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# Neonatal dexamethasone accelerates spreading depression in the rat, and antioxidant vitamins counteract this effect

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ABSTRACT

The use of dexamethasone (Dex) to treat chronic lung disease in preterm infants may produce adverse effects in the developing brain. Here, we evaluated the effects of neonatal Dex on the propagation of cortical spreading depression (CSD), and tested the action of vitamins C and E against the effect of Dex. Five groups of Wistar rats received, respectively: [1] no treatment (Naïve); [2] Vehicle (V); [3] tapering doses of Dex (Dex; 0.5 mg/kg, 0.3 mg/kg, and 0.1 mg/kg) on postnatal day (PND) 1–3; [4] Dex plus 200 mg/kg vitamin C and 100 mg/kg vitamin E (DexCE); [5] only vitamins C and E (CE). Vehicle and vitamins were administered on PND 1–6. CSD was recorded after the pups reached maturity (PND 60–70). The Dex-treated group presented with higher CSD velocities (mean values±SD, in mm/min: 4.14±0.22, $n=10$) compared with the control groups (Naïve: 3.52±0.13, $n=8$; V: 3.57±0.18, $n=10$; CE: 3.51±0.24, $n=10$; $p<0.05$ for all). Vitamins C and E antagonized this effect (DexCE group; CSD velocity: 3.43±0.12, $n=9$). No intergroup difference was observed concerning P-wave amplitude and duration. In all groups, after the cortex underwent CSD, the electrocorticogram (ECoG) amplitude increased approximately 50% compared with the baseline amplitude for the same animal (CSD-induced ECoG potentiation); however, no intergroup difference was observed. Data suggest that coadministration of antioxidant vitamins with Dex may be a helpful therapeutic strategy to reduce brain adverse effects of dexamethasone.



## 1. Introduction

Dexamethasone is a synthetic glucocorticoid hormone that is largely applied to preterm infants to prevent or treat chronic lung diseases (Choi et al., 2004; Doyle et al., 2014a, 2014b). However, neonatal treatment with glucocorticoids can produce unwanted side effects in the developing nervous system of children (Hitzert et al., 2014; Shinwell et al., 2000; Stark et al., 2001). Evidence from laboratory animals also indicates such adverse effects (Bhatt et al., 2013; Duksal et al., 2009;

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Ichinohashi et al., 2013; Kim et al., 2013; Menshanov et al., 2014; Neal et al., 2003; Sze et al., 2013; Zuloaga et al., 2011). These reports address the brain effects of dexamethasone on behavioral, biochemical, and morphological parameters. However, some other studies have demonstrated electrophysiological changes in animals previously treated with dexamethasone, including the appearance (Davidson et al., 2011) or modulation of epileptiform activity (Yılmaz et al., 2014). Electrophysiological alterations also include the modulation of synaptic plasticity-dependent phenomena, such as long-term potentiation (LTP) (Kamphuis et al., 2003; Lin et al., 2006; Wang et al., 2010). In this scenario, experimental investigation based on electrophysiological phenomena related to epilepsy and LTP is highly desirable. This is the case regarding the phenomenon known as cortical spreading depression (CSD), which has been studied by our group (Batista-de-Oliveira et al., 2012a; Torrente et al., 2014b; see Guedes, 2011 for a review) and by others (Footitt and Newberry, 1998; Dreier, 2011).

CSD has been experimentally described as a reversible and propagated wave of reduction of the spontaneous electrical activity of the cerebral cortex (Leão, 1944). This phenomenon occurs in response to electrical, chemical, or mechanical stimulation applied to one point of the cortical surface. Simultaneously with the depression of brain activity, a slow direct current (DC) potential change of the tissue has been described (Leão, 1947). CSD has been widely used to evaluate brain processes that depend on neural excitability (Batista-de-Oliveira et al., 2012b; Guedes et al., 2005; Lima et al., 2013), and a causal association between CSD and an LTP-like potentiation of spontaneous and evoked cortical electrical activity has been demonstrated both *in vitro* (Footitt and Newberry, 1998) and *in vivo* (Faraguna et al., 2010; Guedes et al., 2005; Souza et al., 2011).

Dexamethasone increases the presence of reactive oxygen species (ROS) in cultured hippocampus and cerebral cortex (McIntosh and Sapolsky, 1996), and reduces the basal activity of brain antioxidant enzymes (McIntosh et al., 1998). Considering the hypothesis that most of the adverse effects of dexamethasone are caused by oxidative stress, studies have employed the antioxidant vitamins C and E to counteract the adverse effects of this synthetic hormone (Camm et al., 2011; Herrera et al., 2010; Niu et al., 2013; Williams et al., 2012). These vitamins are able to minimize neuronal loss, as well as the oxidative stress produced in the brain by the dexamethasone treatment (Camm et al., 2011). However, no information is currently available regarding the effects of dexamethasone combined with antioxidant vitamins on brain electrical activity. In this context, the use of CSD has also been employed to study the actions of antioxidants on the brain (Abadie-Guedes et al., 2008; Guedes et al., 2012; Monte-Guedes et al., 2011).

The current *in vivo* study evaluated, in the albino rat, the long lasting effects of neonatal dexamethasone, combined or not with the administration of antioxidant vitamins C and E, on changes in CSD features and LTP-like electrocorticogram (ECoG)-potentiation associated with CSD.

## 2. Results

### 2.1. Body weight

The five experimental groups did not differ with respect to body weight on PND 1 and PND 2. From PND 3 to PND 6, rats previously treated intraperitoneally with dexamethasone and dexamethasone plus vitamins C and E (the Dex and DexCE groups, respectively) exhibited lower body weight than groups that were untreated (Naïve), vehicle-injected (V), and treated with both vitamin C and vitamin E (CE). However, no weight difference was observed when the pups reached PND 60 (Table 1).

### 2.2. CSD parameters

The 1-min application of a cotton ball (1–2 mm diameter) soaked with 2% KCl (approximately 270 mM) to a point of the occipital cortical surface was very effective in eliciting a single CSD episode that was propagated and sequentially recorded at two points on the parietal cortex. This is illustrated in the recordings shown in Fig. 1.

Measurements of the amplitude and duration of the CSD negative potential change revealed no intergroup difference; the mean amplitudes ranged from 7.50±1.45 mV to 8.22±2.24 mV, and the mean durations ranged from 72.18±3.98 s to 75.14±6.77 s (Table 2).

**Table 1 – Body weights of the five experimental groups of rats, intraperitoneally injected with dexamethasone (Dex), antioxidant vitamins (CE), or both (DexCE). Two additional control groups were injected only with the vehicles in which these drugs were dissolved (V) or received no treatment (Naïve).**

| | Body weight (g) | | | | | | |
|---|---|---|---|---|---|---|---|
| | PND1 | PND2 | PND3 | PND4 | PND5 | PND6 | PND60 |
| Naïve | 6.70±0.82 | 7.69±0.70 | 9.17±0.90 | 10.94±1.38 | 12.70±1.87 | 14.94±1.13 | 255.00±22.69 |
| V | 6.91±0.89 | 7.85±0.82 | 9.50±0.94 | 11.20±1.32 | 12.50±1.65 | 14.28±1,73 | 240.30±30.20 |
| Dex | 6.80±0.54 | 6.89±0.49 | 7.10±0.70* | 8.10±0.84* | 8.90±1.24* | 10.25±1.60* | 246.94±12.05 |
| DexCE | 6.95±0.76 | 7.20±0.79 | 7.80±1.06* | 9.20±1.16* | 10.35±1.13* | 11.75±1.06* | 244.44±24.96 |
| CE | 6.90±0.94 | 8.00±1.04 | 9.20±1.23 | 11.00±1.30 | 12.35±1.83 | 14.05±1.85 | 251.21±16.77 |

**PND**: postnatal day.
Data are reported as mean±S.D.
* Significantly different from the Naïve, V, and CE groups at the same age ($P<0.05$; one-way ANOVA plus the Holm–Sidak test).

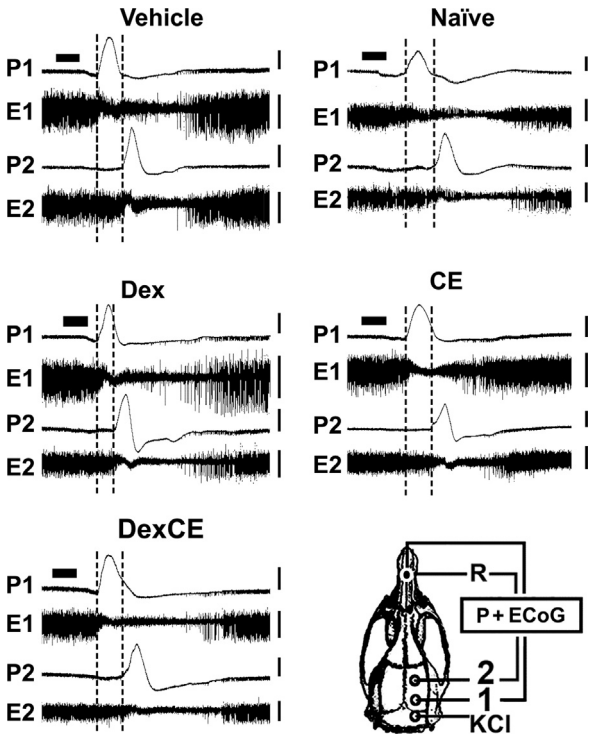


**Fig. 1 – Electrocorticogram (ECoG) and slow potential change (P) recordings during cortical spreading depression (CSD) in five rats representative of the experimental groups: Naïve, Vehicle, Dexamethasone (Dex), Dexamethasone plus vitamins C and E (DexCE), and only vitamins C and E (CE). The horizontal bars, which correspond to 1 min, show the time of KCl application to elicit CSD at one occipital cortical point in the right hemisphere. CSD elicitation by KCl was repeated at intervals of 30 min. CSD propagation in the occipital-to-frontal direction was monitored for 4 h at the two recording points in the parietal region of the same hemisphere. The bottom right skull diagram shows recording positions 1 and 2 and the position of the reference electrode (R), on the nasal bones, as well as the position of the KCl stimulus. The vertical calibration bars indicate 5 mV for the P recordings and 500 μV for the ECoG recordings (negative upwards).**

The Dex group exhibited higher CSD propagation velocity (4.14 mm/min±0.22, $n=10$) than the three control groups (Naïve: 3.52 mm/min±0.13, $n=8$; V: 3.57 mm/min±0.18, $n=10$; CE: 3.51±0.24 mm/min, $n=10$) ($p<0.05$). Treatment with Dex plus vitamins C and E (group DexCE) resulted in CSD velocity (3.43 mm/min±0.12, $n=9$) comparable to that of the controls (Fig. 2).

### 2.3. *ECoG potentiation associated with CSD*

In all groups, the amplitudes of the digitized ECoG (one 10-min sample from each hour) during the baseline period were compared with those of samples from the CSD period. The ECoG amplitude became higher in the CSD period compared with the baseline period (Fig. 3). Quantification of ECoG amplitudes revealed a CSD-related increase of approximately 50–60% ($p<0.05$; paired *t*-test). Although the Dex group exhibited a non-significant trend toward displaying fewer amplitude increases, no intergroup difference was observed (Table 3).

**Table 2 – Amplitude and duration of the slow potential change (P) during cortical spreading depression in young adult rats (PND 60-70) distributed in five experimental groups according to the previous (PND 1-6) hormonal and antioxidant treatments: (1) Naïve control (Naïve); (2) Vehicle control (V); (3) dexamethasone (Dex); (4) dexamethasone plus vitamins C and E (DexCE); and (5) control with only vitamins C and E (CE).**

| | CSD features | |
|---|---|---|
| | *P* amplitude (mV) | *P* duration (s) |
| Naïve | 7.50±1,45 | 74.80±8.56 |
| V | 8.22±2.24 | 72.18±3.98 |
| Dex | 7.75±2.80 | 75.14±6.77 |
| DexCE | 7.84±2.24 | 74.67±7.65 |
| CE | 7.78±2.36 | 73.09±6.33 |

Data are presented as mean±SEM of 9 CSD episodes elicited at 30-min intervals by 1-min 2% KCl application during the 4-h recording period. There was no difference between the experimental groups (one-way ANOVA).

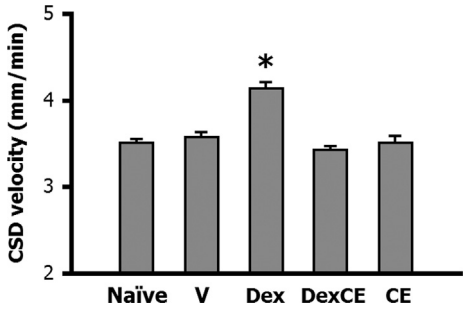


**Fig. 2 – Propagation velocities of cortical spreading depression (CSD) in 60- to 70-day-old rats that previously received no treatment (naïve group, $n=8$), vehicle (V, $n=10$), dexamethasone (Dex, $n=10$), dexamethasone with vitamins C and E (DexCE, $n=9$), or only vitamins C and E (CE, $n=10$). Data are presented as mean±SEM of nine CSD episodes elicited during the 4-h recording period, at 30-min intervals, by 1-min application of a cotton ball (1–2 mm diameter) soaked in 2% KCl. *$P<0.05$ compared with the other groups (ANOVA plus Holm–Sidak test).**

## 3. Discussion

In this study, we demonstrated for the first time that treatment with the glucocorticoid dexamethasone early in life (PND 1–6) clearly accelerates CSD propagation, and the coadministration of antioxidant vitamins with Dex counteracted that effect. Because the CSD acceleration was measured when the animals were quite developed (PND 60–70), we suggest that the effect of Dex on CSD propagation is permanent, or at least long-lasting, as suggested regarding other

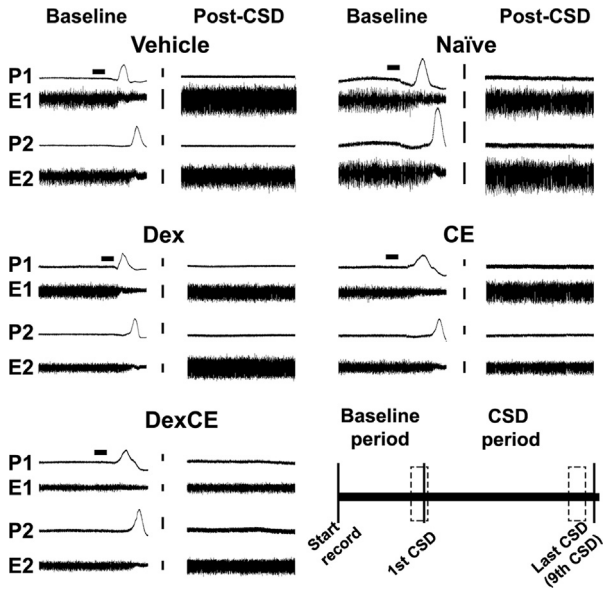


**Fig. 3 – Examples of recordings of spontaneous cortical activity (Electrocorticogram [E] and DC potential recordings [P]) on the right hemisphere of five animals representative of the five groups of this study, which show the potentiation of electrocorticographic activity after cortical spreading depression (CSD). Rats were treated with the vehicle used to dilute dexamethasone and vitamin C (Vehicle, saline solution), and vitamin E (Vehicle, olive oil), no treatment (naïve), dexamethasone (Dex), vitamins C and E (CE), or dexamethasone plus vitamins C and E (DexCE). For each animal, comparison between the baseline recordings (left traces) and the post-CSD recordings (right traces, taken after a series of seven episodes of CSD) revealed the potentiation of the electrocorticogram amplitude. The horizontal bars in P1 traces indicate the period (1 min) during which stimulation with 2% KCl was applied to the occipital region of the same hemisphere to elicit CSD, which was recorded at two parietal points 1 and 2 (see skull diagram in Fig. 1). Vertical bars correspond to −5 mV in P and −0.5 mV in E (negative upwards). The bottom-right time diagram of the recording session indicates the time-points (rectangles formed by interrupted lines) from which the baseline and post-CSD recording samples were taken (respectively, left and right rectangles). The beginning of the record, as well as the first and the last (9th) CSD episode are indicated in the diagram by the vertical solid lines.**

**Table 3 – Increase of the electrocorticogram (ECoG) amplitude after cortical spreading depression (CSD) in developed rats (PND 60–70). ECoG was recorded for 2 h to obtain a baseline value (before regular elicitation of CSD was initiated), followed by 4 h of recording, during which CSD was elicited at 30-min intervals. In each recording hour, one 10-min recording sample was analyzed with an algorithm implemented in MATLAB™. Data (mean ± SD) are presented as relative units (values of the normalized amplitudes in relation to the lowest value, which was considered equal to (1). Compared with the baseline period, the amplitudes after CSD were significantly higher ($P<0.05$; paired $t$-tests) in all groups, as indicated by the asterisks. There was no difference between the experimental groups (one-way ANOVA).**

| | EcoG amplitude (Relative units) | |
|---|---|---|
| | Before CSD | After CSD |
| Naïve | 1.05 ± 0.03 | 1.71 ± 0.20* |
| V | 1.06 ± 0.04 | 1.72 ± 0.33* |
| Dex | 1.06 ± 0.05 | 1.50 ± 0.23* |
| DexCE | 1.05 ± 0.03 | 1.68 ± 0.37* |
| CE | 1.04 ± 0.02 | 1.70 ± 0.42* |

effects of Dex on the brain (Ichinohashi et al., 2013; Li et al., 2014; Vázquez et al., 2012). Mammalian brain development largely occurs early in life, during the perinatal period. In the rat, the lactation period is very sensitive to adverse environmental and nutritional conditions (Morgane et al., 1978; Smart and Dobbing, 1971). It has been demonstrated that Dex exposure early in life can change brain morphology and function in rodents, producing long-term effects (Ichinohashi et al., 2013; Li et al., 2014; Vázquez et al., 2012).

Clinical and experimental studies show that early-life DEX exposure exerts a lasting adverse effect on somatic growth (Flagel et al., 2002; Shrivastava et al., 2000). In line with those results, our DEX-treated rats weighed significantly less than those of the control groups. This adverse effect may be causally linked, at least in part, to inadequate nutritional intake during the postnatal period, to the rise in tissue catabolism, or to protein breakdown after Dex treatment (Leitch et al., 1999; Neal et al., 2004). Interestingly, Dex exposure during the late gestational period of the rat enhances serum levels of the anorexic factor leptin, and lowers the hypothalamic mRNA levels of the orexigenic factor NPY in progeny (Iwasa et al., 2014). Wang and colleagues (Wang et al., 2010) did not observe any differences in maternal care between Dex-treated and vehicle-treated rats; therefore, these authors argue that the somatic growth deficits observed in DEX-treated rat pups can hardly be explained by differences in maternal care versus control pups. This view is also supported by the present data.

CSD has been implicated in important neurological human disorders, such as epilepsy, migraine, and traumatic brain injury (Dreier et al., 2012; Noseda and Burstein, 2013; Torrente et al., 2014a). Abnormal EEG activity, including seizures, is commonly seen in preterm human newborns within 48 h of premature birth, and is associated with adverse outcomes (Hellström-Westas and Rosén, 2005; Logitharajah et al., 2009; Shah et al., 2012). In adult rats, Dex can modulate *status epilepticus*, resulting in better or worse outcome depending on the experimental paradigm (Al-Shorbagy et al., 2012; Duffy et al., 2014; Yılmaz et al., 2014). However, this issue has not yet been addressed in developing rats. Thus, it is critical to understand whether and how common clinical interventions, such as neonatal glucocorticoid therapy, affect brain electrical activity as the brain develops. Several studies have suggested alternative therapeutic approaches that aim to minimize the adverse effects of dexamethasone treatment on the brain early in life. These include handling (Claessens et al., 2012b), pharmacological manipulation of the serotonergic system (Nagano et al., 2012), use of statins (Tijsseling

et al., 2013) and favorable lactation conditions (Wang et al., 2010). In this context, antioxidant vitamins have been successfully employed to attenuate Dex-related physiological disturbances in both the cardiovascular system (Herrera et al., 2010; Niu et al., 2013) and the nervous system (Camm et al., 2011). The present data represent an experimental contribution to that, mainly considering that the antioxidant vitamins C and E reversed the CSD Dex effects.

Our findings using CSD can be explained by different mechanisms. We suggest that the following four factors are most likely involved, and deserve comment: oxidative stress, the glutamatergic system, glial activity, and myelin content. The most plausible hypothesis involves oxidative stress; evidence suggests that glucocorticoid treatment increases the production of ROS in cultured hippocampus and cerebral cortex (McIntosh and Sapolsky, 1996), and decreases the activity of cerebral antioxidant enzymes (McIntosh et al., 1998). Pandya et al. (2007) emphasize that repeated exposure to Dex can significantly influence the oxidative energy metabolism of brain mitochondria in young growing animals, as well as in adults. Camm et al. (2011) demonstrated that neonatal Dex treatment increases oxidative stress indexes in the cerebral cortex, and that the coadministration of Dex and antioxidant vitamins C and E minimizes them, in addition to reducing the loss of neuronal mass induced by Dex treatment. Several reports have related CSD to oxidation processes and/or antioxidant factors in the brain (Guedes et al., 2012). In addition, it has been observed (in accordance with our results) that oxidative stress increases CSD propagation velocity, while antioxidants decrease the velocity (Abadie-Guedes et al., 2008). It is important to highlight that CSD may induce neuronal injury and cell death in young rats from 25 to 35 days old (Jafarian et al., 2010; Sadeghian et al., 2012), but not in adult rats (Nedergaard and Hansen, 1988). At that early age, it is possible that Dex increases these harmful effects, and antioxidant vitamins administered during early life may protect the cerebral cortex against these injuries. This possibility shall be investigated in future experiments.

The glutamatergic system has an important role in brain development and in the functioning and plasticity of the central nervous system (McDonald and Johnston, 1990). It was reported recently that neonatal Dex exposure produces behavioral abnormalities in juvenile and adult mice by altering glutamatergic neurotransmission via the NMDA receptor, and an NMDA receptor antagonist is able to counteract these Dex-induced abnormalities in later life (Li et al., 2014). Therefore, we also consider it reasonable to postulate that Dex-induced disturbances in glutamatergic neurotransmission may result in CSD effects. Interestingly, endogenous release of excitatory amino acids and their action on the NMDA receptor play an important role in the initiation, propagation, and duration of CSD (Marrannes et al., 1988; Zhou et al., 2013). Our group has previously demonstrated a long-lasting facilitation of CSD in rats treated with monosodium glutamate early in life (Lima et al., 2013). Thus, it is tempting to suggest that Dex treatment at early developmental stages may hyperactivate NMDA receptors, contributing to the CSD acceleration that we report here.

Considering that CSD propagation depends largely on the finely balanced electrochemical equilibrium of interactions between neurons and glia, as well as the extracellular milieu (Martins-Ferreira et al., 2000), changes in the number and activity of glial cells might be related to the increased CSD propagation velocity in the present Dex-treated group. Claessens et al. (2012a) reported that neonatal Dex treatment causes significant reductions in astrocyte number and density in the hippocampus and corpus callosum. Unemura et al. (2012) also observed in *in vitro* and *in vivo* conditions that the astrocyte number decreases after glucocorticoids are applied, and suggest that this decrease occurs as a result of reduced expression of the glucocorticoid receptor.

The propagation velocity of CSD correlates inversely with cortical myelin content in rodents: whether toxic or autoimmune-induced, cortical demyelination accelerates CSD, while genetically engineered hypermyelination (neuregulin-1 type I transgenic mice) decelerates CSD (Merkler et al., 2009). Therefore, it is reasonable to associate the increased CSD propagation velocity in the Dex-treated group with the reduction of cortical myelin content. In line with this association, Kim et al. (2013) observed a decrease in myelin content and changes in the oligodendrocyte morphology after administering 0.5 mg/kg/d Dex on PND 1–3. However, hypomyelination was not observed when a lower dose of Dex was used (Camm et al., 2011). Additional studies are necessary to clarify the real role of each of these hypotheses. Also, another interesting hypothesis to be tested is the possible role played by cytokines, which increase after CSD (Kunkler et al., 2004) and activate microglia (Grinberg et al., 2011). The CSD-related increased production of cytokines could be modified by the Dex and/or antioxidant vitamin treatment, as employed in our study, and this must be clarified.

Changes in brain excitability also influence CSD propagation, lending support to the idea that CSD is a useful index of brain excitability (Souza et al., 2011). Because CSD is an excitability-related brain phenomenon, we evaluated the LTP-like ECoG potentiation produced in the cortical tissue by CSD propagation (Footitt and Newberry, 1998; Souza et al., 2011). Significant potentiation occurred in all groups, and no intergroup difference was observed, although the Dex group exhibited a non-significant trend toward displaying less ECoG potentiation. Interestingly, *in vitro* studies have provided evidence that Dex treatment early in life impairs LTP in the hippocampus, as well as memory processes (Kamphuis et al., 2003; Lin et al., 2006; Wang et al., 2010). The non-significant trend in the Dex group may be explained not only by changes in synaptic plasticity, but also via alteration of neuronal activity, which contributes to hyperexcitability after CSD (Ghadiri et al., 2012), and consequently to the present observed increase in ECoG amplitude.

In conclusion, the present *in vivo* study in young rats describes novel and enduring electrophysiological CSD effects that are attributed to previous treatment with Dex and/or the antioxidant vitamins C and E. The results allow us to draw two conclusions. First, Dex treatment increases CSD propagation velocity in a long-lasting manner. Second, this effect is abolished by concomitant treatment with vitamins C and E. We suggest that Dex therapy in premature organisms may benefit from the coadministration of these antioxidant vitamins concerning the adverse effects of glucocorticoids on the brain, although the appropriate extrapolation from

**Table 4 – Description of the treatments (intraperitoneal injections) given to the five experimental groups on postnatal days (PND) 1 to 6.**

| | Intraperitoneal injections | | | |
|---|---|---|---|---|
| | (PND1–PND3) | | (PND4–PND6) | |
| | 1st Injection | 2nd Injection | 1st Injection | 2nd Injection |
| **Naïve** | – | – | – | – |
| **V** | 0.9% NaCl | Olive oil | 0.9% NaCl | Olive oil |
| **Dex** | Dex | Olive oil | 0.9% NaCl | Olive oil |
| **DexCE** | Dex+vitamin C | Vitamin E | Vitamin C | Vitamin E |
| **CE** | Vitamin C | Vitamin E | Vitamin C | Vitamin E |

Animals received no injections (Naïve), vehicle (V; saline in the first injection and olive oil in the second injection), dexamethasone in the first injection and vehicle in the second injection (Dex), Dex+Vitamin C (first injection) and Vitamin E (second injection), or only vitamins C and E (in the first and second injections, respectively). Dexamethasone was administered on PND 1 to 3, and was discontinued from PND 4 to 6.

the rat brain to the human brain still requires further investigation.

## 4. Experimental procedures

### 4.1. Animals

Forty-seven newborn male Wistar rats from litters of 8 to 9 pups were used in this study. The animals were handled in accordance with the standards of the Ethics Committee for Animal Research of our university, which comply with the "Principles of Laboratory Animal Care" (NIH; Bethesda, USA).

Shortly after birth (PND 0), the pups were assigned to five experimental groups according to the hormonal and antioxidant treatments applied: (1) Naive Control (N; without any treatment); (2) Vehicle Control (V; injected only with vehicle solution); (3) Dexamethasone (Dex); (4) dexamethasone with vitamins C and E (DexCE); and (5) Control with Vitamins C and E (CE). During the treatment period (PND 1–6), each rat received two intraperitoneal injections daily (10 μL/g for each injection) due to the different solubilities of dexamethasone (dexamethasone-21-phosphate, disodium salt) and vitamin C [L-ascorbic acid] (both dissolved in 0.9% NaCl) versus vitamin E [(+)-α-tocopherol] (dissolved in olive oil). The three drugs were purchased from Sigma (St. Louis, MO, USA). Dexamethasone was administered on the three first postnatal days in tapering doses: 0.5 mg/kg (PND 1), 0.3 mg/kg (PND 2), and 0.1 mg/kg (PND 3). Vitamin C (200 mg/kg/day) and vitamin E (100 mg/kg/day) were applied daily on PND 1–6 at fixed doses, as previously described (Herrera et al., 2010; Camm et al., 2011; Williams et al., 2012; Niu et al., 2013). The vitamin solutions were prepared daily, shortly before the injections. Table 4 presents the experimental groups included in this study.

After weaning (PND 21), the pups were housed in polypropylene cages (51.0 cm × 35.5 cm × 18.5 cm; 3–4 rats per cage) with free access to water and commercial standard diet (PRESENCE, Purina, Brazil). The animal room was under a 12-h/12-h light/dark cycle (lights on at 6:00 a.m.), and room temperature was set at 23±1 °C. Body weight was recorded daily during the hormonal and antioxidant treatment (PND 1–6) and at PND 60.

### 4.2. CSD recording

When the pups reached 60–70 days of age, they were anesthetized with an intraperitoneal injection of 1000 mg/kg urethane plus 40 mg/kg chloralose, and three trephine holes were drilled on the right side of the skull. These holes were aligned in the frontal-occipital direction and paralleled to the midline. The recording session lasted 6 h; during the initial 2 h (baseline period), no CSD was elicited; during the last 4 h (CSD period), CSD was elicited at 30-min intervals by applying a cotton ball (1–2 mm diameter) soaked with 2% KCl solution (approximately 270 mM) to the posterior hole drilled on the occipital region for 1 min. The other two holes on the parietal region served as recording sites. We simultaneously recorded the DC slow potential change typical of CSD and the ECoG (band pass filters: 1–35 Hz) using two Ag–AgCl Agar-Ringer electrodes (one in each hole on the parietal region) against a common reference electrode of the same type placed on the nasal bones. The spontaneous cortical electrical activity was amplified and digitized with an MP100 or MP150 system (BIOPAC Systems, Inc, USA) and stored in an IBM-compatible computer for further analysis. The amplitude and duration of the typical CSD-negative DC potential change, as well as the CSD propagation velocity, were calculated. One 10-min recording sample of the digitized ECoG was selected per recording hour, and the average ECoG amplitude was calculated with an algorithm implemented in MATLAB™ (The MathWorks, Inc.). For each animal, ECoG amplitude data are presented as relative units (values of the normalized amplitudes in relation to the lowest sample value, which was considered equal to 1).

During the entire recording period, rectal temperature was maintained at 37±1 °C by means of a heating blanket. The electrodes' positions and the gains of the amplifiers remained unchanged throughout.

### 4.3. Statistical analysis

For intergroup comparisons (body weight, amplitude, duration, velocity, and ECoG potentiation) data were analyzed by one-way ANOVA, followed by the Holm–Sidak test. For intragroup comparisons of the ECoG analysis (baseline versus

CSD period) the paired t-test was used. Differences were considered statistically significant when $P \leq 0.05$.

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## REFERENCES

Abadie-Guedes, R., Santos, S.D., Cahú, T.B., Guedes, R.C.A., de Souza Bezerra, R., 2008. Dose-dependent effects of astaxanthin on cortical spreading depression in chronically ethanol-treated adult rats. Alcohol Clin. Exp. Res. 32, 1417–1421.

Al-Shorbagy, M.Y., El Sayeh, B.M., Abdallah, D.M., 2012. Diverse effects of variant doses of dexamethasone in lithium-pilocarpine induced seizures in rats. Can. J. Physiol. Pharmacol. 90, 13–21.

Batista-de-Oliveira, M., Lopes, A.A.C., Mendes-da-Silva, R.F., Guedes, R.C.A., 2012a. Aging-dependent brain electrophysiological effects in rats after distinct lactation conditions, and treadmill exercise: a spreading depression analysis. Exp. Gerontol. 47, 452–457.

Batista-de-Oliveira, M., Monte-Silva-Machado, K.K., Paiva, A.K., Lima, H., Fregni, F., Guedes, R.C.A., 2012b. Favorable and unfavorable lactation modulates the effects of electrical stimulation on brain excitability: a spreading depression study in adult rats. Life Sci. 91, 306–311.

Bhatt, A.J., Feng, Y., Wang, J., Famuyide, M., Hersey, K., 2013. Dexamethasone induces apoptosis of progenitor cells in the subventricular zone and dentate gyrus of developing rat brain. J. Neurosci. Res. 91, 1191–1202.

Camm, E.J., Tijsseling, D., Richter, H.G., Adler, A., Hansell, J.A., Derks, J.B., Cross, C.M., Giussani, D.A., 2011. Oxidative stress in the developing brain: effects of postnatal glucocorticoid therapy and antioxidants in the rat. PLoS One 6, e21142.

Choi, C.W., Hwang, J.H., Shim, J.W., Ko, S.Y., Lee, E.K., Kim, S.S., Chang, Y.S., Park, W.S., Shin, S.M., 2004. Decreasing incidence of chronic lung disease despite the gradual reduction of postnatal dexamethasone use in very low birth weight infants. J. Korean Med. Sci. 19, 514–518.

Claessens, S.E.F., Belanoff, J.K., Kanatsou, S., Lucassen, P.J., Champagne, D.L., de Kloet, E.R., 2012a. Acute effects of neonatal dexamethasone treatment on proliferation and astrocyte immunoreactivity in hippocampus and corpus callosum: towards a rescue strategy. Brain Res. 1482, 1–12.

Claessens, S.E.F., Daskalakis, N.P., Oitzl, M.S., de Kloet, E.R., 2012b. Early handling modulates outcome of neonatal dexamethasone exposure. Horm. Behav. 62, 433–441.

Davidson, J.O., Quaedackers, J.S.L.T., George, S.A., Gunn, A.J., Bennet, L., 2011. Maternal dexamethasone and EEG hyperactivity in preterm fetal sheep. J. Physiol. 589, 3823–3835.

Doyle, L.W., Ehrenkranz, R.A., Halliday, H.L., 2014a. Late (>7 days) postnatal corticosteroids for chronic lung disease in preterm infants. Cochrane Database Syst. Rev. 5 (CD001145).

Doyle, L.W., Ehrenkranz, R.A., Halliday, H.L., 2014b. Early (<8 days) postnatal corticosteroids for preventing chronic lung disease in preterm infants. Cochrane Database Syst. Rev. 5 (CD001146).

Dreier, J.P., 2011. The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. Nat. Med 17, 439–447.

Dreier, J.P., Major, S., Pannek, H.W., Woitzik, J., Scheel, M., Wiesenthal, D., Martus, P., Winkler, M.K., Hartings, J.A., Fabricius, M., Speckmann, E.J., Gorji, A., 2012. Spreading convulsions, spreading depolarization and epileptogenesis in human cerebral cortex. Brain 35 (Pt 1), 259–275.

Duffy, B.A., Chun, K.P., Ma, D., Lythgoe, M.F., Scott, R.C., 2014. Dexamethasone exacerbates cerebral edema and brain injury following lithium-pilocarpine induced status epilepticus. Neurobiol. Dis. 63, 229–236.

Duksal, F., Kilic, I., Tufan, A.C., Akdogan, I., 2009. Effects of different corticosteroids on the brain weight and hippocampal neuronal loss in rats. Brain Res. 1250, 75–80.

Faraguna, U., Nelson, A., Vyazovskiy, V.V., Cirelli, C., Tononi, G., 2010. Unilateral cortical spreading depression affects sleep need and induces molecular and electrophysiological signs of synaptic potentiation in vivo. Cereb. Cortex 20, 2939–2947.

Flagel, S.B., Vázquez, D.M., Watson, S.J., Neal, C.R., 2002. Effects of tapering neonatal dexamethasone on rat growth, neurodevelopment, and stress response. Am. J. Physiol. 282, R55–R63.

Footitt, D.R., Newberry, N.R., 1998. Cortical spreading depression induces an LTP-like effect in rat neocortex in vitro. Brain Res. 781, 339–342.

Ghadiri, M.K., Kozian, M., Ghaffarian, N., Stummer, W., Kazemi, H., Speckmann, E.-J., Gorji, A., 2012. Sequential changes in neuronal activity in single neocortical neurons after spreading depression. Cephalalgia 32, 116–124.

Grinberg, Y.Y., Milton, J.G., Kraig, R.P., 2011. Spreading depression sends microglia on Lévy flights. PLoS One 6 (4), e19294, http://dx.doi.org/10.1371/journal.pone.0019294.

Guedes, R.C.A., Tsurudome, K., Matsumoto, N., 2005. Spreading depression in vivo potentiates electrically-driven responses in frog optic tectum. Brain Res. 1036, 109–114.

Guedes, R.C.A., 2011. Cortical spreading depression: a model for studying brain consequences of malnutrition. In: Preedy, V.R., Watson, R.R., Martin, C.R. (Eds.), Handbook of Behavior, Food and Nutrition. Springer, New York, NY, pp. 2343–2354.

Guedes, R.C.A., Abadie-Guedes, R., Bezerra, R.D.S., 2012. The use of cortical spreading depression for studying the brain actions of antioxidants. Nutr. Neurosci. 15, 111–119.

Hellström-Westas, L., Rosén, I., 2005. Electroencephalography and brain damage in preterm infants. Early Hum. Dev. 81, 255–261.

Herrera, E.A., Verkerk, M.M., Derks, J.B., Giussani, D.A., 2010. Antioxidant treatment alters peripheral vascular dysfunction induced by postnatal glucocorticoid therapy in rats. PLoS One 5, e9250.

Hitzert, M.M., Van Braeckel, K.N.J.A, de Bok, M., Maathuis, C.G.B., Roze, E., Bos, A.F., 2014. Functional outcome at school age of preterm-born children treated with high-dose dexamethasone. Early Hum. Dev. 90, 253–258.

Ichinohashi, Y., Sato, Y., Saito, A., Ito, M., Watanabe, K., Hayakawa, M., Nakanishi, K., Wakatsuki, A., Oohira, A., 2013. Dexamethasone administration to the neonatal rat results in neurological dysfunction at the juvenile stage even at low doses. Early Hum. Dev. 89, 283–288.

Iwasa, T., Matsuzaki, T., Munkhzaya, M., Tungalagsuvd, A., Kawami, T., Murakami, M., Yamasaki, M., Kato, T., Kuwahara, A., Yasui, T., Irahara, M., 2014. Prenatal exposure to glucocorticoids affects body weight, serum leptin levels, and hypothalamic neuropeptide-Y expression in pre-pubertal female rat offspring. Int. J. Dev. Neurosci. 36C, 1–4.

Jafarian, M., Rahimi, S., BEHNAM, F., Hosseini, M., Haghir, H., Sadeghzadeh, B., Gorji, A., 2010. The effect of repetitive

spreading depression on neuronal damage in juvenile rat brain. Neuroscience 169, 388–394.
Kamphuis, P.J.G.H., Gardoni, F., Kamal, A., Croiset, G., Bakker, J.M., Cattabeni, F., Gispen, W.H., van Bel, F., Di Luca, M., Wiegant, V. M., 2003. Long-lasting effects of neonatal dexamethasone treatment on spatial learning and hippocampal synaptic plasticity: involvement of the NMDA receptor complex. FASEB J 17, 911–913.
Kim, J.W., Kim, Y.J., Chang, Y.P., 2013. Administration of dexamethasone to neonatal rats induces hypomyelination and changes in the morphology of oligodendrocyte precursors. Comp. Med. 63, 48–54.
Kunkler, P.E., Hulse, R.E., Kraig, R.P., 2004. Multiplexed cytokine protein expression profiles from spreading depression in hippocampal organotypic cultures. J. Cereb. Blood Flow Metab. 24, 829–839.
Leão, A.A.P., 1944. Spreading depression of activity in the cerebral cortex. J. Neurophysiol. 7, 359–390.
Leão, A.A.P., 1947. Further observations on the spreading depression of activity in the cerebral cortex. J. Neurophysiol. 10, 409–414.
Leitch, C.A., Ahlrichs, J., Karn, C., Denne, S.C., 1999. Energy expenditure and energy intake during dexamethasone therapy for chronic lung disease. Pediatr. Res. 46, 109–113.
Li, S., Fujita, Y., Zhang, J., Ren, Q., Ishima, T., Wu, J., Hashimoto, K., 2014. Role of the NMDA receptor in cognitive deficits, anxiety and depressive-like behavior in juvenile and adult mice after neonatal dexamethasone exposure. Neurobiol. Dis. 62C, 124–134.
Lima, C.B., Soares, G.S.F., Vitor, S.M., Castellano, B., Andrade da Costa, B.L.D.S., Guedes, R.C.A., 2013. Neonatal treatment with monosodium glutamate lastingly facilitates spreading depression in the rat cortex. Life Sci. 93, 388–392.
Lin, H., Huang, C., Hsu, K., 2006. Effects of neonatal dexamethasone treatment on hippocampal synaptic function. Ann. Neurol. 59, 939–951, http://dx.doi.org/10.1002/ana.20885.
Logitharajah, P., Rutherford, M.A., Cowan, F.M., 2009. Hypoxic-ischemic encephalopathy in preterm infants: antecedent factors, brain imaging, and outcome. Pediatr. Res. 66, 222–229.
Marrannes, R., Willems, R., De Prins, E., Wauquier, A., 1988. Evidence for a role of the N-methyl-D-aspartate (NMDA) receptor in cortical spreading depression in the rat. Brain Res. 457, 226–240.
Martins-Ferreira, H., Nedergaard, M., Nicholson, C., 2000. Perspectives on spreading depression. Brain Res. Rev. 32, 215–234.
McDonald, J.W., Johnston, M.V., 1990. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. Brain Res. 15, 41–70.
McIntosh, L.J., Hong, K.E., Sapolsky, R.M., 1998. Glucocorticoids may alter antioxidant enzyme capacity in the brain: baseline studies. Brain Res. 791, 209–214.
McIntosh, L.J., Sapolsky, R.M., 1996. Glucocorticoids increase the accumulation of reactive oxygen species and enhance adriamycin-induced toxicity in neuronal culture. Exp. Neurol. 141, 201–206.
Menshanov, P.N., Bannova, A.V., Dygalo, N.N., 2014. Dexamethasone suppresses the locomotor response of neonatal rats to novel environment. Behav. Brain Res. 271C, 43–50.
Merkler, D., Klinker, F., Jürgens, T., Glaser, R., Paulus, W., Brinkmann, B.G., Sereda, M.W., Stadelmann-Nessler, C., Guedes, R.C.A., Brück, W., Liebetanz, D., 2009. Propagation of spreading depression inversely correlates with cortical myelin content. Ann. Neurol. 66, 355–365.
Monte-Guedes, C.K.R., Alves, E.V.S., Viana-da-Silva, E., Guedes, R. C.A., 2011. Chronic treatment with ascorbic acid enhances cortical spreading depression in developing well-nourished and malnourished rats. Neurosci. Lett. 496, 191–194.
Morgane, P.J., Miller, M., Kemper, T., Stern, W., Forbes, W., Hall, R., Bronzino, J., Kissane, J., Hawrylewicz, E., Resnick, O., 1978. The effects of protein malnutrition on the developping nervous system in the rat. Neurosci. Biobehav. Rev. 2, 137–230.
Nagano, M., Liu, M., Inagaki, H., Kawada, T., Suzuki, H., 2012. Early intervention with fluoxetine reverses abnormalities in the serotonergic system and behavior of rats exposed prenatally to dexamethasone. Neuropharmacology 63, 292–300.
Neal, C.R., VanderBeek, B.L., Vázquez, D.M., Watson, S.J., 2003. Dexamethasone exposure during the neonatal period alters ORL1 mRNA expression in the hypothalamic paraventricular nucleus and hippocampus of the adult rat. Dev. Brain Res. 146, 15–24.
Neal, C.R., Weidemann, G., Kabbaj, M., Vázquez, D.M., 2004. Effect of neonatal dexamethasone exposure on growth and neurological development in the adult rat. Am. J. Physiol. 287, R375–R385.
Nedergaard, M., Hansen, A.J., 1988. Spreading depression is not associated with neuronal injury in the normal brain. Brain Res. 449, 395–398.
Niu, Y., Herrera, E.A., Evans, R.D., Giussani, D.A., 2013. Antioxidant treatment improves neonatal survival and prevents impaired cardiac function at adulthood following neonatal glucocorticoid therapy. J. Physiol. 591, 5083–5093.
Noseda, R., Burstein, R., 2013. Migraine pathophysiology: anatomy of the trigeminovascular pathway and associated neurological symptoms, CSD, sensitization and modulation of pain. Pain 154 (Suppl 1), S44–S53.
Pandya, J.D., Agarwal, N.A., Katyare, S.S., 2007. Dexamethasone treatment differentially affects the oxidative energy metabolism of rat brain mitochondria in developing and adult animals. Int. J. Dev. Neurosci. 25, 309–316.
Sadeghian, H., Jafarian, M., Karimzadeh, F., Kafami, L., Kazemi, H., Coulon, P., Ghabaee, M., Gorji, A., 2012. Neuronal death by repetitive cortical spreading depression in juvenile rat brain. Exp. Neurol. 233, 438–446.
Shah, D.K., Boylan, G.B., Rennie, J.M., 2012. Monitoring of seizures in the newborn. Arch. Dis. Child. Fetal Neonatal Ed. 97, F65–F69.
Shinwell, E.S., Karplus, M., Reich, D., Weintraub, Z., Blazer, S., Bader, D., Yurman, S., Dolfin, T., Kogan, A., Dollberg, S., Arbel, E., Goldberg, M., Gur, I., Naor, N., Sirota, L., Mogilner, S., Zaritsky, A., Barak, M., Gottfried, E., 2000. Early postnatal dexamethasone treatment and increased incidence of cerebral palsy. Arch. Dis. Child Fetal Neonatal Ed. 83, 177–181.
Shrivastava, A., Lyon, A., McIntosh, N., 2000. The effect of dexamethasone on growth, mineral balance and bone mineralisation in preterm infants with chronic lung disease. Eur. J. Pediatr. 159, 380–384.
Smart, J.L., Dobbing, J., 1971. Vulnerability of developing brain. Vi. Relative effects of foetal and early postnatal undernutrition on reflex ontogeny and development of behaviour in the rat. Brain Res. 33, 303–314.
Souza, T.K.M., e Silva, M.B., Gomes, A.R., de Oliveira, H.M., Moraes, R.B., de Freitas Barbosa, C.T., Guedes, R.C.A., 2011. Potentiation of spontaneous and evoked cortical electrical activity after spreading depression: in vivo analysis in well-nourished and malnourished rats. Exp. Brain Res. 214, 463–469.
Stark, A.R., Carlo, W.A., Tyson, J.E., Papile, L.-A., Wright, L.I.L., Shankaran, S., Donovan, E.F., Oh, W.I., Bauer, C.R., Saha, S., Poole, W.K., Stoll, B.J., 2001. Adverse effects of early dexamethasone treatment in extremely-low-birth-weight infants. N. Engl. J. Med. 344, 95–101.
Sze, C., Lin, Y., Lin, Y., Hsieh, T., Kuo, Y.M., Lin, C., 2013. The role of glucocorticoid receptors in dexamethasone-induced apoptosis

of neuroprogenitor cells in the hippocampus of rat pups. Mediators Inflamm. 2013, 628094, http://dx.doi.org/10.1155/2013/628094.

Tijsseling, D., Camm, E.J., Richter, H.G., Herrera, E.A., Kane, A.D., Niu, Y., Cross, C.M., de Vries, W.B., Derks, J.B., Giussani, D.A., 2013. Statins prevent adverse effects of postnatal glucocorticoid therapy on the developing brain in rats. Pediatr. Res., 1–7.

Torrente, D., Cabezas, R., Avila, M.F., García-Segura, L.M., Barreto, G.E., Guedes, R.C.A., 2014a. Cortical spreading depression in traumatic brain injuries: is there a role for astrocytes? Neurosci Lett. 565, 2–6.

Torrente, D., Mendes-da-Silva, R.F., Lopes, A.A.C., González, J., Barreto, G.E., Guedes, R.C.A., 2014b. Increased calcium influx triggers and accelerates cortical spreading depression in vivo in male adult rats. Neurosci. Lett. 558, 87–90.

Unemura, K., Kume, T., Kondo, M., Maeda, Y., Izumi, Y., Akaike, A., 2012. Glucocorticoids decrease astrocyte numbers by reducing glucocorticoid receptor expression in vitro and in vivo. J. Pharmacol. Sci. 119, 30–39.

Vázquez, D.M., Neal, C.R., Patel, P.D., Kaciroti, N., López, J.F., 2012. Regulation of corticoid and serotonin receptor brain system following early life exposure of glucocorticoids: long term implications for the neurobiology of mood. Psychoneuroendocrinology 37, 421–437.

Wang, Y., Huang, C., Hsu, K., 2010. The role of growth retardation in lasting effects of neonatal dexamethasone treatment on hippocampal synaptic function. PLoS One 5, e12806.

Williams, D.B., Wan, Z., Frier, B.C., Bell, R.C., Field, C.J., Wright, D. C., 2012. Dietary supplementation with vitamin E and C attenuates dexamethasone-induced glucose intolerance in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 302, R49–R58.

Yılmaz, T., Akça, M., Turan, Y., Ocak, H., Kamaşak, K., Yildirim, M., 2014. Efficacy of dexamethasone on penicillin-induced epileptiform activity in rats: an electrophysiological study. Brain Res. 1554, 67–72.

Zhou, N., Rungta, R.L., Malik, A., Han, H., Wu, D.C., MacVicar, B.A., 2013. Regenerative glutamate release by presynaptic NMDA receptors contributes to spreading depression. J. Cereb. Blood Flow Metab. 33, 1582–1594.

Zuloaga, D.G., Carbone, D.L., Hiroi, R., Chong, D.L., Handa, R.J., 2011. Dexamethasone induces apoptosis in the developing rat amygdala in an age-, region-, and sex-specific manner. Neuroscience 199, 535–547.