al. 2015), multiple sclerosis (Pusic et al. 2015), and epilepsy (Fabricius et al. 2008; Wei et al. 2014). Thus, CSD is an interesting and useful experimental model for evaluating the proper functioning of neural tissue (Guedes 2011; Miura et al. 2013; Petrusic and Zidverc-Trajkovic 2014).

Our group previously described in rats the CSD effects of administration of other amino acids early in life (Frazão et al. 2008; Lima et al. 2009; Maia et al. 2009), as well as in adulthood (Trindade-Filho et al. 2009). In addition, our group has investigated the CSD effects under conditions of nutritional alterations occurring early in life (Guedes 2011). Proper nutrition during early life stages is crucial for brain development in humans and other mammals because at this time point, developmental processes such as hyperplasia, hypertrophy, myelination, and organization of synapses occur rapidly. The brain is thus more vulnerable to environmental challenges, including unfavorable lactation conditions (Morgane et al. 1978; Rocha-de-Melo et al. 2006). In the rat, increasing the number of pups to be suckled by one dam is an easy way of creating unfavorable lactation conditions (Alamy and Bengelloun 2012). Litters with an increased number of pups enhance the demand for the dam's milk, which can result in a moderate state of malnutrition that can affect the animal's behavior and the electrical activity of the brain.

This study aimed to investigate the possible action of taurine and alanine on anxiety-like behavior and CSD. Considering that there is no information in the literature about the influence of neonatal taurine and alanine administration on the CSD features, we decided to characterize it in the cerebral cortex of young rats, suckled under normal and unfavorable conditions (respectively, litters with 9 and 15 pups). Additionally, in these young rats, we measured the blood glucose levels. We also compared amino acid effects on CSD in groups treated early in life (young groups) and in adulthood (adult groups). Our hypotheses were that treatment with taurine and alanine would modulate behavior and CSD propagation and that the unfavorable lactation condition would modify the amino acid effects.

Materials and methods

Animals

All experimental procedures were previously approved by the Institutional Ethics Committee for Animal Research of our University (Approval protocol no. 23076.021609/2013-67), whose norms comply with those established by the National Institutes of Health Guide for Care and Use of Laboratory Animals (Bethesda, MD, USA).

Newborn male Wistar rats, born from distinct dams, were pooled and assigned to be suckled under normal or unfavorable conditions, represented, respectively, by litters with 9 pups (L_9 groups; $n = 97$) and litters with 15 pups (L_{15} groups; $n = 52$).

After weaning (postnatal day 25), the pups had free access to water and the same commercial lab chow, with 23 % protein, that was offered to their dams (Purina Ltd). They were housed in polyethylene cages (51 cm × 35.5 cm × 18.5 cm) under controlled temperature at 22 ± 1 °C with a 12-h light:12-h dark cycle (lights on at 6:00 a.m.).

Taurine and alanine treatment early in life (young groups)

From postnatal days 7 to 27, L_9 and L_{15} pups received per gavage taurine and/or L-alanine (purchased from Sigma, St. Louis, MO, USA), as follows: only taurine (300 mg/kg/day; 11 L_9 and 11 L_{15} pups); only alanine (300 mg/kg/day; 11 L_9 and 11 L_{15} pups); and taurine plus alanine (300 mg/kg/day each; 10 L_9 and 10 L_{15} pups). Amino acid doses were based on the literature (Liu et al. 2012). Two additional control groups received no gavage (naïve group; 11 L_9 and 10 L_{15} pups) or vehicle (distilled water; 11 L_9 and 10 L_{15} pups). The initial volume administered per gavage was 0.5 mL/day in the second and third weeks of life; it was increased to 1.0 mL/day in the fourth week of life, as previously described (Lima et al. 2009). We measured body weight at postnatal days 7, 14, 21, 28, and 33–35 (when we performed the electrophysiological recordings).

Taurine and alanine treatment at adulthood (adult groups)

From postnatal days 90 to 110, an additional set of five groups of L_9 rats received 300 mg/kg/day taurine ($n = 9$), 300 mg/kg/day L-alanine ($n = 9$), taurine plus alanine

($n = 8$), vehicle ($n = 8$), or no treatment (naïve condition; $n = 9$). We compared these groups with the five corresponding L_9 groups treated early in life. In these adult groups, the volume administered per gavage was 1.0 mL/100 g body weight/day (Liu et al. 2012). We measured body weight on the day of the electrophysiological recording (116–120 days of life).

For treated young and adult groups, amino acids were dissolved in distilled water immediately before administration. The gavage occurred between 12 a.m. and 2 p.m.

Elevated plus-maze test

We conducted the elevated plus-maze test on the 28th postnatal day. The cross-shaped elevated plus-maze apparatus consisted of four arms (two closed arms and two open arms (each measuring 49 cm long × 10 cm wide) raised 55 cm above the floor). A central squared platform (10 × 10 cm wide) connected the open and closed arms. For each 5-min session, under dim light and in a sound-attenuated room, we initially placed the rat in the central platform facing an open arm. A video camera recorded the behavioral activity of the animal. The recorded activity was stored in a computer and subsequently analyzed with the software ANY-maze (version 4.99 m). After each test, we cleaned the arms and the central platform with a 70:30 ethanol:water solution. The following parameters were analyzed: total distance traveled, duration of immobility, number of entries into the open arms, and time spent in the open arms. We considered that the animal entered one open or one closed arm when its four paws entered the arm.

Analysis of blood glucose

After the behavioral test, the animals were fasted for 6 h. A drop of blood was collected from the animal's tail and used for measuring the blood glucose level employing a portable glucose meter (G-TECH free).

CSD recording

On the day of the electrophysiological recording, each animal was anesthetized with a mixture of 1 g/kg urethane plus 40 mg/kg chloralose injected intraperitoneally. Three trephine holes were drilled on the right side of the skull, aligned in the frontal-to-occipital direction and parallel to the midline. One hole was positioned on the frontal bone (2 mm in diameter) and used to apply the stimulus (KCl) to elicit CSD. The other two holes were positioned on the parietal bone (3–4 mm in diameter) and used to record the propagating CSD wave. CSD was elicited at 20-min intervals by a 1-min application of a cotton ball (1–2 mm in diameter) soaked with 2 % KCl solution (approximately 270 mM) to the anterior hole drilled at the frontal region. Rectal temperature was continuously monitored and maintained at 37 ± 1 °C by means of a heating blanket. The DC slow potential change accompanying CSD was recorded for 4 h using two Ag–AgCl agar–Ringer electrodes (one in each hole) against a common reference electrode of the same type, placed on the nasal bones. We calculated the CSD velocity of propagation from the time required for a CSD wave to pass the distance between the two cortical electrodes. In the two cortical recording places, we used the initial point of each DC-negative rising phase as the reference point to calculate the CSD velocities. In addition, we calculated amplitude and duration of the CSD waves, as previously reported (Mendes-da-Silva et al. 2014).

Statistics

Results in all groups are expressed as means ± standard deviations. Body weights, anxiety-like behavioral activity and CSD propagation rates were compared between groups using ANOVA, including as factors lactation conditions (L_9 and L_{15}), gavage treatment (naïve, water, taurine, alanine, both), and ages (young and adult L_9 condition) followed by a post hoc test (Holm–Sidak), where indicated. A p value less than 0.05 was considered significant.

Results

Body weight and blood glucose level

In the five young groups, ANOVA showed a main effect of the lactation condition for body weight during the whole suckling period ($p < 0.001$). The L_{15} animals presented with lower ($p < 0.001$) body weights compared with the L_9 groups. The weight reduction ranged from 20.1 to 36.5 % and was independent of the gavage treatment. In the animals treated in adulthood, the mean body weights (in g) were 381.5 ± 45.6 for the naïve group ($n = 9$), 367.8 ± 24.3 for the water group ($n = 8$), 391.0 ± 20.8 for the taurine group ($n = 9$), 383.7 ± 20.3 for the alanine group ($n = 9$), and 331.5 ± 21.9 for the taurine plus alanine group ($n = 9$).

In the L_{15} groups, glycemia was significantly lower (mean values ranged from 85.8 ± 9.6 to 98.5 ± 7.0 mg/dL) than the corresponding L_9 groups (mean values ranged from 104.6 ± 10.6 to 117.9 ± 9.9 mg/dL; $p < 0.05$). The L_9 and L_{15} groups treated with taurine plus alanine displayed lower glycemia compared with the corresponding groups treated with alanine only.

Data on body weights and on glycemia are illustrated in Fig. 1.

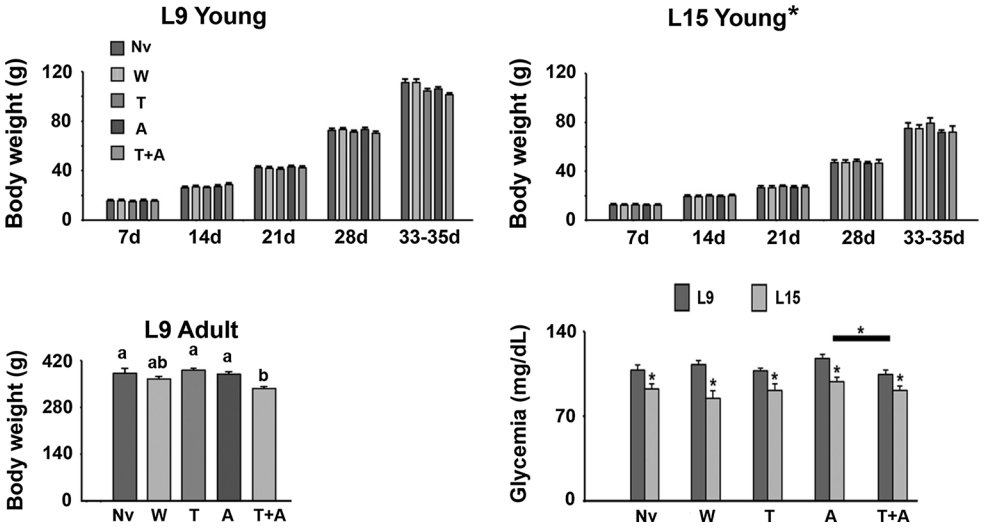

Fig. 1 Body weight of young (*upper panels*) and adult rats (*left lower panel*), and glycemia of young rats (*right lower panel*). Rats were previously suckled in litters with 9 and 15 pups (respectively, L_9 and L_{15} condition). Data are expressed as mean ± SEM for 48 L_9 and 45 L_{15} young rats and 35 L_9 adult rats. *Asterisks* indicate significant differences between L_{15} and the corresponding L_9 groups ($p < 0.05$; one-way ANOVA followed by the Holm–Sidak test)

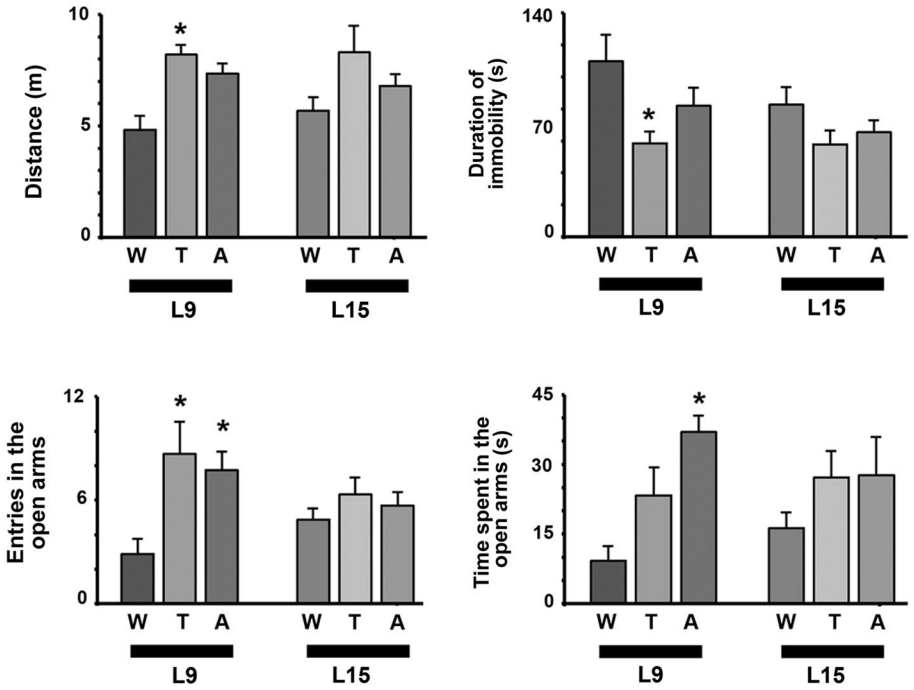

Fig. 2 Behavioral activity in the elevated plus maze of young rats that were previously suckled in litters with 9 and 15 pups (respectively L_9 and L_{15} condition). *W*, *T*, and *A* are groups treated with water, taurine, and alanine, respectively. *Bars* represent mean values ± standard error of the mean for 5 to 9 rats per group. *Asterisks* indicate values that are significantly different from the W-control groups ($p < 0.001$; ANOVA followed by the Holm–Sidak test)

Behavioral activity in the elevated plus maze

The effect of administration of taurine or alanine on the anxiety-like behavioral activity in the elevated plus-maze test is shown in Fig. 2.

Compared with the water-treated controls, the L_9 group treated with taurine traveled a greater distance in the maze (8.21 ± 1.13 versus 4.82 ± 1.81 m), displayed a shorter immobility time (58.3 ± 19.6 versus 109.7 ± 47.1 s) and entered a higher number of times in the open arms (8.7 ± 4.5 versus 2.9 ± 2.4) ($p < 0.05$). The L_9 group treated with alanine displayed higher number of entries in the open arms (7.8 ± 3.0) and spent a longer time in the open arms (37.0 ± 8.6 versus 9.3 ± 7.5 s) ($p < 0.05$).

CSD velocity of propagation

In all groups, stimulation with 2 % KCl (approximately 270 mM) at one point of the frontal cortical surface for 1 min elicited, as a rule, a single CSD wave that propagated without interruption and was recorded at the two parietal recording points (Fig. 3; see the recording points in the skull diagrams). During the 4-h recording, the appearance

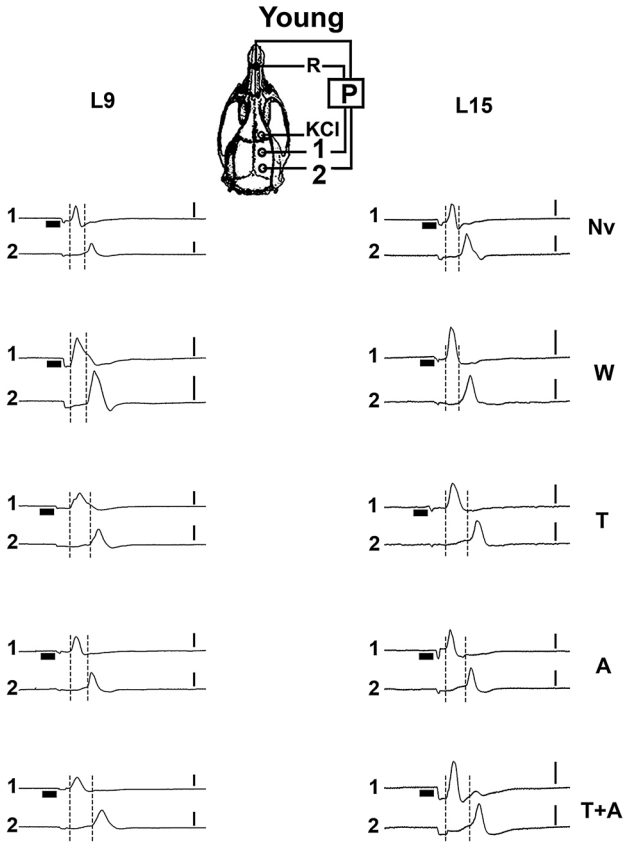

Fig. 3 Recordings of the slow potential changes (*P*) during cortical spreading depression (CSD) at two cortical points (1 and 2) in 10 young rats (5 L_9 rats and 5 L_{15} rats). The diagram of the skull shows recording positions 1 and 2 from which the traces marked at the *left* with the same numbers were obtained. The position of the common reference electrode (*R*) on the nasal bones and the application point of the CSD-eliciting stimulus (KCl) are also shown. *Nv* naïve group; *W*, *T*, *A*, and *T+A* are rats treated per gavage with water, taurine, alanine, and taurine plus alanine, respectively. The vertical bars indicate 10 mV for *P* (negative upwards). CSD was elicited in the frontal cortex by chemical stimulation (a 1- to 2-mm diameter cotton ball soaked with 2 % KCl) applied for 1 min on the intact dura mater, as indicated by the *horizontal bars*. The *vertical dashed lines* indicate the latency for a CSD wave to cross the inter-electrode distance. The latencies were shorter in the L_{15} groups compared with the corresponding L_9 groups. The latencies were larger in the groups treated with *T*, *A*, and *T+A* under both lactation conditions when compared with the respective *Nv* and *W* controls

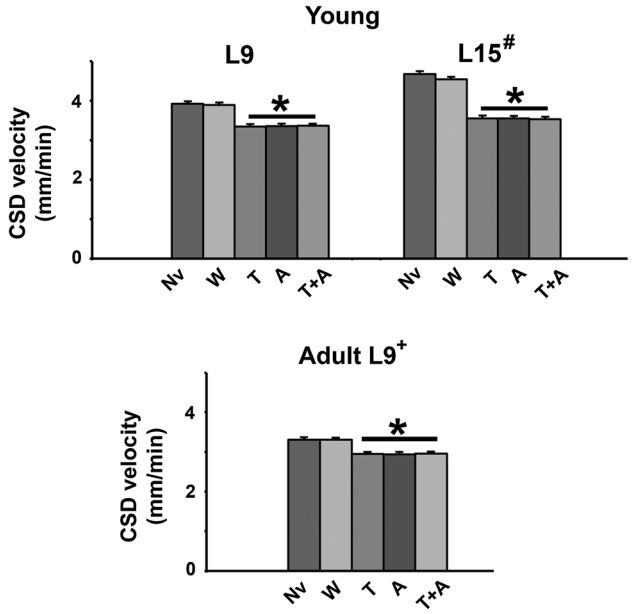

Fig. 4 CSD velocity of young rats (33- to 35-day old; *upper panel*) and adult rats (116- to 120-day old; *lower panel*). At the young age, rats were suckled in two distinct lactation conditions represented by litters with 9 and 15 pups (respectively, L_9 and L_{15} groups). The adult rats were studied only in the L_9 condition. *Nv* naïve group; *W*, *T*, *A*, and *T+A* are rats treated per gavage with water, taurine, alanine, and taurine+alanine, respectively. Data are mean ± SEM. *Asterisks* indicate values that are significantly different from the Nv- and W-control groups ($p < 0.001$; ANOVA followed by the Holm–Sidak test). *Ash* indicates all L_{15} groups that were significantly different from the corresponding L_9 groups ($p < 0.01$). *Plus* denotes age difference (adult < young; $p < 0.01$)

of the slow potential change confirmed the presence of CSD after KCl stimulation.

In the young rats, ANOVA indicated intergroup differences, and post hoc (Holm–Sidak) test comparisons showed that the velocities were higher ($p < 0.001$) in the L_{15} groups compared to the L_9 groups. Concerning gavage treatment, ANOVA detected a main effect, and post hoc testing revealed that treatment with taurine, alanine, or both significantly lowered the CSD propagation velocities ($p < 0.001$) compared with the naïve and water controls. In the early-treated groups, the amino acid decelerating effect on CSD propagation was more intense in L_{15} than L_9 rats ($p < 0.001$), indicating an interaction between the treatment with taurine and/or alanine and the lactation condition. In the L_9 young animals, the CSD velocities (mean ± SD in mm/min) in the naïve and water controls were, respectively, 3.86 ± 0.14 and 3.86 ± 0.07. In groups treated with taurine, alanine, or both, the CSD velocity was significantly lower (respectively, 3.34 ± 0.15, 3.36 ± 0.12, and 3.36 ± 0.09; $p < 0.001$). In L_{15} young animals, the CSD velocities in the control groups were higher than in the L_9 condition (4.68 ± 0.21 and 4.54 ± 0.13 for the naïve and water groups, respectively). Treatment with taurine, alanine, or both resulted in lower CSD velocity compared with L_{15} controls (3.56 ± 0.25 for the taurine group, 3.56 ± 0.10 for the alanine group, and 3.53 ± 0.17 for the group treated with both; $p < 0.001$). Data on CSD velocity in young animals are shown in the upper panel of Fig. 4.

The lower panel of Fig. 4 shows the CSD velocity of the groups treated in adulthood. Experiments confirmed the CSD effects of taurine, alanine, or both amino acids observed in the young groups. In the adult rats,

the velocities were 3.31 ± 0.07 for the naïve condition, 3.32 ± 0.07 for the water group, 2.95 ± 0.11 for the taurine-treated animals, 2.94 ± 0.15 for the alanine group, and 2.99 ± 0.08 for the taurine plus alanine group.

Amplitude and duration of CSD waves

Tables 1 and 2, respectively, show data on amplitude and duration of the negative slow potential change, the hallmark of CSD. The mean amplitude varied from 9.20 ± 1.74 to 12.66 ± 4.53 mV in the L_9 groups and from 10.70 ± 3.12 to 14.03 ± 2.87 mV in the L_{15} groups (Table 1), and no intergroup differences were observed. The mean duration varied from 67.71 ± 2.98 to 71.30 ± 2.61 s in the L_9 groups and from 67.27 ± 1.06 to 69.12 ± 1.53 s in the L_{15} groups (Table 2). ANOVA revealed shorter durations in L_{15} compared with L_9 rats.

Table 1 Amplitude of the slow potential change of cortical spreading depression in young (33–35 days of life) and adult rats (116–120 days)

Treatment groups	Amplitude (mV)		
	L_9—young	L_{15}—young	L_9—adult
Nv	11.07 ± 2.56 (11)	13.20 ± 3.26 (7)	10.47 ± 3.15 (7)
W	11.11 ± 4.49 (11)	14.03 ± 2.87 (8)	9,41 ± 1.94 (7)
T	12.12 ± 3.71 (11)	12.28 ± 3.62 (7)	10.66 ± 3.73 (8)
A	12.45 ± 4.42 (11)	12.79 ± 3.57 (8)	9.64 ± 2.30 (8)
T+A	12.66 ± 4.53 (10)	10.70 ± 3.12 (10)	9.20 ± 1.74 (8)

Treatments were per gavage

L_9 and L_{15} are groups previously suckled under normal or unfavorable lactation conditions (respectively, in litters with 9 and 15 pups). Data are expressed as mean ± standard deviation, with the number of animals in parentheses

No intergroup significant differences were observed

Nv naïve (no treatment), *W* receiving distilled water, *T* received 300 mg/kg/day of taurine, *A* received 300 mg/kg/day of alanine, *T+A* received 300 mg/kg/day of taurine+300 mg/kg/day of Alanine

Table 2 Duration of the negative slow potential change of the cortical spreading depression in young (33–35 days of life) and adult rats (116–120 days)

Treatment groups	Duration (s)		
	L_9—young	L_{15}—young	L_9—adult
Nv	69.73 ± 4.68 (9)	66.92 ± 2.45 (8)*	68.10 ± 2.23 (8)
W	71.01 ± 4.63 (8)	69.01 ± 1.63 (7)*	69.76 ± 4.03 (7)
T	69.43 ± 5.63 (10)	68.38 ± 2.46 (9)*	69.56 ± 3.59 (7)
A	71,30 ± 2.61 (8)	69.12 ± 1.53 (7)*	69.62 ± 6.64 (8)
T+A	70.07 ± 2.73 (7)	67.27 ± 1.06 (7)*	69.83 ± 1.16 (8)

Treatments were per gavage

L_9 and L_{15} are groups previously suckled under normal or unfavorable lactation conditions (respectively, in litters with 9 and 15 pups). Data are expressed as mean ± standard deviation, with the number of animals in parentheses

The asterisk indicates that the L_{15} values are significantly lower than the corresponding L_9 values ($p < 0.007$)

Nv naïve (no treatment), *W* receiving distilled water, *T* received 300 mg/kg/day of taurine, *A* received 300 mg/kg/day of alanine, *T+A* received 300 mg/kg/day of taurine+300 mg/kg/day of Alanine

Discussion

Our data clearly demonstrate that chronic treatment with taurine and alanine produces anxiolytic-like behavior (higher number of entries in the open arms in the elevated plus-maze) and reduces the propagation velocity of CSD. It is possible that these amino acids act as an anti-anxiety agent at the central level in the nervous system, as postulated (Murakami and Furuse 2010; Yu et al. 2015). The effect on CSD occurred regardless of lactation conditions (normal versus unfavorable lactation) or age at amino acid treatment (young versus adult). The stress of the gavage procedure cannot be the cause of the CSD deceleration because the groups that received distilled water by gavage showed a CSD propagation velocity similar to the naïve groups that received no gavage. These results reinforce the importance of these amino acids for the proper electrophysiological functioning of the brain and suggest that chronic treatment with taurine and/or alanine can modulate, at least in part, the brain's ability to propagate CSD. Interestingly, no additive effect was observed in the group treated simultaneously with both amino acids, suggesting that this type of cooperation is not important for the action of both amino acids on the CSD; indeed, a lack of additive effects has been already reported for action potentials in cerebellar slices (Okamoto and Quastel 1976).

Blood glucose levels, body weights, and anxiety-like reactions were lower in the L_{15} groups compared with the corresponding L_9 groups, confirming the effectiveness of increasing litter size in producing malnutrition, which impairs behavior (Hernandes et al. 2005; Belluscio et al. 2014). Although in this study we did not monitor the blood and brain levels of amino acids, based on evidence in the literature, we consider it reasonable to assume that chronic treatment with taurine and/or alanine might have caused an imbalance in some cerebral areas in the concentrations of these and other amino acids. Specifically, the chronic (Murakami and Furuse 2010) and acute administration of taurine (Molchanova et al. 2007) results in an increased concentration of taurine and other amino acids in distinct regions of the rat brain. Notably in humans, continuous L-alanine/L-glutamine infusion (0.75 g/kg/day up to 5 days) significantly increases levels of both glutamine and alanine

in the plasma and brain of patients with severe traumatic brain injury (Nägeli et al. 2014).

Taurine affects the distribution of other amino acids in the brain, acts on GABA receptors, and modulates synaptic transmission (Wu and Prentice 2010). All of these actions can affect CSD features (e.g., Miura et al. 2013). However, based on our findings and with support from the literature, we can speculate about two types of mechanisms that deserve particular attention: direct mechanisms based on primary actions of the amino acids and indirect mechanisms by which taurine and alanine act via a secondary metabolic pathway.

Concerning the direct mechanisms, one possibility is that the amino acids counteract oxidative stress in the brain. It is important to note that taurine and alanine can protect the CNS from damage caused by oxidative stress in a variety of conditions (Li et al. 2012; Rosemberg et al. 2010; Sinha et al. 2008; Grosser et al. 2004). These findings collectively lead us to consider a possible role for taurine and alanine in the effective elimination of free radicals, aiding in promoting an oxidative balance in the organism.

It is interesting to note also that increasing reactive oxygen species levels in the CNS can elicit CSD both in vitro (Netto and Martins-Ferreira 1989) and in vivo (El-Bachá et al. 1998). Our group has studied the CSD effects of other antioxidant molecules such as astaxanthin (Abadie-Guedes et al. 2008) and ascorbic acid (Mendes-da-Silva et al. 2014). Thus, we suggest that taurine and alanine might have contributed to a reduction in the speed of propagation of CSD because of their possible role as antioxidant molecules in the CNS. However, further investigation is needed to clarify the role of taurine and alanine as possible antioxidant molecules in biological systems, including their action via indirect mechanisms.

Concerning indirect mechanisms, both in vitro and in vivo evidence demonstrates the action of taurine as a regulator of neuronal excitability and activity by interacting with glycinergic and GABAergic receptors (Okamoto et al. 1983; Wu and Prentice 2010; Song et al. 2012). Alanine appears to act differentially on glutamatergic and GABAergic neurons (Schousboe et al. 2003). Although the enzymatic activity of alanine aminotransferase is relatively modest in glutamatergic neurons (Westergaard et al. 1993), GABAergic neurons express the entire enzymatic machinery necessary to perform the metabolic reaction constituting the GABA shunt. In an operating GABA shunt, alanine and glutamate can be used as metabolic fuel for GABA synthesis; alanine donates the amino group to α-ketoglutarate, yielding L-glutamic acid, which undergoes decarboxylation by glutamic acid decarboxylase, resulting in GABA (Schousboe et al. 2003). Therefore, we suggest that chronic treatment with alanine reduces excitability by increasing synthesis of GABA.

Nutritional inadequacy remains a major non-genetic factor that affects the developing brain (Alamy and Bengelloun 2012). Nutrient intake in insufficient quantity and/or quality can disrupt the biochemical and morphological organization of the brain (Morgane et al. 2002) and accelerate CSD propagation (Guedes 2011; Mendes-da-Silva et al. 2014). Our L_{15} CSD data confirm the facilitating action of early malnutrition. Concerning the mechanisms by which malnutrition facilitates CSD propagation, we know that a larger extracellular space volume in the brain hinders CSD elicitation and propagation (Mazel et al. 2002). Inadequate nutrient intake early in life increases cell-packing density and reduces the extracellular space, leading to facilitation of CSD. Another important factor that modulates CSD propagation is cortical myelination. Previous work has demonstrated an inverse correlation between the degree of cortical myelination and CSD propagation velocity (Merkler et al. 2009). Nutritional deficiency reduces brain myelination and increases CSD propagation velocity (De Luca et al. 1977; Rocha-de-Melo et al. 2006). Furthermore, malnutrition impairs glial function (Morgane et al. 1978), and this glial impairment facilitates CSD (Largo et al. 1997). In addition, malnourished rats present increased levels of the enzyme glutamic acid decarboxylase (Díaz-Cintra et al. 2007). This condition, in association with reduced brain glutamate uptake (Feoli et al. 2006), enhances extracellular glutamate in the malnourished brain, which might contribute to CSD facilitation.

Our findings also suggest that unfavorable lactation itself (L_{15} condition) can modulate the action of taurine and alanine on CSD, impairing its velocity of propagation more intensely than in the L_9 condition. This kind of malnutrition-associated CSD modulation has been previously demonstrated for the convulsant drug pilocarpine (Vasconcelos et al. 2004). We found that the L_{15} condition enhanced the effects of taurine and alanine on CSD. Taken together, these data suggest different responsiveness of the CSD to distinct classes of compounds. If this inference could be confirmed in the human brain concerning the neural action of therapeutic drugs, then the effectiveness of such drugs might possibly oscillate as a function of the early nutritional status of the patient, an idea that warrants further investigation.

The CSD propagation velocity decreases with age (Guedes et al. 1996), indicating that the aging process is related to the enhancement of the brain resistance to CSD (Batista-de-Oliveira et al. 2012), which the present study confirms. In comparison to young animals, our adult rats presented lower velocities of propagation of CSD. Although several mechanisms could explain these findings, we consider that oxidative stress and age-related impairment of cerebral blood flow are more likely involved, as previously discussed (Batista-de-Oliveira et al. 2012).

In conclusion, this study supported the hypothesis of a beneficial action of taurine and alanine on brain processes related to anxiety-like behavior in rats. Also, this study describes a novel effect of the amino acids taurine and alanine on the excitability-related CSD phenomenon. The in vivo CSD data support four conclusions. First, amino acid administration at either young or adult ages decelerates CSD. Second, the effects of taurine and alanine are not additive. Third, increasing litter size accelerates CSD and increasing age decelerates it, confirming previous studies. Fourth, in young rats, an unfavorable lactation condition intensifies the amino acid effects on CSD. The present data might advance understanding of the relationship among amino acids, anxiety-like behavior, CSD, neuronal excitability, and nutrition in the brain.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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