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Favorable and unfavorable lactation modulates the effects of electrical stimulation on brain excitability: A spreading depression study in adult rats

M. Batista-de-Oliveira [a,b], K.K. Monte-Silva-Machado [a], A.K. Paiva [a], H. Lima [a], F. Fregni [b,*], R.C.A. Guedes [a,**]

[a] Laboratory of Nutrition Physiology, Department of Nutrition, Universidade Federal de Pernambuco, 50670901, Recife, PE, Brazil
[b] Spaulding Rehabilitation Hospital, Harvard Medical School, Boston, MA, USA

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ABSTRACT

Aims: We investigated how different nutritional states resulting from distinct lactation conditions modulate the effects of cortical electrical stimulation (CES) on the excitability-related phenomenon known as cortical spreading depression (CSD).
Main methods: Wistar rats were reared in different litter sizes with 12, 6 or 3 pups, designated as malnourished (M), well-nourished (W) and overnourished (Ov), respectively. CSD was recorded for 4 h on 2 cortical points of each cerebral hemisphere at baseline and after CES. CES was applied for 20 min on the left cortex using a bipolar electrode placed between the CSD recording electrodes. Paired Student *t* test and ANOVA followed by Tukey test were used for statistical analysis ($p<0.05$).
Key findings: The lactation conditions significantly influenced body weight (the M and Ov groups presented the lowest and largest average weight, respectively) and modified the CSD velocities of propagation in adulthood (Ov<W<M CSD velocity). CES increased CSD velocity of propagation in the stimulated hemisphere in all groups, and in the non-stimulated hemisphere (8.66% ± 1.38) in the Ov group only. We observed nutritional-dependent CES effects on cortical excitability as evaluated by the different CSD velocities across the three groups (mean ± sem, M (10.13% ± 1.70), Ov (14.65% ± 1.10) and W (25.70% ± 5.05)).
Significance: These findings suggest valuable mechanisms of action for the brain stimulation techniques, which have gained importance because of their increasing use for the treatment of neuropsychiatric diseases. Data also suggest modulation of CES-effects by baseline excitability (as determined by the early nutritional state).

Introduction

Noninvasive cortical electric stimulation (CES) has been increasingly explored to modulate plasticity experimentally and in neuropsychiatry. CES effects depend on the baseline cortical excitability. Because nutritional status profoundly affects brain activity through different mechanisms, it is a useful variable to understand how baseline cortical excitability can influence CES effects.

A previous study (Durand et al., 2006) showed that electrical stimulation applied to hippocampal slices can block epileptiform activity through non-synaptic mechanisms of increased extracellular potassium concentrations. Nutritional state might also influence cortical excitability through non-synaptic mechanisms. Ketogenic diet increases the levels of adenosine — a metabolite that has a non-synaptic release and can significantly influence activity in local neural regions (Masino et al., 2009). Moreover, rats previously submitted to favorable conditions of lactation displayed body composition and histochemical brain alterations (Rocha-de-Melo et al., 2004), as well as electrophysiological modifications (Rocha-de-Melo et al., 2006). This method of producing malnutrition or overnutrition is very simple and consists in manipulating the number of pups to be suckled by one dam (Plagemann et al., 1998; Rocha-de-Melo et al., 2006). Additionally, undernourished animals show a decrease in glial cells — an important component for the non-synaptic modulation of neural activity (Morgane et al., 1978).

Here, we explored the effects of CES on cortical spreading depression (CSD) in order to find out whether its effects can be influenced by the modification of brain excitability (by manipulating nutritional status), whether alternative, non-synaptic mechanisms might explain CES effects, and also if they would be restricted to the stimulated hemisphere. CSD was first described as a fully reversible, slowly propagating wave of depression of the spontaneous cortical electrical activity (Leão, 1944). Simultaneous to the depression of the spontaneous electrical activity, a slow DC-potential change (SPC) appears in the cortical region where the CSD is observed (Leão, 1947).

* Correspondence to: Laboratory of Neuromodulation, Spaulding Rehabilitation Hospital, Harvard Medical School, 125 Nashua Street #727, Boston, MA 02114, USA. Tel.: +1 617 573 2195.
** Correspondence to: Avenida Prof. Moraes Rego, 1235-Cidade Universitária, Recife, PE, CEP 50670-901, Brazil. Tel: +55 81 2126 8936; fax: +55-81-2126-8473.
E-mail addresses: fregni.felipe@mgh.harvard.edu (F. Fregni), guedes.rca@gmail.com, rguedes@ufpe.br, rc.guedes@terra.com.br (R.C.A. Guedes).

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Leão suggested that CSD "does not depend upon synaptic transmission of impulses" (Leão, 1944). This conclusion was based on its characteristics such as slow time course, prolonged recovery time and marked vasodilation. Grafstein (1956) also suggested that the likely mechanism of CSD operates through non-synaptic transmission.

It is known that alternating electrical stimulation can change cortical excitability either by synaptic mechanisms (Rothkegel et al., 2010), or by non-synaptic mechanisms (Durand et al., 2006). Therefore, by investigating the effects of CES on CSD, we would provide further insight on whether CES might also be involved in modulation of cortical excitability in association with non-synaptic mechanisms.

This study continues our previous investigation in which we showed preliminary effects of CES on CSD: accelerated CSD propagation in nourished rats after 1 Hz CES, applied for two consecutive days (Fregni et al., 2005). We aimed to investigate in the young adult rat: (1) Does a single session of CES acutely influence CSD propagation? (2) Is this effect modulated by malnutrition and overnutrition? (3) Are these effects restricted to the stimulated hemisphere? We hypothesized that CES, nutritional state and combination of both factors would significantly alter CSD.

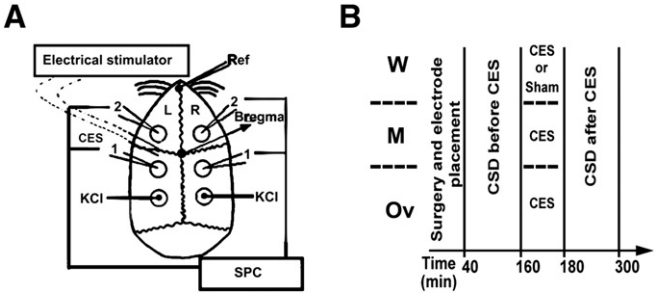

Fig. 1. A: Places of the four recording electrodes for the cortical spreading depression (CSD). Each hemisphere received two electrodes, on cortical areas 1 and 2 (directly on the intact dura-mater) separated by a distance of 5.6 mm. A fifth electrode of the same type was placed on the nasal bones and served as a common reference (Ref) for the recording electrodes. The bipolar electrode for the epidural cortical electrical stimulation (CES) is shown between the recording points 1 and 2 in the left hemisphere. In each hemisphere, the place of epidural KCl application is in the posterior parietal hole. B: Diagram summarizing the timeline and group distribution of our experiment (timeline is out of scale for graphical presentation). Under anesthesia, the animals' skulls were trepanated for placing the CSD-recording and CES electrodes. After recording baseline CSD, we applied CES (or sham) and recorded CSD after CES-stimulation, according to the timeline. W, M and Ov refer to the well-nourished, malnourished and overnourished groups, respectively.

Methods

Animals and diet

Wistar newborn male rats were submitted to three lactation conditions, as previously described (Rocha-de-Melo et al., 2006; Frazão et al., 2008). Briefly, the pups were suckled in litters of three different sizes, designated as large-, medium- and small-size litters (composed by 12, 6 and 3 pups, respectively), originating the malnourished (M; $n=10$), well-nourished (W; $n=23$) and overnourished groups (Ov; $n=12$). Animals were raised from birth until the day of the electrophysiological recording (60–75 days old) in a room with 23 ± 1 °C with a 12 h/12 h light/dark cycle (lights on at 7:00 am), with free access to food and water. After weaning, all pups were housed in groups of 3–4 per cage ($51\times35.5\times18.5$ cm), and kept on the commercial laboratory chow diet. All experiments were carried out at the *Universidade Federal de Pernambuco* in accordance with the guidelines of the Institutional Ethics Committee for Animal Research, which comply with the "Principles of Laboratory Animal Care" (National Institutes of Health, Bethesda, USA).

CSD recording

When the pups were 60–75 days old, they were intraperitoneally anesthetized with a mixture of 1 g/kg urethane plus 40 mg/kg chloralose (both from Sigma Co., USA). Six trephine holes (2–4 mm diameter; three holes in each hemisphere) were drilled. In each hemisphere, the holes were aligned in the anteroposterior direction and parallel to the midline (dura mater left intact).

At the cortical region exposed on the posterior parietal bone, CSD was elicited by applying a cotton pledget (1–2 mm in diameter) soaked with 2% KCl solution (approximately 0.28 M) that remained for 1 min on the cortical surface. The interval between two consecutive stimuli in the same hemisphere was 20 min. At the other two holes in each hemisphere, drilled anteriorly to the stimulation hole, the slow DC-potential changes typical of the propagating CSD episodes were continuously recorded for a total of 4 h, by means of Ag–AgCl, agar-Ringer type electrodes. The recording electrodes were placed on motor cortical areas, as previously described (Fregni et al., 2005). A fifth electrode of the same type was placed on the nasal bones and served as a common reference for the other 4 recording electrodes (see Fig. 1A for electrode placement and Fig. 1B for experiment timeline).

In each hemisphere, CSD-velocity of propagation was calculated from the time required for a CSD wave to cross the distance between the two cortical recording points. This time was measured using the beginning of the rising phase of the negative slow DC-potential change as the initial point. Besides the propagation velocity of CSD, the amplitudes of the CSD slow potential shifts as well as the incidence of "multiple" CSD, i.e., two or more CSD episodes appearing after a single KCl stimulation, were also quantified. During surgery and CSD recording, the animal breathed spontaneously and rectal temperature was continuously monitored and kept at 37 ± 1 °C by a heating pad.

Cortical electrical stimulation (CES)

CES was applied during the CSD-recording session. After the 2 initial recording hours of CSD (baseline recording), the recording was interrupted and CES was applied on the left cortex surface through a Teflon-coated, stainless steel (100-μm diameter wires), bipolar stimulating electrode with the tips separated by 0.5 mm. This bipolar electrode was gently and epidurally placed between the two recording electrodes, on the left frontal cortex surface (4.2 mm anterior to bregma and 3.5–4.0 mm lateral to the midline). The two wires of the bipolar electrode were arranged in the antero-posterior direction, parallel to the midline, and the anode was always the more posterior wire of the pair (Fig. 1A). We preferred epidural stimulation to diminish the risk of cortical lesion; this method has also been used for human brain stimulation (Brown et al., 2003).

Electrical stimulation was delivered by an electrical stimulator (Insight, *Ribeirão Preto*, Brazil) and consisted of pulses of 1 Hz and 600 μA for 20 min (total of 1200 pulses). After CES, the CSD recording was restarted and continued for 2 more hours. Possible effects of CES on CSD were identified by comparing, in the same animal, the CSD-velocities of propagation before and after CES. In these experiments the animal served as control for itself. In the W condition, possible effects of the electrode placement on CSD-propagation were excluded by comparing the stimulated animals ($n=13$) with a sham (placebo, $n=10$) group, in which the CES electrodes were placed on the cortical surface, thereafter connected to the electrical stimulator, but this remained switched off during the entire CSD-recording. At the end of the CSD recording session (post-CES), the animals, while still anesthetized, were killed by lesioning the bulbar region with a sharp needle, promptly provoking cardio-respiratory arrest.

Table 1
Body weight of malnourished (M), overnourished (Ov) and well-nourished (W) rats, respectively suckled in litters performed by 12, 3 and 6 pups. The dams received a standard diet (LABINA, 23% of protein, PURINA, Brazil). Data are presented as mean ± standard deviation, with the number of animals in parentheses. M and Ov-values marked with low-case letters are significantly different from the respective values in the groups marked with the same letters on the left column. ($p \leq 0.05$; ANOVA plus Tukey test).

Body weight (g)						
Days old	7th	14th	21st	30th	60th	CSD recording
Group						
N[a] (9)	20.0 ± 3.4	36.8 ± 2.9	56.3 ± 3.3	103.5 ± 4.7	277.8 ± 32.6	284.0 ± 15.2
M[b] (7)	14.0 ± 1.4[a]	23.4 ± 1.8[a]	37.0 ± 3.4[a]	77.0 ± 6.8[a]	249.0 ± 9.2	272.4 ± 13.8
Ov (14)	23.3 ± 3.1[a, b]	41.9 ± 5.0 [a,b]	63.7 ± 6.0[a, b]	110.6 ± 8.2[b]	296.9 ± 17.5 [b]	299.0 ± 20.6 [b]

Body weights

Body weights were measured at postnatal days 7, 14, 21, 30 and 60, as well as on the day of CSD recording.

Statistical analysis

Body weight and CSD-velocity intergroup differences were compared by using ANOVA in which the main dependent variable was body weight (or CSD velocity and amplitude) and the independent variable was the lactation/nutrition condition. In this case, ANOVA was followed by the post-hoc Tukey test where necessary. Intragroup differences (before *versus* after CES stimulation, and stimulated- *versus* contralateral hemisphere) were analyzed by the paired *t*-test. Differences in the incidence of CSD episodes were analyzed by the Fisher's exact probability test. Differences were considered significant when $p \leq 0.05$.

Results

Body weights

Confirming previous data with this model of changing the nutritional status during lactation (Plagemann et al., 1998; Maia et al., 2006; Rocha-de-Melo et al., 2006), in the present study the mean body weights of the three distinct nutritional groups (M, W and Ov) were found to be inversely proportional to their litter sizes, i.e., M<W<Ov. The M group presented mean body weight significantly lower than the W and Ov groups at 7, 14, 21 and 30 days of life ($p<0.05$). The animals with the opposite nutritional condition (Ov) were significantly heavier then the W rats at 7, 14 and 21 postnatal days ($p<0.05$). These results are shown in Table 1.

Nutritional status, acute cortical electrical stimulation (CES) and CSD

In all groups, application of 2% KCl on a cortical point for 1 min usually elicited a single CSD wave, which was recorded by the two electrodes located more anteriorly in both hemispheres. Fig. 2 shows examples of CSD recordings in four adult rats (from the M, W, Ov and W-sham groups, respectively). The early manipulation of the nutritional status influenced significantly the CSD velocity of propagation in adulthood in the baseline recording (before CES). This nutrition-dependent effect was observed both in the contralateral (non-stimulated, right hemisphere), and in the stimulated-hemisphere (left) according to the following crescent order of the velocity values across the experimental groups: Ov<W<M. In the right hemisphere, the mean velocities (in mm/min) for the three nutritionally distinct groups were 3.28 ± 0.05, 3.53 ± 0.2 and 3.92 ± 0.18, respectively for Ov, W and M. In the left hemisphere, the velocities were respectively 3.30 ± 0.06, 3.61 ± 0.14, and 3.84 ± 0.16). After CES, the CSD velocities increased in the stimulated (left) hemisphere in all groups ($p<0.05$), when compared to the pre-CES values. In addition, in the Ov animals the CSD accelerating effect of CES was also observed in the non-stimulated (right) hemisphere. In the left hemisphere, the post-CES velocities of CSD were respectively 3.78 ± 0.13, 4.53 ± 0.59 and, 4.22 ± 0.23 mm/min for the Ov, W and M, respectively. In the right hemisphere, the mean velocities were respectively 3.56 ± 0.17, 3.57 ± 0.19 and 4.64 ± 0.31. No CSD differences were observed in the sham group. These results are presented in Fig. 3.

The greatest CES-induced average increase in CSD velocity of propagation in the stimulated hemisphere was noticed in W rats (25.70%), when compared to the Ov (14.65%) and M groups (10.13%). The intergroup difference was statistically significant ($p<0.05$; ANOVA plus Tukey test); showing that the increase in CSD velocity was more pronounced in the animals with normal nutrition early in life (W group).

The incidence of "multiple" CSD after a single KCl stimulation is presented in Table 2. After CES, KCl elicited a greater number of CSD-episodes in the W and M group, as compared to the respective CSD appearance during baseline recordings ($p<0.05$).

The amplitudes of the slow potential changes of the CSD are presented in Table 3. No intra- or intergroup significant differences in CSD amplitude could be found.

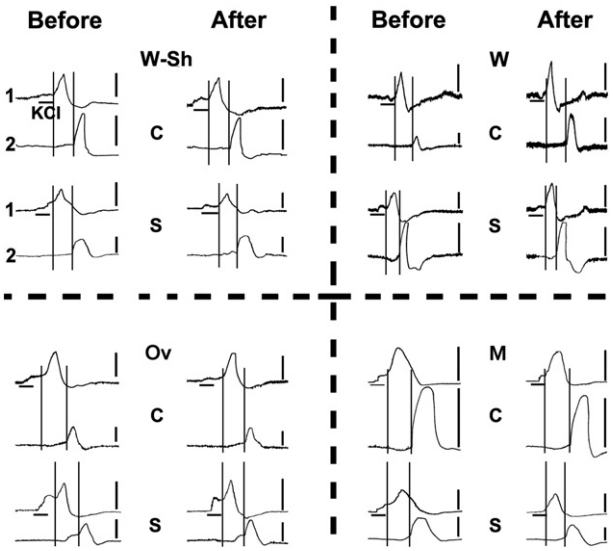

Fig. 2. Recordings of the slow potential change of KCl-elicited CSD in four adult rats, previously suckled in normal, favorable and unfavorable lactation conditions, denominated respectively well-nourished (W), overnourished (Ov) and malnourished (M). The horizontal bars under traces marked with 1 indicate the time (1 min) of topical application of 2% KCl, necessary to elicit a single CSD wave, which was recorded by the two electrodes located anteriorly to the stimulation point, as show in the scheme of Fig. 1A. The vertical bars indicate 10 mV (negative upwards). Before and after refer to the recordings prior- and subsequent to cortical electrical stimulation (CES). **Sh** is a W-rat in the sham-stimulated condition. **C and S** represent the contralateral (right) and stimulated (left) hemispheres, respectively.

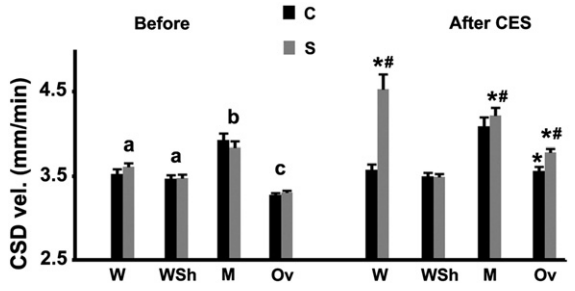

Fig. 3. CSD velocity of propagation (in **mm/min**) before and after cortical electric stimulation (CES) in rats previously suckled in normal, favorable and unfavorable lactation conditions, denominated respectively as well-nourished (W), overnourished (Ov) and malnourished (M); **Sh** is a W-rat from the sham-stimulated condition. **C and S** represent the contralateral (right) and stimulated hemisphere (left), respectively. The values are presented as mean ± sem. In the basal period, groups with distinct letters are significantly different ($p \leq 0.05$, ANOVA + Tukey test). * Statistically different from the respective basal values (before CES); the symbol # indicates interhemispheric difference ($p < 0.05$; paired *t*-test).

Table 3
Amplitudes of the CSD slow potential shifts in the malnourished (M), well-nourished (W and W-Sham) and overnourished (Ov) groups. The values were obtained from left (LH) and right hemispheres (RH), during CSD recordings, before and after cortical electrical stimulation (CES). CES was applied only on the LH. In the W-sham group, the CES electrodes were placed on the cortical surface, but the electrical stimulator remained switched off. Data are expressed as mean ± standard deviation. The number of rats per group is in parentheses. Intergroup, and intragroup differences (before *versus* after CES) were analyzed with ANOVA and paired *t*-test, respectively. No significant differences were found.

Amplitudes of CSD slow potential shifts (mV)			
Lactation condition		Hemispheres	
		Right	*Left*
W-Sham (6)	Before	8.85 ± 3.91	6.45 ± 2.57
	After-CES	9.66 ± 3.68	6.98 ± 3.19
W (5)	Before	9.78 ± 3.12	7.62 ± 2.55
	After-CES	12.14 ± 5.31	9.34 ± 2.71
M (7)	Before	8.59 ± 3.43	10.97 ± 5.99
	After-CES	8.38 ± 3.63	10.64 ± 4.91
Ov (9)	Before	8.76 ± 2.49	8.43 ± 3.43
	After-CES	9.57 ± 2.98	8.27 ± 3.46

Discussion

Despite its increasing therapeutic application, the mechanisms underlying CES remain elusive. Previous evidence has demonstrated the clinical relevance of electrical stimulation for the treatment of neuropsychiatric diseases (Fregni and Pascual-Leone, 2007). In these cases, the therapeutic and beneficial effects have been causally linked to an enhancement in cortical excitability. These effects can be theoretically explained by synaptic and non-synaptic mechanisms.

Regarding the synaptic mechanisms, for instance, transcranial magnetic stimulation (TMS) has been shown to change the excitability of various neuronal circuits at the motor cortical level in humans through mechanisms that are LTP and LTD like (Fisher et al., 2002). In rats, alternating low frequency stimulation, a technique that mimics TMS, demonstrates long-lasting synaptic enhancement in hippocampal circuits (Habib and Dringenberg, 2009). For CES, on the other hand, most studies show that this technique can strengthen synaptic connectivity inducing plastic changes. Therefore, in this study, we suggest an association with another potential mechanism — a non-synaptic mechanism. Based on the present findings, we discuss here the possibility that CES is associated with non-synaptic effects and also demonstrate that these effects are modulated by the nutritional status during the critical brain development period. We used CSD as a tool to speculate the association with non-synaptic mechanisms. The CSD can index measurement of ion flow, extracellular volume and biphasic changes in pH. For instance, by means of higher extracellular $[K^+]$ it is possible to facilitate CSD propagation (Somjen, 2001) and also increase cortical excitability after brain electrical stimulation (Klueva et al., 2003). Thus, CSD appears to be a useful tool to investigate non-synaptic effects associated with CES action on cortical excitability. Because we observed in our study an increase in CSD velocity after CES, we first discuss potential non-synaptic mechanisms that could possibly be related to these effects.

CES seems to increase CSD susceptibility, influencing the neuron–glia interactions aforementioned, instead of affecting only synaptic transmission. One could suggest that CES, besides influencing extracellular potassium concentration, would influence the calcium levels related to glial activity and capable to participate in the CSD propagation. CES can promote the expression of neurotrophins (c-Fos and BDNF) in the cerebral cortex of rats (Zhang et al., 2007), and given that these neurotrophins use distinct Ca^{2+} signaling pathways, they might modulate neuron–glia interactions in an acute manner (Todd et al., 2007; Pfrieger, 2010). Thus, one possibility is that CES would increase cortical excitability by changing neurotrophin expression, as shown by higher CSD velocity of propagation.

On the other hand, some of the CES effects on CSD might also be explained by modulation of synaptic transmission. For instance, the use of MK-801, a NMDA antagonist, impairs CSD propagation (Guedes et al., 1988). In addition, serotonin also seems to be involved in CSD propagation, since the increase of serotoninergic activity decelerates CSD (Amâncio-dos-Santos et al., 2006). Therefore it is possible that CES enhances glutamatergic transmission and/or reduces serotoninergic activity; however these effects could be secondary to non-synaptic effects. For instance, transcranial direct current stimulation (tDCS) is an example of a technique of brain stimulation in which the main effect is based on modification of neuronal membrane that leads secondarily to changes in synaptic activity (Nitsche et al., 2003).

The second question of this study was whether manipulating nutritional status would change the effects of CES on CSD. The present data allow us to conclude that changes in lactation conditions altered the nutritional status, which also confirm results of previous studies (Maia et al., 2006; Rocha-de-Melo et al., 2006). As underscored by other reports, besides having increased body weights, the rats suckled in the Ov-condition present hyperphagia, hyperglycemia, hyperinsulinemia, higher triglycerides levels and insulin resistance (Plagemann et al., 1999). The disturbed body weight evolution caused by malnutrition or overnutrition is accompanied respectively by a decrease (Fisher et al., 2002) or increase (Todd et al., 2007) in brain weight, when the nutritional manipulation occurs during the "brain growth spurt period". In this period, the nervous system is more vulnerable to external aggressions,

Table 2
Multiple CSD episodes in adult rats before *versus* after unilateral CES. During lactation, the pups were suckled in litters designated as large-, medium- and small-size litters (composed by 12, 6 and 3 pups, respectively), originating the malnourished (M; n = 10), well-nourished (W; n = 23) and overnourished groups (Ov; n = 12). Values marked with (*) are significantly different from the corresponding pre-CES values (Fisher's test). The number of rats per group that presented greater number of CSD episodes is in parentheses.

		No. of multiple CSD episodes	
Nutritional groups	Before/After CES	Right hemisphere (non-stimulated)	Left hemisphere (stimulated)
W	**Before (a)**	2 (2)	2 (1)
	After (b)	1 (1)	37* (5)
W-sham	**Before (c)**	2 (2)	5 (3)
	After (d)	5 (3)	8 (3)
M	**Before (e)**	7 (4)	8 (3)
	After (f)	5 (3)	26* (7)
Ov	**Before (g)**	3 (3)	12 (7)
	After (h)	10 (5)	26 (8)

represented in this study by the malnutrition (Dobbing, 1968) or by overnutrition (Plagemann et al., 1999). Such brain weight oscillation probably resulted from the lesser/higher number and/or size of glial and neuronal cells, as well as from alterations in the events that cause neuronal maturation, which include reduction of processes such as dendritic development, synapse formation and myelination for malnourished rats (Morgane et al., 1993; Almeida et al., 2002). By the same logic, one can expect the opposite reaction for the overnourished animals. Rocha-de-Melo et al. (2004) assessed morphometric features of nitric oxide activity in rats. In the malnourished group, the labeled-cell density in white matter of area 17 was higher compared to the overnourished rats. According to these authors, there was a clear trend, in the malnourished group, to lower values of soma area, dendrite field and branches per neuron.

Following the above described evidence, it is reasonable to postulate that part of the changes in cortical excitability demonstrated by CSD in malnourished and overnourished rats could be due to such developmental modifications in brain structure that are dependent on nutrition state, as already stressed by others (Morgane et al., 1978; Rocha-de-Melo et al., 2004; Frazão et al., 2008). In this scenario, the lactation condition was a useful variable to induce changes in baseline excitability (similar to what occurs in neuropsychiatric disorders) and therefore also to study the effects of CES on CSD in animals with different cortical activity as induced by baseline nutritional status. In fact, CES effects were modulated by nutritional state induced brain alterations.

On the other hand, some neurologic disorders such as epilepsy present developmental origins (Qureshi and Mehler, 2010). CSD mechanisms have been postulated as sharing common features with those of human neural diseases (Leão, 1944; Dreier et al., 2012). Moreover, electrical cortical stimulation presents a suppressive effect on epileptogenicity when the stimulation is applied on the seizure onset zone (Kinoshita et al., 2005). Therefore, we believe CSD becomes an interesting model to evaluate the effects of CES on brain electrical activity. By the same logic, CSD can be considered as a useful tool to investigate the relationship between CES and underlying mechanisms of epilepsy, what can provide further information to ameliorate the effects or progression of this disease on the organism life span.

In the present study, the well-nourished rats demonstrated the greatest increase of CSD velocity of propagation after CES. Although the electrical stimulation technique leads to an increase of CSD velocity of propagation in the stimulated hemisphere in the M and Ov groups, the velocity increases were significantly different after CES, according to the following crescent order Ov<W<M. This lower velocity of CSD propagation in Ov rats confirms previous results (Rocha-de-Melo et al., 2006). The amount of glial cells seems to influence these differences on CSD process in malnourished and overnourished rats. As mentioned previously, it is known that glial cells modulate CSD characteristics (Gardner-Medwin, 1981), thus the fact that the malnourished rats present reduced number of glial cells (Morgane et al., 1978, 1993) would explain differences in CSD velocity of propagation when compared to the other groups.

Besides these mechanisms related to early nutritional state and the CES effect on CSD propagation, it is important to underscore that CES effects on CSD propagation were seen in both hemispheres only in overnourished rats. With respect to the bilateral CES effect in Ov rats, it is known that the inter-hemispheric communication influences the CSD propagation (Pinto and Guedes, 2008). This inter-hemispheric communication or transcallosal inhibition can restrict the effects of CES to the ipsilateral hemisphere as observed in M and W rats, but in the Ov rats, we observed CES effects on CSD in the contralateral hemisphere, as observed by the lower CSD velocity in the contralateral hemisphere. Two potential explanations are that (1) in overnourished animals the possible increased amount of glial cells and also increased energetic demand would facilitate CES effects on cortical excitability and consequently affecting CSD velocity of propagation also in the contralateral hemisphere or, (2) alternatively, overnourishment in these animals might disrupt inhibitory inter-hemispheric callosal connections.

Another potential mechanism to explain the differential effects of CES in CSD according to the nutritional state might be related to the influence of increased energetic demand. In other words, overnourished rats present hyperglycemia, higher triglyceride levels and insulin resistance (Plagemann et al., 1999). It has been shown that hyperglycemia (Costa-Cruz and Guedes, 2001) and high-fat diet (Paixão et al., 2007) impair CSD propagation. In fact, the hyperlipidemia can also be provoked by a ketogenic diet, and this diet can also increase the levels of adenosine — a metabolite with a non-synaptic release, which can influence local neural activity (Masino et al., 2009). Thus, reduced CSD velocity of propagation found in overnourished rats could be explained by hyperglycemia and possibly by the increased levels of adenosine in these animals. On the other hand, Costa-Cruz and Guedes (2001) also demonstrated that hypoglycemia facilitates CSD velocity of propagation. In summary, these two conditions (over and malnourishment) can induce profound changes in cortical excitability and activity resulting in a significant change in CSD.

Conclusion

Our results from this exploratory approach suggest that CES can change cortical excitability also through non-synaptic mechanisms as observed by the modification of CSD. These effects are influenced by the nutritional state as well. As brain stimulation techniques such as rTMS, tDCS and CES have been used for the treatment of neuropsychiatric diseases accompanied by cerebral excitability alterations, it is important to consider whether these diseases are also associated with changes that can influence non-synaptic regulation of activity similarly to what have been seen by changes in nutritional status observed in this study. The results of this investigative approach acknowledge the putative effects of CES on brain spontaneous electrical activity and contribute to the understanding of these effects on disease progression, and, consequently, on the organism life span.

Conflict of interest statement

The authors declare that they have no competing interests.

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