Brain effects of the lectin from *Canavalia ensiformis* in adult rats previously suckled in favorable and unfavorable conditions: A spreading depression and microglia immunolabeling study

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Objective: To evaluate in adult rats, previously suckled under favorable and unfavorable conditions, the brain electrophysiological and microglial effects of the treatment early in life with the lectin (ConA) from *Canavalia ensiformis*.

Methods: Male Wistar newborn rats ($n = 89$) were suckled under favorable or unfavorable conditions, represented by litters with 6–7 pups or 12–14 pups (groups N6 and N12, respectively). From postnatal days 5–24, they were treated intraperitoneally with 1 or 10 mg/kg ConA (groups L1 and L10, respectively), or with saline solution (group Sal), or no treatment (group Naïve). At 90–120 days of age, cortical spreading depression (CSD) was recorded at two parietal points for 4 hours, and CSD parameters (velocity of propagation and amplitude and duration of the DC slow potential change) were measured. Fixative-perfused brain sections were reacted with anti-Iba1 antibodies to quantify immunolabeled microglia.

Results: Compared with the control groups, ConA-treated animals dose-dependently presented with reduced CSD propagation velocities and increased amplitude and duration of the CSD slow potential change. Microglia Iba-1 immunoreactivity was lower in both nutritional groups treated with ConA, in comparison with the control groups. The CSD hemisphere presented with higher immunoreactivity compared with the CSD-free hemisphere.

Discussion: Attenuation in CSD propagation and microglia reaction was associated in adulthood with ConA treatment during brain development, indicating that the lectin can affect the electrophysiological and microglial development, and suggesting long-lasting protective action of the lectin on the rat brain, which is not impeded by the unfavorable suckling condition.

Keywords: Lectin, ConA, Brain electrophysiology, Nutritional state, Microglia

Introduction

Lectins are proteins of non-immune origin that have the ability of specifically binding to carbohydrate-based structures and mediate important physiological processes in living organism. They constitute a wide group of structurally diverse proteins, present in plants, animals, and fungi, which binds reversibly and specifically with free and conjugated carbohydrates or even with complex sugar structures.[1] Because of their remarkable physicochemical properties, lectins are used as biotechnological tool in studies of cell-surface recognition,[2,3] providing a sensitive detection system for changes in glycosylation and carbohydrate expression that may occur during embryogenesis, growth, and disease.[4]

The concanavalin A (ConA) obtained from the seeds of *Canavalia ensiformis* has been chosen as a lectin model in several studies. ConA was the first well isolated and characterized lectin, available commercially in a high pure degree, free of carbohydrate.[5] ConA is a tetrameric protein in physiological environment that binds specifically to α-D-mannose, α-D-glucose, and glucopyranosides,[6] forming a highly

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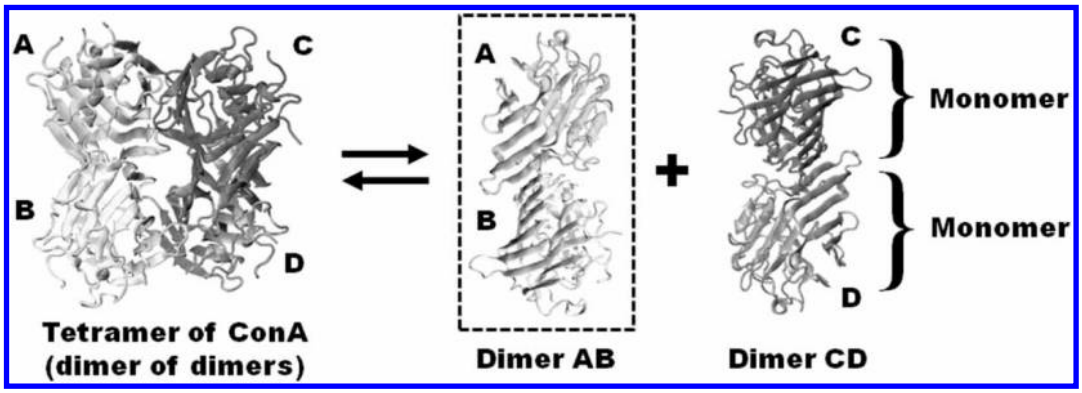

Figure 1 The ConA tetramer, composed of the equal subunits A–D, can be described as a dimer of dimers (AB and CB) in chemical equilibrium under pH change. Both forms have the same affinity by glucose and mannose. PDB file number 3CNA.[5] The structures were built using VMD software.[8]

specific 4:1 carbohydrate–ConA complex.[7] The structure of the ConA, built using visual molecular dynamics (VMD) software[8] is shown in Fig. 1.

ConA can bind specifically to different mannose and glucose residues,[6] modulating several events such as neuroplasticity,[9–11] current responses of ionotropic glutamate receptors,[12,13] presynaptic inhibition in sympathetic neurons,[14] and dopamine transporter desensitization.[15] Lectins also present several immunological effects, exerting a dose-dependent immunomodulation which depends on changes in the balance between pro-inflammatory (IL-4, IL-12, TNF-α, and IFN-γ) and anti-inflammatory (IL-6, IL-10) cytokines.[16]

From their receptor and cell-surface binding properties, it is reasonable to suppose that lectins may modulate neuronal cell communication, or even their differentiation and proliferation. Indeed, the endogenous lectin Galectin-1, a β-galactoside-binding protein, exhibits neuroprotective functions[17–21] and potent activity against neuroinflammation.[22,23] Exogenous lectins like ConA are able to modulate glutamatergic receptors in presynaptic neurons,[24,25] and ConBr, the lectin from *Canavalia brasiliensis*, protects hippocampal slices against glutamate neurotoxicity,[26] displaying neuroprotective and antidepressant activity. However, the possible effects of exogenous lectins on the microglia reaction (as an indicator of inflammatory response), as well as brain excitability have not been much investigated.

The excitability-related phenomenon known as cortical spreading depression (CSD), initially reported by Leão,[27] has been characterized as a phenomenon in which the neuronal excitability of the cerebral cortex is reversibly altered. CSD occurrence depends on the neuron–glial interactions. CSD can be provoked by electrical, mechanical, or chemical stimulation.[28] This response is characterized by a marked diminution (depression) of the cortical spontaneous or evoked electrical activity, lasting 1–2 minutes. Changes on the CSD wave features (velocity of propagation, intensity, and duration) can be caused by many different classes of drugs and nutritional/physiological conditions.[29] Several reports suggest that CSD seems to be involved in various pathophysiological events with clinical importance for humans, such as ischemia,[30] migraine,[31,32] and epilepsy.[33]

Several studies indicate that malnutrition early in life leads to biochemical,[34] structural,[35] and electrophysiological[36] changes of the nervous system as during development the brain presents a higher degree of plasticity. Early nutritional insults can have important effects on brain electrophysiological activity,[37] including a facilitation effect on the propagation of CSD.[36] The early malnourished brain may also respond differently to pharmacological treatments, concerning the CSD responses as compared with the normal brain responses.[38]

To improve the knowledge about brain lectin effects, we investigated here the microglia and CSD responses produced by the systemic treatment early in life with the lectin ConA in rats subjected to favorable and unfavorable lactation conditions, represented, respectively, by suckling the pups in small-size and large-size litters. Part of these data has been presented elsewhere.[39]

Materials and methods

Animals

Male Wistar newborn rats ($n = 89$) from distinct dams were randomly distributed into two groups regarding the nutritional conditions during the suckling period: (a) suckled in small litters, with 6–7 pups (group N6, with 41 pups), and (b) suckled in large litters, with 12–14 pups (group N12, with 48 pups). Decreasing or increasing the number of pups to be suckled by one dam is an easy and interesting method to produce positive or negative nutritional impact early in life, with modulation of brain electrophysiological and histological organization.[37,40,41]

The animals were handled in accordance with the norms of the Ethics Committee for Animal Research

of the Universidade Federal de Pernambuco, Brazil (Approval Protocol no. 23076.031272/2010-53), which complies with the 'Principles of Laboratory Animal Care' (National Institutes of Health, Bethesda, MD, USA). The animals were housed in polypropylene cages ($51 \times 35.5 \times 18.5$ cm^3) in a room with temperature maintained at 22 ± 1°C with a 12:12-hour light–dark cycle (lights on at 7:00 a.m.), and with free access to water and a lab chow diet with 23% protein (Purina do Brazil Ltd, São Paulo, Brazil).

Experimental groups and lectin treatment

The animals were treated from the 5th to the 24th day of postnatal life with daily intraperitoneal injections of saline (N6-Sal and N12-Sal groups; $n = 10$ for each group) or 1 mg/kg lectin ConA or 10 mg/kg lectin (purchased from Sigma Co., St Louis, MO, USA) dissolved in saline (groups N6-L1 and N12-L1, and groups N6-L10 and N12-L10; $n = 10$, 10, 10, and 17, respectively). Two groups of 'naïve' (non-injected) pups were used as additional controls (groups N6-Nv and N12-Nv; $n = 11$ and 11, respectively).

CSD recording

When the pups became adults (postnatal days 90–120), CSD was recorded, for a 4-hour period, as previously described.[42] Briefly, under anesthesia (1 g/kg urethane plus 40 mg/kg chloralose, intraperitoneal), three trephine holes (2–3 mm diameter) were drilled on the right side of the skull. In the first hole, on the frontal bone, CSD was triggered at 20 minutes intervals by applying a cotton ball (1–2 mm diameter) soaked in 2% KCl solution for 1 minute. The amplitude of electrocorticographic depression and the slow potential change, typical of a propagating CSD wave, were recorded simultaneously at two parietal points on the cortical surface using a pair of Ag/AgCl agar-Ringer electrodes separated by a fixed distance for each pair (4–5.5 mm for different pairs). These electrodes are plastic conic pipettes (5 cm length, 0.5 mm tip inner diameter) filled with Ringer solution and solidified with the addition of 0.5% agar, into which a chlorided silver wire was inserted. A third electrode of the same type was placed on the nasal bones and used as a common reference. During the recording session, rectal temperature was continuously monitored and maintained at 37 ± 1°C with a heating blanket. For all CSD episodes, we calculated the amplitude and duration of the negative slow potential shifts of the CSD waves, as well as their velocity of propagation, based on the time spent for a CSD wave to cross the interelectrode distance.

Body and brain weights

Body weights were measured at postnatal days 7, 14, 21, 30, 60, and 90. Brains were weighed on the day of CSD recording (90–120 days).

Iba1 immunolabeling of microglia

Thirty-two rats from the eight experimental groups (four N6 groups and four N12 groups; $n = 4$ from each group) were perfused with 0.9% saline solution followed by 4% paraformaldehyde diluted in 0.1 M phosphate-buffered saline, pH 7.4. After being immersed in the fixative for 4 hours, the perfused brains were transferred to a 30% (w/v) sucrose solution for cryoprotection. Longitudinal serial sections (30 μm thickness) were obtained at −20°C with a cryoslicer (Cryostat Leica CM3050 S; Leica Biosystems, Wetzlar, Germany). Sections were immunolabeled with a polyclonal antibody against the ionized calcium-binding adapter molecule 1 (Iba1) to detect microglia (anti-Iba1, #019-19741; Wako Pure Chemical Industries Ltd, Osaka, Japan). Free-floating sections were subjected to endogenous peroxidase blocking (2% H_2O_2 in 70% methanol for 10 min); then, sections were incubated for 1 hour in blocking buffer solution (BB) containing 0.05 M Tris-buffered saline (TBS), pH 7.4; 10% fetal calf serum; 3% bovine serum albumin; and 1% Triton X-100. Next, sections were incubated overnight at 4°C with rabbit anti-Iba1 (1:1500 diluted in BB solution). After three washes with TBS plus 1% Triton, sections were incubated at room temperature for 1 hour with biotinylated anti-rabbit (1:500) secondary antibodies. Sections were then rinsed in TBS plus 1% Triton and incubated with horseradish peroxidase-conjugated streptavidin (1:500). The peroxidase reaction was visualized by incubating the sections in Tris buffer containing 0.5 mg/ml 3,3′-diaminobenzidine and 0.33 μl/ml H_2O_2. Finally, sections were mounted, dehydrated in graded alcohols and xylene, and coverslipped in Entellan®. Densitometric analysis of the cerebral cortex was performed bilaterally for each animal. The immunolabeling pattern of the right hemisphere, which suffered repeated episodes of CSD, was compared with that of the left hemisphere (control). In each section, we analyzed photomicrographs of four fields within the parietal cortex using Image J software (National Institutes of Health; version 1.46r). A Leica-DMLS microscope coupled to a Samsung high-level color camera (model SHC-410NAD) was used to obtain digital images from brain sections. Images from the parietal cortex immunoreacted for Iba1 were obtained with a 20× microscope objective. Care was taken to obtain the digital images using the same light intensity conditions. We analyzed the percentage of the area occupied by

Iba1-labeled cells, as well as their total immunoreactivity expressed in arbitrary units.

Statistics

Body and brain weights, as well as CSD propagation rates, amplitudes, and durations, and quantitative immunohistochemical analysis were compared between groups using two-way analysis of variance (ANOVA), including litter size (N6 and N12) and treatment (naïve, saline, L1, and L10) as factors, followed by a *post hoc* (Holm–Sidak) test when indicated. Differences were considered significant when $P \leq 0.05$. All values are presented in the text as mean ± standard deviations. The Sigmastat® version 3.10 statistical software was used for all analysis.

Results

Impact of the suckling conditions on the body and brain weight

In the N12 condition, the body weights were lower ($P < 0.05$) than in the N6 condition up to the 30th day of postnatal life (Fig. 2A and B). In each suckling condition, saline-treated and naïve animals displayed similar body weights, indicating no effect of saline injection. At 90 days of life, L10, but not L1, treatment was associated with lower body weights compared with the corresponding control groups. The brain weights were reduced in the N12 condition (range: from 1.5760 ± 0.0879 to 1.6075 ± 0.0854 g) compared with the N6 groups (range: from 1.6620 ± 0.0192 to 1.7343 ± 0.0346 g). Lectin treatment did not affect the brain weights of adult animals (Fig. 2C).

Impact of the early lectin treatment and suckling conditions on CSD propagation

CSD was successfully elicited in all groups by KCl 2% solution applied for 1 minute through the hole (2–3 mm in diameter) drilled on the frontal bone. The KCl-elicited CSD wave propagated and was recorded by the two electrodes (marked as 1 and 2 in the diagram of Fig. 3). In all recordings, the CSD slow potential change returned to the baseline value after 1–2 minutes. The early systemic treatment with 1 and 10 mg/kg lectin resulted, in both nutritional states, in longer latencies for a CSD wave to cross the interelectrode distance, as compared with the saline and naïve conditions; these latencies are delimited by the interrupted lines in the CSD traces of Fig. 3.

For each group, the CSD velocity of propagation was calculated as a mean of 10–12 consecutive CSD episodes (elicited at intervals ≥20 minutes) in each animal. The mean CSD velocities are shown in the Fig. 4. At 90–120 days of life, CSD propagation velocities (mean ± standard deviation, in mm/minute) in the small-size litter groups were: N6-Sal = 3.41 ± 0.10, N6-Nv = 3.40 ± 0.09, N6-L1 = 3.12 ± 0.14, and N6-L10 = 2.82 ± 0.18. Compared with the corresponding L6 groups, the L12 animals presented with higher CSD velocities ($P < 0.05$), confirming the facilitating effect of the unfavorable suckling condition.

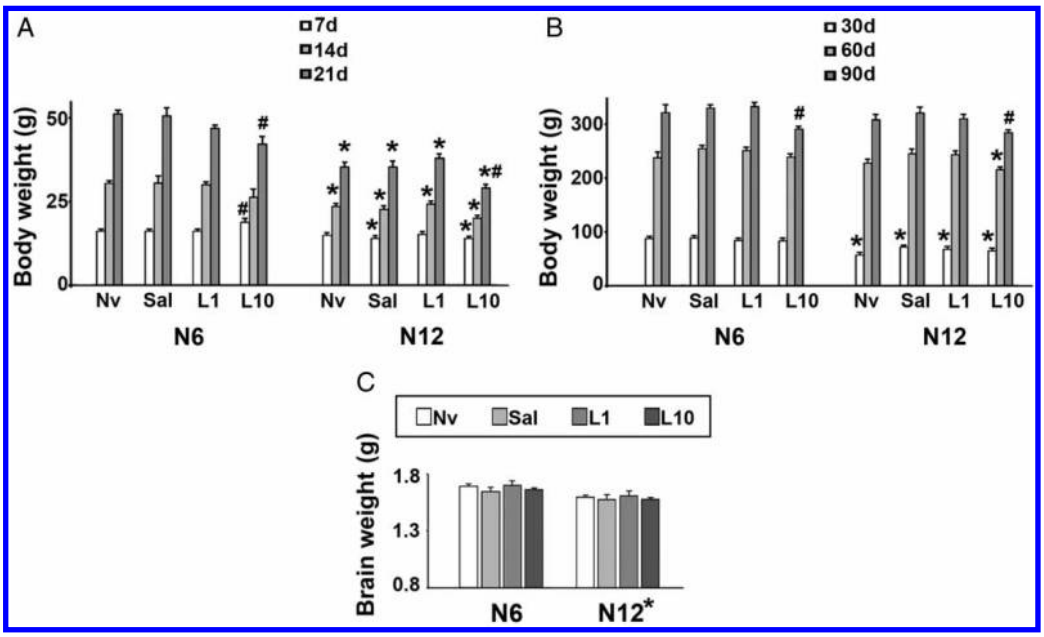

Figure 2 Body weights (mean ± SD) of rats suckled in litters formed by 6–7 pups or 12–14 pups (respectively, N6 and N12 groups) that were submitted to systemic lectin ConA administration (1 mg/kg – L1; or 10 mg/kg – L10), or saline (NaCl 0.9%) solution, or not submitted to any treatment (Naive group). The weights were measured at the postnatal days 7, 14, and 21 (A), and 30, 60, and 90 (B). The brain weights (C) were obtained at adulthood (90–120 days of life). Data are expressed as mean ± SEM. $^{*}P < 0.05$ compared with the corresponding N6 group. $^{\#}P < 0.05$ compared with the other treatment group in the same suckling condition (two-way ANOVA followed by Holm–Sidak test).

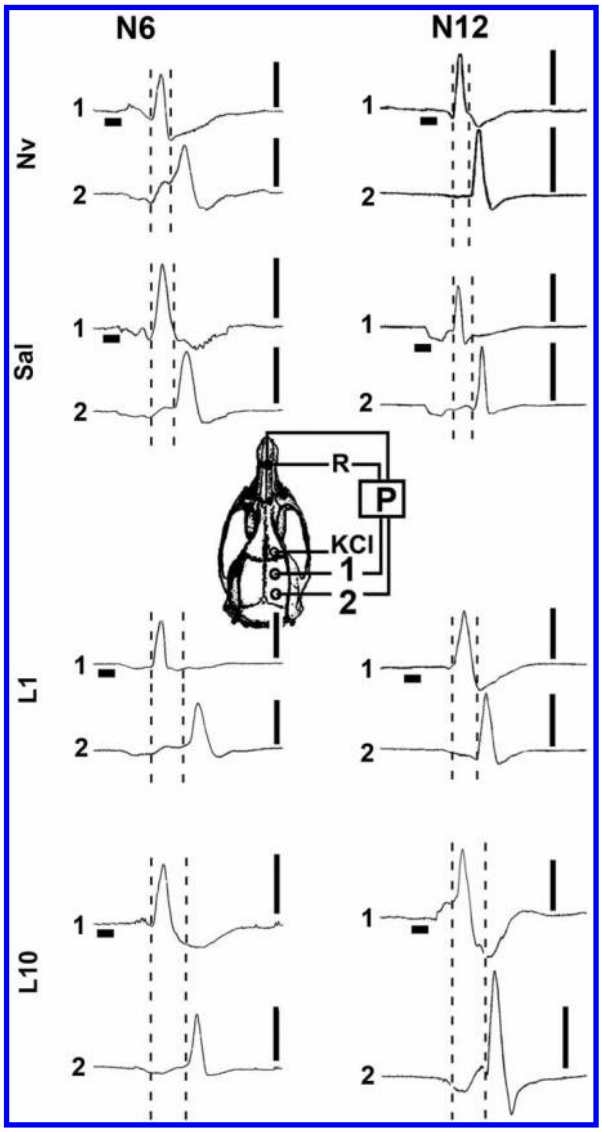

Figure 3 Examples of recordings of the slow potential change (P), typical of CSD, on two points of the right hemisphere of eight 90–120-day-old rats representative of the eight groups of this study. N6 and N12 are the two suckling conditions (suckling in litters with 6–7 and 12–14 pups, respectively). Nv, naïve group (no treatment); Sal, L1 and L10 are groups injected intraperitoneally with saline, 1 mg/kg/day lectin and 10 mg/kg/day lectin, respectively. The horizontal bars indicate the period (1 minute) of stimulation with 2% KCl on the frontal region of the right hemisphere, to elicit CSD. The vertical bars correspond to 10 mV (negative upwards). The vertical dashed lines delimitate the time spent for a CSD episode to propagate from recording points 1 to 2. The skull diagram shows the point of KCl application, the point where the reference electrode (R) was placed, and the recording positions 1 and 2, from which the traces marked with the same numbers were obtained. The distance between the two recording points is 4.5 mm in all cases.

The values for the L12 groups (in mm/minute) were: N12-Sal = 4.26 ± 0.16, N12-Nv = 4.22 ± 0.19, N12-L1 = 3.74 ± 0.13, and N12-L10 = 3.25 ± 0.16. ANOVA showed that in both L6 and L2 suckling conditions, the early systemic lectin-treatment dose-dependently decreased the CSD velocity ($P < 0.05$) when

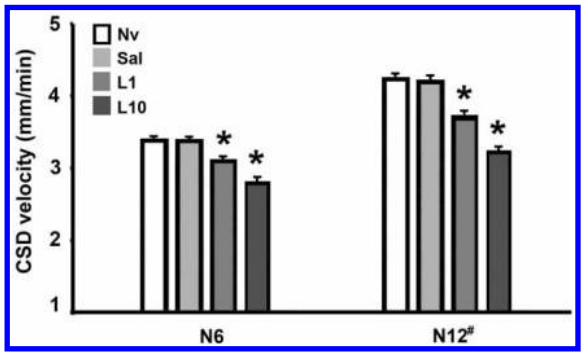

Figure 4 CSD velocities of propagation in adult rats (90–120-day-old) suckled in litters with 6–7 or 12–14 pups (respectively, groups N6 and N12), and treated during the lactation period with saline (Sal), 1 mg/kg/day lectin (L1) and 10 mg/kg/day lectin (L10). Nv, naïve group. Values are means ± SEM. *$P < 0.05$ compared with the controls (Sal and Nv) in the same suckling condition. #$P < 0.05$ compared with the N6 groups. (ANOVA plus Holm–Sidak test).

compared to the corresponding saline-treated and naïve controls.

The mean CSD amplitudes (in mV) and durations (in seconds) are presented in Table 1. In the N12 condition, ANOVA revealed that treatment with 10 mg/kg/day lectin (group L10) increased the amplitude and duration of CSD compared with the L1, and the saline and naïve controls. In addition, all N12 groups presented with shorter duration compared with the corresponding N6 group.

Iba1 immunohistochemistry and densitometric analysis

Fig. 5 depicts representative photomicrographs of specific immunolabeling of the calcium-binding protein Iba1 in the microglial cells of the parietal cortex from adult rats ($n = 4$ for each group) treated systemically early in life with saline solution, ConA (1 mg/kg, L1; or 10 mg/kg, L10) or no injection

Table 1 Amplitudes and durations of the negative slow potential shifts of CSD in adult rats previously suckled under favorable and unfavorable conditions (respectively N6 and N12) and treated with 1 mg/kg (L-1) and 10 mg/kg ConA lectin (L-10).

Groups	Treatment	Amplitude (mV)	Duration (s)
N6	Nv ($n = 8$)	10.4 ± 1.8	57.7 ± 6.2
	Sal ($n = 10$)	11.5 ± 2.9	58.8 ± 5.8
	L1 ($n = 9$)	10.5 ± 2.7	58.5 ± 5.4
	L10 ($n = 8$)	10.4 ± 2.0	57.1 ± 5.3
N12	Nv ($n = 8$)	10.2 ± 3.3	42.8 ± 5.0#
	Sal ($n = 9$)	10.9 ± 3.6	41.4 ± 3.2#
	L1 ($n = 8$)	11.5 ± 2.9	46.5 ± 4.1#
	L10 ($n = 10$)	15.5 ± 3.8*	53.4 ± 4.7#

They were compared with control groups treated with saline (Sal), or without any treatment (naïve; Nv). Data are expressed as mean ± S.D. The number of rats per group is in parentheses. *$p < 0.05$ compared with the other treatment groups in the same suckling (N12) condition. #$p < 0.05$ compared with the corresponding N6 value (ANOVA plus Holm-Sidak test).

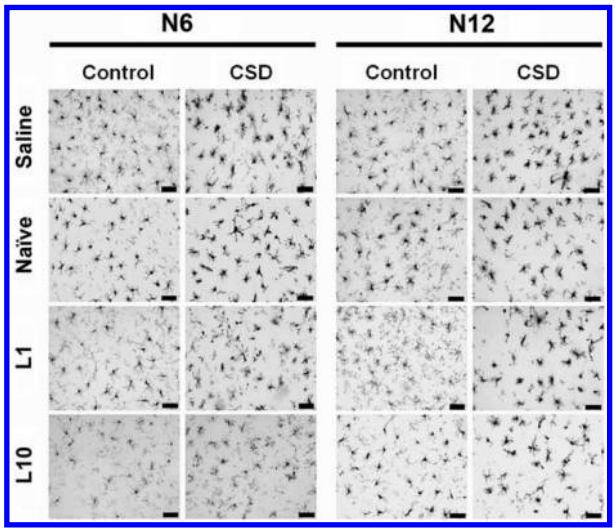

Figure 5 Representative gray-scale digital images of Iba1-immunolabeled microglia in coronal sections through the neocortex of eight adult rats, representative of the eight groups of this study. N6 and N12 are the two suckling conditions (suckling in litters with 6–7 and 12–14 pups, respectively). Nv, naïve group (no treatment); Sal, L1, and L10 are groups injected intraperitoneally with saline, 1 mg/kg/day lectin and 10 mg/kg/day lectin, respectively. CSD, the brain hemisphere in which CSD was elicited. Control, the contralateral hemisphere, in which CSD was not elicited. Scale bar, 20 μm.

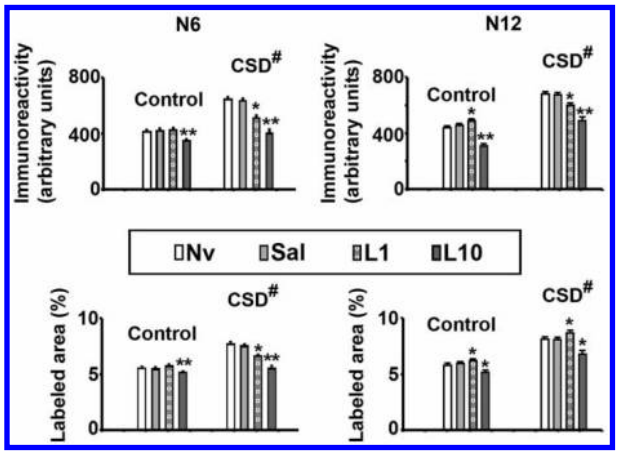

Figure 6 Quantitative measures of immunoreactivity (expressed as arbitrary units) and percent area occupied by the Iba1-labeled microglia in the neocortex of adult rats (90–120-day-old) suckled in litters with 6–7 or 12–14 pups (respectively, groups N6 and N12), and treated during the lactation period with saline (Sal), 1 mg/kg/day lectin (L1) and 10 mg/kg/day lectin (L10). Nv, naïve group. Data are expressed as means ± SEM of four animals per group (four brain sections per animal; four microscopic fields per section). The CSD values refer to the brain hemisphere in which CSD was elicited, while control values refer to the contralateral hemisphere, in which CSD was not elicited. **P* < 0.05 compared with the saline and naïve controls. ***P* < 0.05 compared with the other three groups. # indicates a significant difference in the CSD hemisphere compared with the control hemisphere.

(naïve group). Quantitative analysis of both hemispheres revealed an interhemispheric difference: the hemisphere in which CSD was elicited was more reactive than the contralateral hemisphere (Fig. 6). Compared with the controls, animals treated with 10 mg/ml ConA presented with a lower percentage of labeled area and lower immunoreactivity (expressed in arbitrary units) ($P < 0.05$; ANOVA followed by the Holm–Sidak test).

Discussion

In the present study, we extended our previous investigation on the identification of electrophysiological and microglial changes produced in the rat cortex by ConA, the lectin from *C. ensiformis*. Our findings confirm the effectiveness of ConA in decelerating CSD and reducing immunolabeling of Iba1-containing microglia, and demonstrate that such effects also occur in animals previously suckled in unfavorable (N12) condition. These two factors (i.e. ConA and unfavorable suckling) seem to be important determinants of the reduction in body weight, and in the CSD and microglia alteration as reported here, and they were not previously combined in a single study involving CSD. Data reinforce our previous suggestion that ConA administration during CNS development altered brain processes that are involved in the observed electrophysiological (CSD) and immunohistochemical (microglia labeling) effects. As ConA treatment occurred early in life and CSD acceleration, as well as the microglial reaction was observed at adulthood, we suggest that these effects of ConA are permanent, or at least long-lasting.

The ConA treatment early in life contributed also for body weight impairment, but did not affect the brain weight. Despite the absence of changes in brain weight, the ConA treatment during the suckling period might have altered glial development as indicated by changes in the Iba1 immunolabeling pattern presently observed. The suckling period is a critical phase for structural and functional maturation of the rat brain. The rapid execution of the normally programmed brain developmental processes results in adequate neuronal migration, myelination, and gliogenesis;[37] under the lactation conditions of the present study, the ConA treatment might have attenuated microglia reaction, as our Iba1 findings suggest. Changes in glial cells are relevant for the CSD findings as there is evidence[43] indicating that glial cells participate in the resistance that the cortical tissue normally presents to CSD propagation. Experimental manipulations can reduce or augment the cortical resistance to CSD, resulting, respectively, in higher or lower propagation rates.[36] Therefore, the estimation of changes in brain CSD susceptibility can be easily achieved by determining the velocity of CSD propagation under CSD-facilitating or CSD-impairing

conditions, and this contributes for the understanding of the electrophysiological processes that underlie brain excitability changes and related diseases (e.g. epilepsy).[33,44] The present data reinforce the implication of ConA treatment as a CSD-impairing and microglia reaction reducing factor.

To the best of our knowledge, there is no available information in the literature on the long-lasting electrophysiological effects of ConA on the CSD phenomenon. The mechanisms by which ConA influences CSD are unknown. One possible mechanism would be via modulation of the glutamatergic system. The regulation of brain excitability in both humans[45] and laboratory animals[46,47] is a process dependent on the glutamatergic system. Recent experimental evidence suggests that the lectin from *C. brasiliensis* can protect hippocampal slices against glutamate neurotoxicity.[26] Interestingly, ConA can modulate the glutamatergic system,[24] which supports the glutamate hypothesis for the ConA action on CSD. It is important to mention that both glutamate receptors modulation[42,48] and glial activity[49] can influence the CSD phenomenon. However, a pure glutamatergic mechanism does not explain the increase in CSD amplitudes in the high-dose ConA-treated group. At the moment one cannot exclude the possibility of other mechanisms, as for example the direct or indirect lectin action on nitrergic- and free radical formation processes,[50] as well as modulation on ionic buffering[51] and neurotransmitter modulating mechanisms.[52]

Under brain energy-demanding conditions such as CSD, microglia reportedly can be transformed into *reactive glia.*[50] Our Iba1 labeling data confirm this assertion: in all groups, the hemisphere that suffered CSD presented with higher Iba1 immunoreactivity when compared with the contralateral, CSD-free hemisphere (see Figs. 5 and 6). In this scenario, the treatment with ConA in the lactation period attenuated microglial Iba1 immunolabeling, suggesting a long-lasting protective action of ConA that could be detected at adulthood. Our novel Iba1 data in the cortex of ConA-treated rats compellingly require a new line of investigation to elucidate whether the CSD changes described here are a consequence of alterations in microglial reactivity.

In our study, suckling in large litters (N12 groups) certainly led to some degree of nutritional deficiency, confirming previous reports.[37] We can conclude that the increase in the number of pups during the lactation period was effective in producing malnutrition, which was confirmed by the lower brain and body weights, observed in the malnourished groups, when compared to their respective well-nourished controls. The decreased number and/or size of cell elements, as well as alterations in the events that cause neuronal maturation (e.g. reduction of processes like dendritic development, synapse formation, and myelination), are probably the causal factors of early malnutrition.[37,53] In the malnourished rat brain, CSD propagates with higher CSD velocities compared with the normally suckled animal.[54] Early malnutrition also increases brain cell packing density, impairs myelin formation and gliogenesis,[37] and reduces brain glutamate uptake,[54] which increases the extracellular glutamate. All these malnutrition-induced effects facilitate CSD propagation,[36] and this has been confirmed presently.

Regarding the possible application of the present findings to human health, the protective action of lectins via the glutamatergic system[26] deserves some comment. It is important to highlight that about 80% of all synapses are glutamatergic.[55] Therefore, excitotoxicity can be generated from excessive glutamatergic activation, leading to neurological diseases, and lectin molecules that counteract excessive glutamatergic activity can be important putative tools to treat or prevent brain diseases. This appears to be the case of ConA and ConBr, the lectin of *C. brasiliensis*, a lectin with the same carbohydrate affinity and with extensive amino acid sequence similarity to ConA.[56] We believe that searching for the cellular and molecular mechanisms underlying the action of ConA and other carbohydrate-binding lectins on the brain's electrophysiological and microglial properties is a very important task that shall be continued.

In summary, we characterized brain electrophysiological (CSD) and immunohistochemical (Iba1 microglia labeling) effects of the lectin ConA, from *C. ensiformis*, comparing them in favorable and unfavorable suckling conditions. We conclude that ConA dose-dependently decelerates CSD propagation, and attenuates immunoreactivity of the Iba1-containing microglia. These effects are not impeded by unfavorable suckling (N12) condition. Finally, the presence of CSD in one cerebral hemisphere was associated with increased microglial reaction. As data advance the understanding of the mechanisms of cerebral electrophysiological and histological alterations induced by lectins, they might be helpful in developing novel therapeutic strategies devoted to treat excitability-related brain disorders.

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Disclaimer statements

Contributors

G.S.F.S.: planning and executing the experiments; helping in analyzing the data and in writing the manuscript; C.B.L.: planning and executing the experiments; helping in analyzing the data; L.C.C.: execution of experiments; taken care of experimental animals; N.V.: planning and executing the immunohistochemistry experiments; helping in analyzing the data; B.C.: planning, and supervising the execution of part of the experiments; analyzing and interpreting part of the data; help in writing the manuscript; R.C.A.G.: planning the experiments and supervising its execution; analyzing the data and writing the manuscript.

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Conflicts of interest

None.

Ethics approval

The experiments were approved by the Ethics Committee for Animal Research of the Universidade Federal de Pernambuco, Brazil (Approval Protocol no. 23076.031272/2010-53).

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