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ORIGINAL ARTICLE

Behavioral and electrophysiological brain effects of aspartame on well-nourished and malnourished rats

Paula Catirina Germano Magalhães[1] · Ricardo Abadie-Guedes[2] · Manoel Augusto Barbosa da Costa Mendonça[1] · Aline Duarte de Souza[1] · Rubem Carlos Araújo Guedes[1]

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Abstract

The non-caloric sweetener aspartame can be potentially harmful to the developing brain, as some studies suggest an association between aspartame intake and adverse neural effects. This study aimed to evaluate the possible effects of aspartame, with or without associated early nutritional deficiency, on behavioral parameters suggestive of anxiety and electrophysiological features of the excitability-related phenomenon known as cortical spreading depression (CSD). Newborn Wistar rats ($n = 80$) were suckled under favorable (L_9; $n = 40$) or unfavorable lactation conditions (L_{15}; $n = 40$), consisting of litters with 9 or 15 pups, respectively. In each lactation condition, animals were divided into 4 groups that received per gavage, from postnatal day 8 to 28, 75 mg/kg/d or 125 mg/kg/d aspartame (groups ASP75 and ASP125), or water (vehicle group), or no treatment (naive group). Behavioral tests (elevated plus-maze [EPM]) were performed at postnatal days 86–95 and CSD was recorded between postnatal days 96–115. Compared to the control groups, aspartame dose-dependently reduced body weight, suggesting a negative impact on animal development; aspartame also caused behavioral changes suggestive of anxiety (shorter stay in the open arms in the EPM) and decelerated CSD (lower propagation speed). Some of these parameters were more affected in L_{15} animals, suggesting an interaction among aspartame and lactation condition. We concluded that early consumption of aspartame adversely affects development of the organism (weight loss), with actions on behavioral (anxiety-like) and cerebral electrophysiological (CSD) parameters. The data suggest caution in aspartame consumption by lactating mothers and their infants.

Keywords Aspartame · Anxiety · Brain excitability · Lactation conditions · Nervous system · Spreading depression

Introduction

Aspartame (L-aspartyl-L-phenylalanine methyl ester) has been used in food products as an alternative to sugar. Currently, the sweetener can be found in soft drinks, powdered drinks, chewing gums, gelatins, dessert mixes, puddings and fillings, frozen desserts, yogurt, tabletop sweeteners, and some pharmaceutical products such as vitamins and cough tablets (Aspartame Information Center 2005). Some authors have postulated that the use of aspartame could be associated with various neural disturbances such as those involved in Parkinson's disease (see the review by Humphries et al. 2008), panic attacks (Drake 1986), behavior impairment (Christian et al. 2004) and brain-excitability-related diseases, such as migraines (Newman and Lipton 2001) and convulsions (Camfield et al. 1992). Aspartame can reduce extracellular dopamine levels (Bergstrom et al. 2007), a condition that is associated with Parkinson's disease and schizophrenia. In view of these findings, some researchers consider the consumption of aspartame by developing organisms as a matter of great concern (von Poser Toigo et al. 2015). In animals, aspartame influences behavioral responses such as learning behavior (Potts et al. 1980) and anxiety-like responses (Ashok et al. 2015), although some conflicting results are in the literature (Ashok and Sheeladevi 2014). In rats, the developing brain is substantially vulnerable to external challenges such as nutritional deficiency and pharmacological agents (Amaral et al. 2009).

Malnutrition affects a considerable portion of the world population that has no access to adequate food for the growth and development of physiological systems (see Morgane et al.

✉ Rubem Carlos Araújo Guedes
guedes.rca@gmail.com; rguedes@ufpe.br

[1] Departamento de Nutrição, Universidade Federal de Pernambuco, Recife, PE 50670-901, Brazil

[2] Departamento de Fisiologia e Farmacologia, Universidade Federal de Pernambuco, Recife, PE 50670-901, Brazil

1993 for a review). However, the chronic effects of aspartame on the malnourished and developing brain have not been well investigated in terms of behavioral and electrophysiological aspects. Here we addressed this issue using the elevated plus-maze paradigm and the excitability-related phenomenon known as cortical spreading depression (CSD).

CSD is a neuronal depolarization response that can be elicited via electrical, mechanical or chemical stimulation of one point of the cortical tissue (Leão 1944). CSD has been demonstrated in various species of mammals, including the human species (Fabricius et al. 2008). The cortical tissue usually resists the propagation of CSD, and certain experimental treatments can either increase or decrease such tissue resistance, which results respectively in CSD deceleration and acceleration (Guedes 2011). Therefore, measuring CSD velocity of propagation along the cortex of experimental animals is an easy and useful way to evaluate the brain's susceptibility to CSD. This parameter may help us understand brain excitability-dependent physiological phenomena, as well as neurological diseases related to brain excitability, such as migraines and epilepsy (Guedes 2011).

In this study, we analyzed the possible influence of neonatal chronic treatment with aspartame on anxiety-like behavior in rats by evaluating behavioral reactions in the elevated plus-maze; in addition, we analyzed cortical excitability by investigating how aspartame affects CSD parameters (velocity of propagation, amplitude and duration of the negative slow potential shift).

Materials and methods

Animals and experimental groups

Experimental procedures were approved by the institutional Ethics Committee for Animal Research of our university (approval protocol no. 23076014638/2013-72), in compliance with the "Principles of Laboratory Animal Care" (National Institutes of Health, Bethesda, USA). Newborn Wistar rats of both sexes, from distinct dams (i.e. from distinct original litters), were assigned to be suckled under normal or unfavorable conditions according to litter size. Litter size was nine pups (L_9 groups) for normal conditions and 15 pups (L_{15} groups) for unfavorable conditions. Each L_9 and L_{15} litter was formed by pups from at least three original litters (maximum of 3 and 5 pups per original litter in the L_9 and L_{15} condition, respectively). Weaning occurred on postnatal day 21. Weaned pups were separated by sex and housed in polypropylene cages (51 cm × 35.5 cm × 18.5 cm; three rats per cage) under controlled temperature (23 ± 1 °C) with a 12-h light:12-h dark cycle (lights on at 6:00 a.m.). They had free access to water and the same commercial lab chow, with 23% protein, that was offered to their dams (Purina Ltd.). In this study, we analyzed data from male pups only: 37 L_9 and 39 L_{15} rats originated from eight and seven litters, respectively. The animals were weighed on days 7, 14, 21, 25 and on the day of the CSD recording (96–115 days of life).

Gavage treatment

Each litter group was divided into 4 sub-groups, three of which were treated by gavage with either 75 mg/kg/day of aspartame, 125 mg/kg/day of aspartame (Sigma, St Louis, USA; groups Asp75 and Asp125, respectively), or vehicle (water; group W), as described by others (Vences-Mejía et al. 2006; Iyyaswamy and Rathinasamy 2012). The fourth group was not treated (naïve; group Nv). Considering that rats metabolize aspartame faster than humans (Fernstrom, 1989), aspartame doses used in the first species are usually increased by a factor of five. Therefore, the doses presently used in our rats can be considered comparable to those used by humans (see discussion in Vences-Mejía et al. 2006). The gavage procedure (modified from Francisco and Guedes 2015) was carried out daily, from postnatal day 8 to 28, between 7 and 9 a.m. The gavage volume was 0.5 ml in the first week of treatment (which corresponded to the second week of life), and 1.0 ml in the second and third weeks of treatment (respectively third and fourth week of life).

Elevated plus-maze test

The elevated plus-maze is frequently used for evaluating risk assessment and anxiety behavior (Wall and Messier 2001). 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. When the rats were 86–90 days old, they were individually placed onto the central platform facing one of the open arms and were observed for 5 min while they freely explored the maze. A video camera recorded behavioral activity. Recordings were stored in a computer and subsequently analyzed with the software ANYmaze™ (version 4.99 m). The animal was considered to have entered one arm when its four limbs were inside the arm. The time spent in open arms, number of entries into the open arms and number of fecal boluses that were expelled by the animal were measured during the 5-min test period. After each test, we cleaned the apparatus with a 70:30 ethanol:water solution.

CSD recording

On postnatal days 96–115, each animal was anesthetized with an intraperitoneal injection of a mixture of 1 g/kg urethane plus 40 mg/kg chloralose, and three trephine holes were drilled in the right side of the skull. This anesthetic mixture

has been routinely used in our laboratory because in the rat it provides a very stable and long-lasting anesthesia, which is very convenient for CSD recording in acute experiments (in which the recovery of the animal from anesthesia is not required). Furthermore, this anesthetic mixture does not block CSD, in contrast to other anesthetic agents, such as ketamine (Hernández-Cáceres et al. 1987). The trephine holes were aligned in the frontal-to-occipital direction and parallel to the midline. One hole (2–3 mm diameter) was positioned on the frontal bone and was used to apply the stimulus (KCl) to elicit CSD. The other two holes (3–4 mm in diameter) were drilled in the parietal bone and were used to record the CSD propagating wave. Rectal temperature was continuously monitored and remained at 37 ± 1 °C. CSD was elicited at 20-min intervals by 1 min application of a cotton ball (1–2 mm in diameter) soaked with 2% KCl solution (approximately 270 mM) to the anterior hole. The direct current (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. Velocity of propagation was calculated from the time required for a CSD wave to pass the distance between the two cortical electrodes. 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 (Francisco and Guedes 2015).

Statistics

The data were compared between groups by using a two-way analysis of variance (ANOVA), including as factors lactation conditions (L_9 and L_{15}) and gavage treatment (naïve, water, Asp75 and Asp125) followed by a post hoc test (Holm-Sidak), where indicated. We considered p values less than 0.05 as significant.

Results

Body weight

Figure 1 shows body weight at different time points, as described in materials and methods. L_{15} animals displayed lower body weights than the L_9 groups ($p < 0.05$). In the L_9 animals, aspartame treatment reduced body weight in comparison to the control groups ($p < 0.05$). On postnatal days 14 and 25, the Asp-125 L_9 group presented with significantly lower body weight compared to the Asp-75 L_9 group ($p < 0.05$).

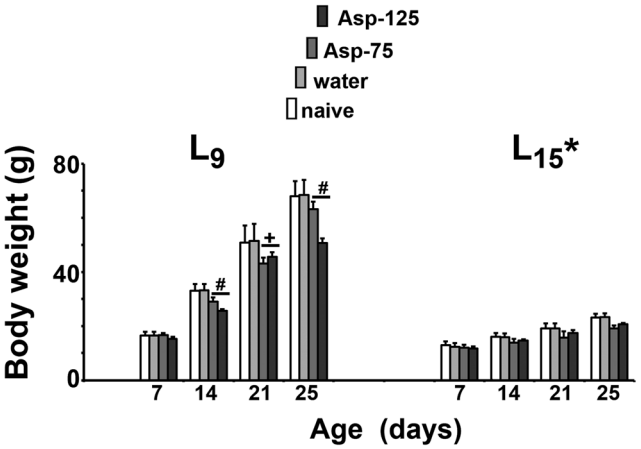

Fig. 1 Body weight of L_9 and L_{15} rats (suckled in litters formed by 9 and 15 pups, respectively). In both suckling conditions, four groups were studied: naïve (no gavage treatment), and water, Asp-75 and Asp-125 that received per gavage water, 75 mg/kg/d aspartame and 125 mg/kg/d aspartame, respectively, from postnatal day 8 to 28. Body weights were measured on days 7, 14, 21 and 25. Data are expressed as the mean ± standard deviation. The asterisk indicates that all L_{15} values are significantly lower than the corresponding L_9 controls. + different from the controls (naïve and water). # significant intergroup differences, as follows: Asp-125 < Asp-75 < water = naïve ($p < 0.05$; ANOVA plus Holm-Sidak test)

Elevated plus-maze test

In the L_9 rats, the mean ± SD time spent in the open arms (as percentage of the total duration of the test; Fig. 2a) was 27.85 ± 4.51%, 29.44 ± 5.29%, 18.40 ± 6.30% and 14.78 ± 2.74% for the naïve, water-treated, ASP75 and ASP125 groups, respectively. In the L_{15} rats the values were respectively 9.77 ± 3.86%, 11.72 ± 3.68%, 8.31 ± 1.43% and 10.50 ± 6.45%. An ANOVA indicated a significant reduction in the values of the L_9 aspartame-treated groups compared to the L_9 naïve and water controls ($F[3, 60] = 10.599$; $p < 0.001$). In the L_{15} condition, no intergroup difference in the percentage of time spent in the open arms was observed ($p > 0.05$). The L_9 versus L_{15} comparison revealed that the L_{15} naïve, water and ASP75 groups remained in the open arms for shorter times than the corresponding L_9 groups ($p < 0.05$).

The number of entries in the open arms (Fig. 2b) was also reduced ($p < 0.05$) in the L_9 ASP75 and ASP125 (which entered 3.50 ± 0.93 and 2.67 ± 1.63 times, respectively) in comparison with the L_9 naïve and water controls (which entered 7.60 ± 3.21 and 8.63 ± 3.14 times, respectively). In the L_{15} condition, no intergroup difference was observed ($p > 0.05$). Compared with the respective L_9 groups, the L_{15} ASP75 and ASP125 groups displayed a higher number of entries in the open arms (5.57 ± 0.98 and 6.00 ± 1.41, respectively), while the L_{15} naïve and water groups entered a reduced number of times in the open arms (5.14 ± 1.46 and 6.25 ± 1.04, respectively) ($p < 0.05$).

During the 5-min observation, the L_9 ASP75 and ASP125 animals expelled a greater number of fecal boluses (3.22 ±

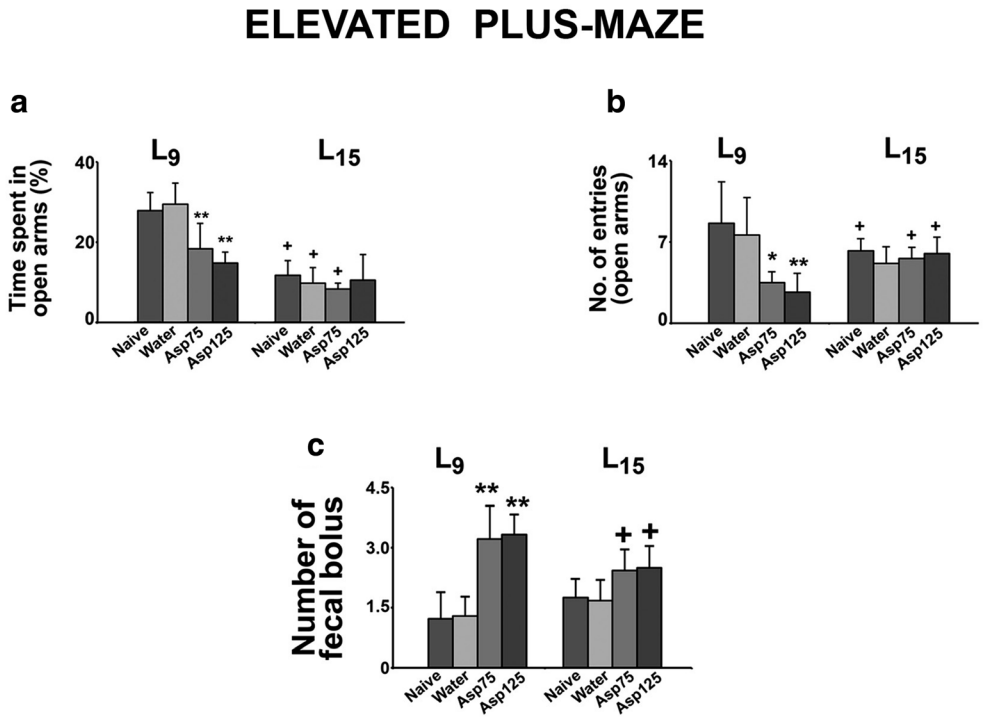

Fig. 2 Behavioral activity (elevated plus maze) of adult rats that were previously suckled in litters with 9 and 15 pups (respectively L_9 and L_{15} condition). In both suckling conditions, four groups were studied: naïve (no gavage treatment), and water, Asp-75 and Asp-125 that received per gavage water, 75 mg/kg/d Aspartame and 125 mg/kg/d Aspartame, respectively, from postnatal day 8 to 28. A- percentage of time spent in the open arms, B- number of open arm entries, C- number of fecal boluses. Data are expressed as the mean ± SD. * different from the naive group; ** different from the naive and water groups. + different from the corresponding L_9 group. ($p < 0.05$; ANOVA plus Holm-Sidak test)

0.83 and 3.33 ± 0.50, respectively) than the L_9 naïve and water controls (respectively 1.22 ± 0.67 and 1.29 ± 0.49) ($p < 0.05$). In the L_{15} condition, no intergroup difference was observed ($p > 0.05$). The L_9 versus L_{15} comparison revealed that the L_{15} ASP75 and ASP125 groups expelled fewer fecal boluses (respectively 2.43 ± 0.53 and 2.50 ± 0.55) than the corresponding L_9 groups ($p < 0.05$). These results are in Fig. 2c.

CSD features

In all groups, topical application of 2% KCl for 1 min at the frontal cortex (on a circular area with 2–3 mm diameter) reproducibly elicited a single CSD wave, which was recorded by the two electrodes, located 5–12 mm posteriorly, at the parieto-occipital region, in the stimulated hemisphere. At the end of the recording session, visual exploration of meninges at the KCl application point revealed only minor signs of hyperemia. Figure 3 depicts electrophysiological recordings showing the slow potential change accompanying CSD on the cortical surface of four L_9 and four L_{15} animals. The slow potential recordings confirmed the presence of CSD after each KCl-stimulation.

In the L_9 rats, the mean ± SD CSD velocities (in mm/min) were 3.27 ± 0.19 and 3.31 ± 0.07 for the water and naive groups, respectively, and 3.16 ± 0.14 and 2.87 ± 0.12 for the 75 mg/kg/day and 125 mg/kg/day Aspartame-treated groups. In the L15 rats, the mean ± SD CSD-velocities (in mm/min) were 4.35 ± 0.14 and 4.34 ± 0.10 for the water and naive groups, respectively, and 4.04 ± 0.10 and 3.76 ± 0.11 for the 75 mg/kg/day and 125 mg/kg/day aspartame-treated groups. The data for all groups are presented in Fig. 4.

The CSD effect of Aspartame was dose-dependent and was modified by the nutritional status of the animals ($L_{15} > L_9$). In the L_9 condition, only the aspartame dose of 125 mg/kg significantly reduced the velocity of propagation of CSD when compared to the control groups ($p < 0.05$). In the L_{15} condition, however, both doses of Aspartame (75 mg/kg/day and 125 mg/kg/day) reduced the CSD velocities significantly in comparison with the L_{15} controls (Fig. 4).

Amplitude and duration of CSD waves

Table 1 shows amplitude and duration of the negative slow potential change, which is the hallmark of CSD. The mean amplitude varied from 7.1 ± 1.9 mV to 10.3 ± 2.6 mV in the L_9 groups and from 10.0 ± 1.9 mV to 13.4 ± 2.9 mV in the L_{15} groups (Table 1). The mean duration varied from 49.8 ± 4.8 s to 65.30 ± 5.1 s in the L_9 groups and from 45.0 ± 3.4 s to 63.9 ± 3.6 s in the L_{15} groups. An ANOVA revealed that aspartame treatment was associated with lower amplitude and longer

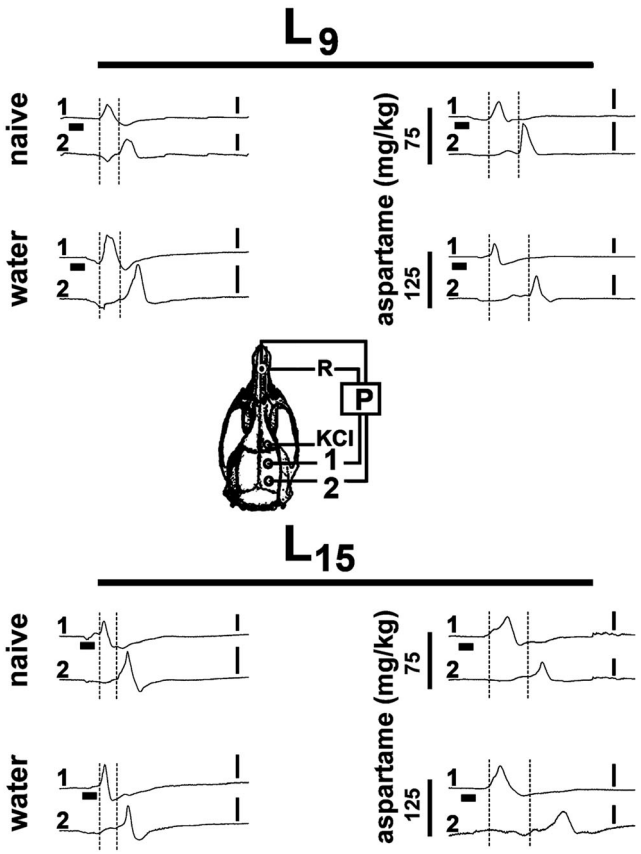

Fig. 3 Slow potential change, recorded during cortical spreading depression (CSD), in 96–115-day-old L_9 and L_{15} rats, which received, from the 8th to the 28th postnatal days, 75 mg/kg/day or 125 mg/kg/day of Aspartame, or distilled water, or were gavage-free (naïve). The horizontal bars show the period of stimulation (1 min) with 2% KCl necessary to elicit CSD. The vertical bars (at the right end of the traces) equal −10 mV (negative upwards). The place of KCl application and of the reference electrode (R) is indicated in the skull diagram, which also shows the recording points 1 and 2 (from which the traces marked with the same numbers were recorded). The interelectrode distance was 6.5 mm in all cases

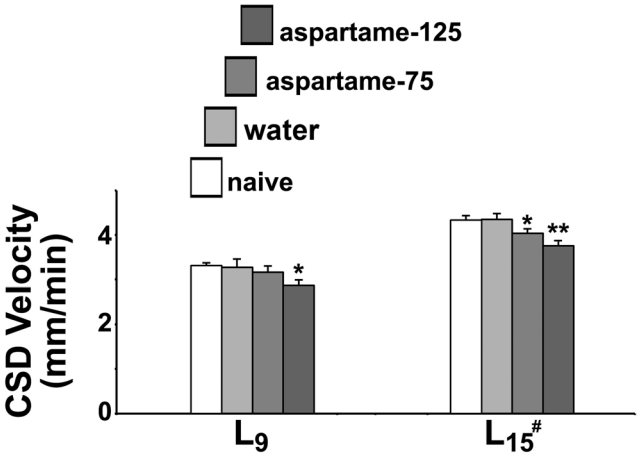

Fig. 4 Velocity of propagation of cortical spreading depression (CSD; mean ± SD) of L_9 and L_{15} 96–115-day-old rats (previously suckled in litters formed by 9 and 15 pups, respectively), which received, per gavage from the 8th to the 28th postnatal days, 75 mg/kg/day or 125 mg/kg/day of aspartame, or distilled water (water-control). Another control group did not receive any gavage (naïve group). * different from the controls. ** different from the control and from the Asp75 groups ($p < 0.05$; ANOVA plus Holm-Sidak test)

duration in comparison with the respective controls ($F[3,74] = 85.900$; $p < 0.001$). An ANOVA also indicated higher CSD amplitudes and shorter durations in L_{15}, in comparison with L_9 rats ($F[1,74] = 16.930$; $p < 0.001$).

Discussion

In this experiment, we explored the behavioral and electrophysiological effects that were associated with a 21-day administration of two distinct doses of aspartame in developing rats. By testing rats in the elevated plus-maze we characterized behavioral responses suggestive of increased anxiety. By recording CSD propagation in anesthetized animals, we demonstrated a CSD decelerating effect of aspartame that is suggestive of excitability changes in the cerebral cortex. To the best of our knowledge, these findings, observed in developing animals, have not been reported before.

Reports in the literature on the effect of aspartame on body weight are rather conflicting, with some authors describing aspartame-associated increases in weight gain, while others report reduced weight gain in relation to controls (von Poser Toigo et al. 2015). Our data indicate that the consumption of aspartame early in life reduced body weight gain in developing rats compared with control groups (Fig. 1). This could be the consequence of reduced milk ingestion by the aspartame-treated rats, as aspartame may interfere with metabolism, liver function and appetite of animals, increasing satiety and thus reducing food intake (Alkafafy et al. 2015). Regarding discrepancies between studies, these may be due to methodological factors such as distinct aspartame doses, different methods of drug administration, varied animal species and distinct periods of treatment over different phases of life (Beck et al. 2002).

In the elevated plus-maze paradigm, a decrease in open arm entries indicates enhanced anxiety of the animal (Zhao et al. 2017). Increased emotional activity and fear result in increased defecation as a consequence of activation of parasympathetic activity (Sanberg et al. 2001). In the present study, data from the elevated plus maze test revealed more anxious behavior in the L_9, but not L_{15} groups that were treated with aspartame, as indicated by the shorter permanence in the open arms, and higher number of expelled fecal boluses (Fig. 2). There is substantial evidence that high levels of fear and anxiety are indicated by fewer visits to the open arms and high defecation scores (Ashok et al. 2015). Therefore, it is reasonable to suggest that our aspartame-treated animals are experiencing more anxiety than the control groups. It is possible that aspartame (or resulting metabolites) acts at the central nervous system as an anxiogenic agent by activating N-

Table 1 Amplitude and duration of the negative slow potential change of cortical spreading depression in adult rats (96–115 days) in different experimental groups

Treatment groups	Amplitude (mV)		Duration (s)	
	L9	L15	L9	L15
Nv	10.2 ± 2.1 (9)	13.3 ± 2.7 (9)+	49.8 ± 4.8 (9)	45.3 ± 2.0 (10)+
W	10.3 ± 2.6 (10)	13.4 ± 2.9 (10)+	51.0 ± 4.7 (9)	45.0 ± 3.4 (10)+
ASP 75	10.1 ± 2.4 (9)	11.0 ± 1.8 (8)	59.0 ± 4.2 (9) *	56.2 ± 1.8 (8)*
ASP 125	7.1 ± 1.9 (10) **	10.0 ± 1.9 (8)*+	65.3 ± 5.1 (10)**	63.9 ± 3.6 (10)**

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 the mean ± standard deviation, with the number of animals in parentheses. +, intergroup difference (L_{15} different from L_9). * different from the two control groups in the same lactation condition. ** different from the two controls and from the Asp75 group ($p < 0.05$; ANOVA plus Holm-Sidak test)

methyl-D-aspartate (NMDA)-receptors, as recently suggested (Collison et al. 2016). This possibility shall be tested in the future by checking some anxiety biomarkers that can be triggered via NMDA receptors (Li et al. 2014). An alternative interpretation of the behavioral findings could include aspartame-induced decrease of monoamine synthesis and imbalance of ionic concentration in the brain (Abhilash et al. 2014). Various studies suggest that aspartame consumption produces disturbances in ionic homeostasis (Abhilash et al. 2014) and modulates Na+, K + -ATPase activity in the rat cortex (Simintzi et al. 2008). However, changes were prominent at a high dose of aspartame (1000 mg/kg; Abhilash et al. 2014). All of the evidence notwithstanding, the possibility also exists, that ions other than potassium are altered with long-term aspartame consumption (see discussion in Christian et al. 2004). Further specific investigation is needed to test those possibilities.

Our group previously described the facilitating effects of nutritional deficiency on CSD propagation early in life in rats (Francisco and Guedes 2015; Guedes 2011). In the present study, the higher CSD velocity and amplitude, as well as the shorter duration, from the L_{15} animals confirms the facilitating action of early malnutrition that was induced by suckling the pups in large litters. In addition, we were able to demonstrate, for the first time, that chronic aspartame administration early in life impairs CSD propagation later in adulthood (lower CSD velocity and amplitude, and longer duration). This novel, long-lasting neural action of aspartame is dose-dependent (Asp125 > Asp75) and is influenced by the lactation condition ($L_{15} > L_9$; Fig. 4). If this last observation could be shown to occur in the human species, then the possibility would exist that a determined anti-migraine or an anti-epileptic drug could have different degrees of effectiveness, depending on previous nutritional status (Guedes et al. 1992). Regarding the underlying mechanisms that mediate the CSD effects of malnutrition, discussions frequently involve morphological changes in the brain that increase cell-packing density (Rocha-de-Melo et al. 2006), reduce the extracellular space (Mazel et al. 2002) and cortical myelination (Merkler et al. 2009) and impair glial function (Largo et al. 1997). Interestingly, all these factors facilitate CSD and, more importantly, CSD velocity is modulated dichotomously, with unfavorable lactation (L15 condition) accelerating CSD in comparison with normal lactation (L_9 condition), and favorable lactation (suckling in litters with three pups only; Rocha-de-Melo et al. 2006) decelerating CSD.

Our CSD findings in the aspartame-treated animals can be explained by a number of mechanisms, the most plausible of which involves redox imbalance that occurs in the brain when aspartame is metabolized (Oyama et al. 2002). Although in the present study we have not measured brain redox status, it is reasonable to suppose that daily administration of aspartame over 21 days might have produced some degree of redox imbalance. In fact, repeated aspartame administration to mice may cause impaired memory performance and enhanced brain oxidative stress as indicated by increased levels of malondialdehyde and nitric oxide, and decreased levels of reduced glutathione (Abdel-Salam et al. 2012; Iyyaswamy and Rathinasamy 2012). Interestingly, aspartame has been causally linked to migraine triggering via redox imbalance (Borkum 2016) and CSD has long been postulated as being the physiological mechanism underlying migraines (Charles and Baca 2013) and perhaps other headache disorders (Chen and Ayata 2016). Furthermore, by treating rats with the antioxidant ascorbic acid we were able to modulate CSD propagation (Mendes-da-Silva et al. 2014). In this context, one important question to be answered would be: 'given that redox imbalance can alter CSD propagation, would this effect be counteracted by restoring the redox balance?' We tested this possibility in cuprizone-treated rats. It is known that dietary cuprizone treatment in rodents for five weeks produce brain demyelination and increased CSD propagation velocity (Merkler et al. 2009), and increased reactive oxygen species (Sanadgol et al. 2018). After five weeks in the cuprizone diet, switching part of the animals back to the normal, cuprizone-free diet restored the myelination, and returned the CSD

velocity of propagation to the control levels (Merkler et al. 2009). However, our data do not allow us to exclude the possible involvement of other mechanisms such as aspartame action on cellular elements (Rycerz and Jaworska-Adamu 2013), neurotransmitters (Abhilash et al. 2014; Simintzi et al. 2007), or neurotransmitter receptors (Collison et al. 2016).

In conclusion, our findings support the hypothesis of an anxiogenic action of aspartame on the brain of developing rats. Additionally, this study describes a novel effect of aspartame on the excitability-related CSD phenomenon. Our CSD data allow us to draw four conclusions. First, aspartame administration early in life decelerates CSD at adult age, suggesting a long-lasting action. Second, the aspartame effect on CSD is dose-dependent. Third, unfavorable lactation (suckling in large litters) accelerates CSD, which confirms previous reports. Fourth, an unfavorable lactation condition intensifies the aspartame effect on CSD, suggesting changes in the electrophysiological features of the brain. The data suggest caution in the aspartame consumption by lactating mothers and their infants.

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