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Vatairea macrocarpa Lectin (VML) Induces Depressive-like Behavior and Expression of Neuroinflammatory Markers in Mice

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Abstract Lectins are proteins capable of reversible binding to the carbohydrates in glycoconjugates that can regulate many physiological and pathological events. Galectin-1, a β-galactoside-binding lectin, is expressed in the central nervous system (CNS) and exhibits neuroprotective functions. Additionally, lectins isolated from plants have demonstrated beneficial action in the CNS. One example is a lectin with mannose-glucose affinity purified from *Canavalia brasiliensis* seeds, ConBr, which displays neuroprotective and antidepressant activity. On the other hand, the effects of the galactose-binding lectin isolated from *Vatairea macrocarpa* seeds (VML) on the CNS are largely unknown. The aim of this study was to verify if VML is able to alter neural function by evaluating signaling enzymes, glial and inflammatory proteins in adult mice hippocampus, as well as behavioral parameters. VML administered by intracerebroventricular (i.c.v) route increased the immobility time in the forced swimming test (FST) 60 min after its injection through a carbohydrate recognition domain-dependent mechanism. Furthermore, under the same conditions, VML caused an enhancement of COX-2, GFAP and S100B levels in mouse hippocampus. However, phosphorylation of Akt, GSK-3β and mitogen-activated protein kinases named ERK1/2, JNK1/2/3 and p38^{MAPK}, was not changed by VML. The results reported here suggest that VML may trigger neuroinflammatory response in mouse hippocampus and exhibit a depressive-like activity. Taken together, our findings indicate a dual role for galactose binding lectins in the modulation of CNS function.

Keywords Lectin · VML · Brain · Depression · Neuroinflammation

Abbreviations

ANOVA	Analysis of variance
BDNF	Brain derived neurotrophic factor
CNS	Central nervous system
ConA	Concanavalin A
COX	Cyclooxygenase
CRD	Carbohydrate recognition domain
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-regulated kinases
FST	Forced swimming test
Gal-1	Galectin-1
GFAP	Glial fibrillary acid protein
GSK	Glycogen synthase kinase
JNK	c-jun amino-terminal kinase
MAPK	Mitogen-activated protein kinase
TNF-α	Tumor necrosis factor

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Introduction

Glycobiology studies have revealed the importance of sugar chains present in the glycoproteins, glycolipids and cell-surface proteoglycans, as biosignaling molecules in intercellular communication and control of intracellular signaling pathways. These glycans have become recognized as participants in neural cell interactions in the developing and adult central nervous system (CNS) [1, 2]. The information and signals of carbohydrates can be recognized by proteins called lectins. These are a structurally heterogeneous class of proteins that have the ability to bind reversibly to carbohydrates present in the glycoconjugates on the cell surface or in cells [3, 4].

In the brain, endogenous lectins with affinity for mannose/glucose and galactose are reported to play important physiological roles [5–10]. Galectin-1 (Gal-1) is a β-galactoside-specific lectin that belongs to the galectin family and is expressed in the CNS [11]. This protein promotes enhancement of endogenous neural stem/progenitor cell proliferation and may decrease astrogliosis, improving neurological recovery of mice following ischemia [12–15]. Furthermore, gal-1 increases production of brain derived neurotrophic factor, which was associated with the prevention of neuronal death [7, 16]. Recent studies have also shown an important role of gal-1 in the formation of hippocampal memory, and it shows potent activity against neuroinflammation [17, 18].

Lectins from plants have been used as important tools in glycobiology [19, 20]. The lectin Concanavalin A (ConA) obtained from *Canavalia ensiformis* is a lectin with affinity for D-mannose/D-glucose. It has been used in the study of neuroplasticity [21, 22] and the isolation of synaptic glycoproteins and glutamate receptors [20, 23], as well as studies of neurotransmitter release [24] and modulation of receptors and transporters of monoamines [25]. Our group has demonstrated that a lectin with affinity for manose/glucose purified from *Canavalia brasiliensis* seeds (ConBr) reduced seizures and cell death induced by quinolinic acid [26], produced an antidepressant-like effect in the forced swimming test (FST) [27], and protected hippocampal slices against glutamate neurotoxicity [28].

The lectin from seeds of *Vatairea macrocarpa* (VML) is a protein with galactose/N-acetyl-galactosamine (Gal/Gal-Nac)-binding activity that can strongly bind to N-acetyl-galactosamine moieties. VML is constituted of four identical 26-kDa subunits, which, in solution, are folded into a stable tetramer [29]. The peripheral biological effects of VML include induction of leukocyte infiltration in rat paw edema [30], neutrophil migration to peritoneal cavity and macrophage activation via proinflammatory cytokine release, such as the tumor necrosis factor (TNF-α) [31, 32].

Neuroinflammatory and glial responses are associated with many neurological dysfunctions, including Alzheimer's disease and major depression [33–35]. Astrocyte activation in response to brain injury usually involves increase in glial fibrillary acid protein (GFAP) expression and changes in S100B protein levels [36–38]. Moreover, the activation and/or expression increment of cyclooxygenase-2 (COX-2), the rate-limiting enzyme in the formation of prostaglandins, by proinflammatory cytokines [39, 40] have also been implicated in the pathophysiology of depression [41]. The signaling pathways involved in COX-2 expression activation are diverse and include MAPKs and Akt/GSK cascades [42, 43].

The purpose of the present work was to evaluate the action of VML, a plant β-galactoside-binding lectin, on the neural function and on neurochemical parameters. Therefore, our study aimed to contribute with the comprehension of the possible mechanisms triggered by β-galactoside-binding lectin in the CNS function and on the neurochemical parameters of cell signaling as well as glial and inflamatory activity. Our previous studies had demonstrated neuroprotective effects of ConBr, a mannose/glucose plant lectin without signals of neuroinflammatory response [26–28]. However, ConBr also may display (in some models) proinflammatory activity in the peripheral tissue [44]. Therefore, there is no a necessary relationship of peripheral response with the CNS activity regarding the induction of inflammation. At moment, there are no studies in the literature which describe the effect of plant lectins with β-galactoside affinity on the CNS. Using the FST, it was found that VML administered by the intracerebrovetricular (i.c.v) route induces an increase in immobility time, an indicative of a depressive-like behavior. Additionally, an enhancement of COX-2, GFAP and S100B levels was observed in mouse hippocampus, suggesting the neuroinflammatory activity of VML. Hence, our findings provide basic knowledge regarding the central activity of VML and suggest a possible dual role for galactose binding lectins in the modulation of CNS function, considering the neuroprotective effects already demonstrated for galectin-1.

Materials and Methods

Purification of *Vatairea Macrocarpa* Lectin

Vatairea macrocarpa lectin was purificated from the crude extract of the seed by affinity chromatography on a Guar gum gel. The purity of samples was confirmed by 12.5 % electrophoresis gel following procedures described previously [45–47].

Vatairea macrocarpa lectin was dissolved in sterile saline (NaCl 0.9 %). Moreover, aiming to verify the

involvement of carbohydrate recognition domain (CRD) on the behavioral alterations induced by VML, the lectin was dissolved in a sterile saline solution containing galactose 0.1 M and maintained for 15 min at 25 °C [31]. The denatured lectin was prepared by thermal denaturation, boiling the solution content of VML for 10 min.

Animal Treatment

Male Swiss albino 6-week-old mice were used in this study. Animals were maintained at 21–23 °C with free access to water and food, under a 12:12 h light: dark cycle. All experimental procedures were approved by the UFSC Ethics Committee of Animal Experiments.

Vatairea macrocarpa lectin dissolved in sterile saline was administered by intracerebroventricular (i.c.v) route in a constant volume of 5 μL/site. The i.c.v injections were performed under ether anesthesia according to the procedure described previously by [48, 49]. Briefly, a 0.4 mm external diameter hypodermic needle attached to a cannula, which was linked to a 25 μL Hamilton syringe, was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mice. A volume of 5 μL was then administered into the left lateral ventricle. The injection was given over 30 s, and the needle remained in place for another 30 s in order to avoid the reflux of the substances injected. The injection site was 1 mm to the left from the mid-point on a line drawn through to the anterior base of the ears. To ascertain that the drugs were administered exactly into the cerebral ventricle, the brains were dissected and examined macroscopically after the test. Results from mice presenting misplacement of the injection site or any signs of cerebral haemorrhage were discarded from statistical analysis.

The behavioral analyses were performed 60 min after VML i.c.v injection. The animals were submitted to open-field task, and immediately after this, were submitted to the forced swimming test. In another set of experiments, animals received a VML injection and 60 min afterwards, they were immediately euthanized, hippocampi were dissected, and biochemical analyses were carried out.

Open-Field Task

To assess the effects of VML on locomotor activity, mice were evaluated in the open-field paradigm, as previously described [48–51]. Animals were individually placed in a wooden box ($40 \times 60 \times 50$ cm) with the floor divided into 12 rectangles. The numbers of squares crossed with all paws (i.e., crossings) were counted in a 6-min session. The apparatus was cleaned with a solution of 10 % ethanol between tests in order to hide animal clues.

Forced Swimming Test

Mice were individually forced to swim in an open cylindrical container (diameter 10 cm; height 25 cm), containing 19 cm of water (depth) at 25 ± 1 °C. The total duration of immobility was measured during a 6-min period as described previously [50–52]. Each mouse was considered to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water [53].

Western Blotting

To quantify MAPKs, Akt, GSK-3β phosphorylation and COX-2 immunocontent, Western blotting analysis was performed, as previously described [54]. Animals were euthanized by decapitation, brains were excised from the skull, and hippocampi were dissected into cold saline solution, placed in liquid nitrogen and then stored at −80 °C until use. Briefly, samples were mechanically homogenized in 400 μL of Tris 50 mM pH7.0, EDTA 1 mM, NaF 100 mM, PMSF 0.1 mM, Na$_3$VO$_4$ 2 mM, Triton X-100 1 %, glycerol 10 %, and Amresco Protease Inhibitor Cocktail catalog number M222 (Working concentration: AEBSF 0.50 mM, Aprotinin 0.30 μM, Bestatin 10.00 μM, E-64 10.00 μM, Leupeptin 10.00 μM, EDTA 50.00 μM). Lysates were centrifuged ($10,000 \times g$ for 10 min, at 4 °C) to eliminate cellular debris. The supernatants were diluted 1/1 (v/v) in Tris 100 mM pH 6.8, EDTA 4 mM and SDS 8 %, followed by boiling for 5 min. Thereafter, sample dilution (40 % glycerol, 100 mM Tris, bromophenol blue, pH 6.8) in the ratio 25:100 (v/v) and β-mercaptoethanol (final concentration 8 %) were added to each sample. Protein content was estimated by the method described by [55]. The same amount of protein (70 μg per lane) for each sample was electrophoresed in 10 % SDS-PAGE minigels and transferred to nitrocellulose membranes using a semi-dry blotting apparatus (1.2 mA/cm^2; 1.5 h). To verify transfer efficiency process, membranes were stained with Ponceau Stain.

The membranes were blocked with 5 % skim milk in TBS (Tris 10 mM, NaCl 150 mM, pH 7.5). The total and phosphorylated forms of MAPKs, Akt and GSK-3β as well as the total content of COX-2 and β-actin, were detected after overnight incubation with specific antibodies diluted in TBS-T containing 2 % BSA. The primary antibodies were dilluted 1:1,000 for anti-phospho-JNK1/2/3 (Sigma-Aldrich), anti-total-Akt (Sigma-Aldrich), anti-phospho GSK-3β (Cell Signaling), anti-total GSK-3β (Cell Signaling) and anti-COX-2 (Cell Signaling); 1:2,000 for anti-phospho-ERK1/2 (Sigma-Aldrich), anti-phospho-Akt (Cell Signaling); 1:5,000 for anti-total-JNK1/2 (Sigma-Aldrich);

1:10,000 for anti-phospho-p38^{MAPK} (Millipore) and anti-total-p38^{MAPK} (Sigma-Aldrich); 1:40,000 for anti-total-ERK1/2 (Sigma-Aldrich). Moreover, the membranes were incubated for 1 h at room temperature with horse radish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse antibody for detection of the proteins. The reactions were developed by chemiluminescence substrate (LumiGLO). All blocking and incubation steps were followed by washing 3× (5 min) with TBS-T (Tris 10 mM, NaCl 150 mM, Tween-20 0.1 %, pH 7.5). All membranes were incubated with mouse anti-β-actin (1:2,000) antibody to verify that equal amounts of proteins were loaded on the gel. The phosphorylation levels of MAPKs, Akt and GSK-3β were determined as a ratio of optical density (OD) of phosphorylated band/OD of total band, and the expression of COX-2 was determined as a ratio of OD of COX-2 band/OD of β-actin band. The bands were quantified using the Scion Image® software.

The antibody against ERK1/2 detected two bands: one at approximately 44 kDa and the second at approximately 42 kDa, corresponding, respectively, to the two ERK isoforms, ERK1 and ERK2. Anti-p38^{MAPK} detected a single band of approximately 38 kDa. Anti-JNK1/2/3 detected two bands, one at approximately 54 kDa and the second at approximately 46 kDa, corresponding, respectively, to the three JNK isoforms, JNK2/3 and JNK1. Anti-Akt detected a single band of approximately 60 kDa. The GSK-3β antibody detected a single band at 46 kDa, and the COX-2 antibody detected a single band at 70 kDa. The anti-β-actin antibody detected a single band of approximately 45 kDa.

Quantification of S100B and GFAP

S100B content in the hippocampus was measured by ELISA [56]. Fifty μL of sample plus 50 μL of Tris buffer were incubated for 2 h on a microtiter plate previously coated with monoclonal anti-S100B (SH-B1). Polyclonal anti-S100B was incubated for 30 min, and then peroxidase-conjugated anti-rabbit antibody was added for a further 30 min. A colorimetric reaction with o-phenylenediamine was measured at 492 nm. The standard S100B curve ranged from 0.019 to 10 ng/mL, and the results were expressed as a percentage of control group. ELISA for GFAP was carried out by coating the microtiter plate with 100 μL of diluted samples containing 30 μg of protein for 24 h at 4 °C. Incubation with a rabbit polyclonal anti-GFAP for 2 h was followed by incubation with a secondary antibody conjugated with peroxidase for 1 h at room temperature; the standard GFAP curve ranged from 0.1 to 10 ng/mL, and the results were expressed as a percentage of control group [56, 57].

Statistical Analysis

Comparisons between experimental and control groups were performed by one-way ANOVA followed by Duncan's Test. A value of $p < 0.05$ was considered to be significant.

Results

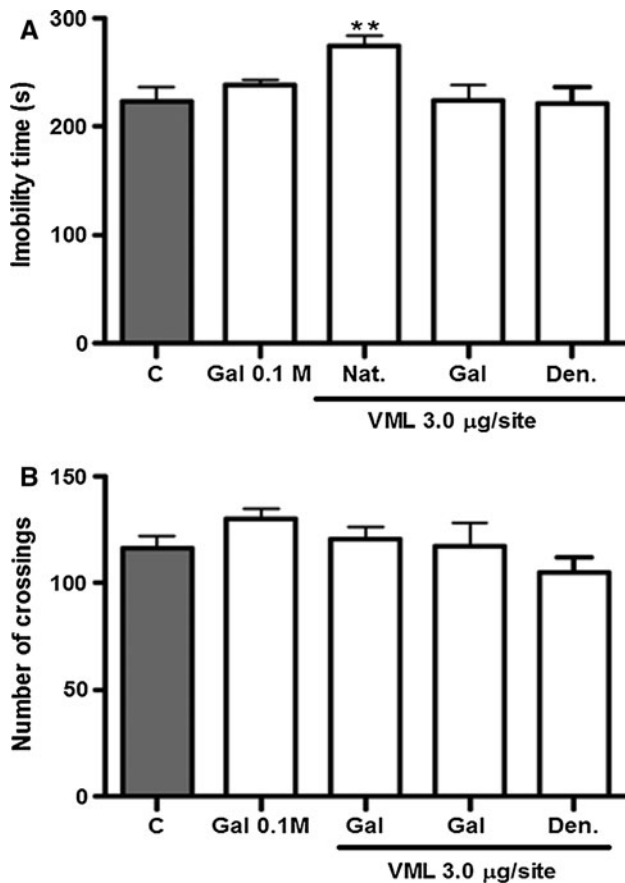### Depressive-Like Effect of VML in the FST

The results shown in Fig. 1a demonstrate that VML administered 60 min before FST increased the immobility time in the FST, as revealed by one-way ANOVA [F(3, 24) = 3.94, $p < 0.05$], indicating a depressive-like effect elicited by the lectin. Figure 1b shows that VML did not cause significant changes in ambulation in the open-field task, as revealed by one-way ANOVA [F(3, 24) = 0.88, $p = 0.47$].
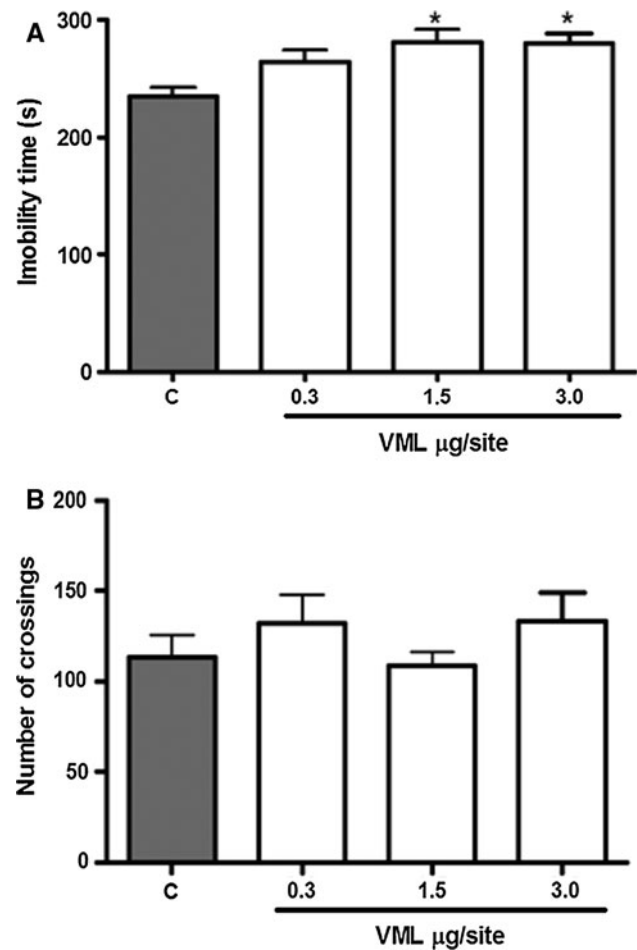
Fig. 1 Effect of VML on immobility time in the FST and number of crossings in the open-field task. Panel **a** shows the immobility time, and Panel **b** shows the number of crossings 60 min after VML i.c.v injection. Values are mean ± SEM. (n = 5–8). *$p < 0.05$, when compared with the control group (Duncan's Test)

Effect of VML Preincubated with Galactose or Denatured Lectin on the Depressive-like Behavior Induced by VML

Figure 2a shows that the incubation of VML preincubated with galactose (0.1 M), a well-known monosaccharide blocker of the lectin CRD, abolished the VML effect in the FST evaluated at 60 min after VML injection [$F_{(4, 31)} = 3.27$, $p < 0.05$]. This result indicates that the effect of VML in FST seems to be mediated by its carbohydrate-binding domain. Furthermore, denatured lectin had no effect on immobility time in the FST. Finally, neither blocked nor denatured lectin produced changes in mouse ambulation in the open-field task [$F_{(4, 31)} = 1.59$, $p = 0.20$], as shown in Fig. 2b.
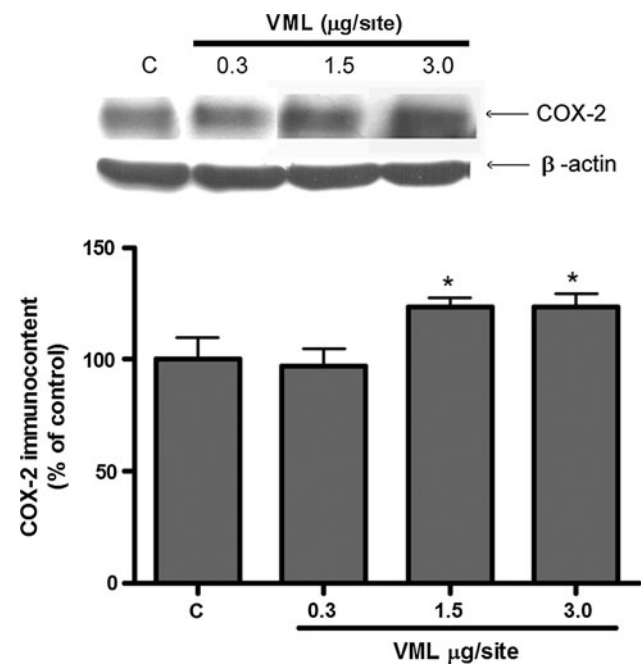
Proinflammatory Effect Induced by VML

To verify the influence of VML in inflammatory response in mouse hippocampus, the immunocontent of COX-2 were evaluated by Western blotting. Figure 3 shows that VML injection at doses of 1.5 and 3.0 μg/site (i.c.v) was able to enhance COX-2 levels in the mouse hippocampus 60 min after its administration [$F_{(3, 17)} = 3.58$, $p < 0.05$].

## Evaluation of GFAP and S100B Expression

Glial activation is associated with many neurodegenerative and neurotoxic effects. Figure 4a, b show, respectively, that 60 min after its administration, VML (3.0 μg/site) induced a remarkable increment in the levels of GFAP [$F_{(3, 19)} = 4.22$, $p < 0.05$] and S100B [$F_{(3, 20)} = 3.11$, $p < 0.05$) in the mouse hippocampus, as evaluated by ELISA.

Cell Signaling Proteins Analysis

To verify the modulation of MAPKs in response to VML administration, the phosphorylation levels of p38^{MAPK}, JNK1/2/3, and ERK1/2 were assessed by Western blotting 60 min after i.c.v administration of VML. The results

Fig. 2 Effect of of blocked and denatured VML on immobility time in the FST and number of crossings in the open-field task. Panel **a** shows the immobility time, and Panel **b** shows the number of crossings 60 min after VML i.c.v injection. Values are mean ± SEM. (n = 5–8). **$p < 0.01$, when compared with all other groups (Duncan's Test). NAT: VML in native structure; VML Gal 0.1: VML blocked with 0.1 M galactose; VML den: Denatured VML; GAL 0.1: Galactose 0.1 M in sterile saline

Fig. 3 Effect of VML in COX-2 immunocontent in mouse hippocampus 60 min after VML i.c.v injection. Assay of COX-2 immunocontent was carried out by Western blotting. The level of COX-2 protein was determined by computer-assisted densitometry as a ratio of the OD of the phosphorylated band over the OD of the β-actin band, and the data are expressed as a percentage of the control. The values are presented as mean ± SEM (n = 5–6). *$p < 0.05$, when compared with control group (Duncan's Test)
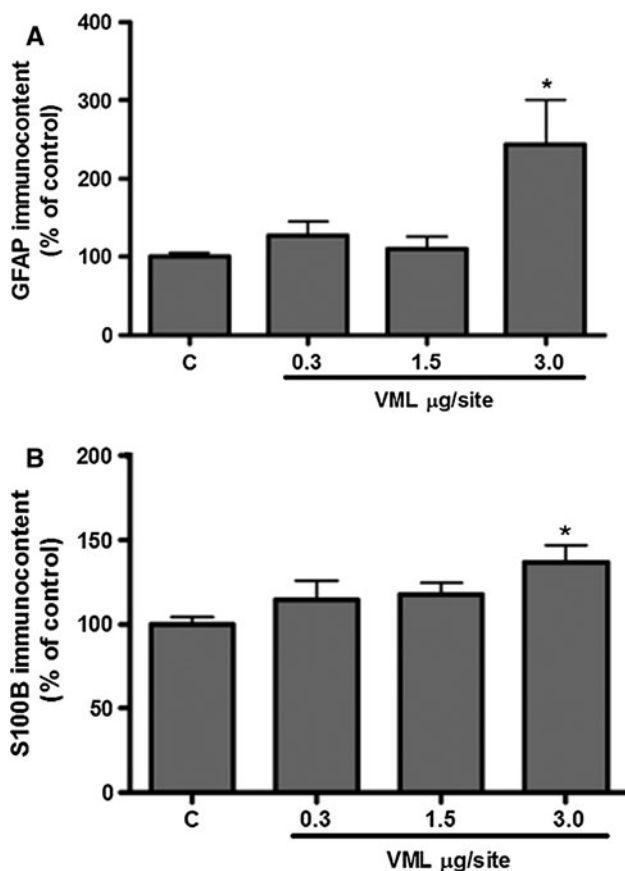
Fig. 4 Effect of VML in GFAP and S100B immunocontent in mouse hippocampus 60 min after VML i.c.v injection. Assay of GFAP and S100B immunocontent was carried out by ELISA, and the data are expressed as a percentage of the control. The values are presented as mean ± SEM (n = 5–6). *$p < 0.05$, when compared with control group (Duncan's Test)

depicted in Fig. 5a–c show that VML did not alter the phosphorylation of p38^{MAPK} [$F_{(3, 20)} = 0.082$, $p = 0.97$], JNK 46 kDa [$F_{(3, 18)} = 0.20$, $p = 0.90$], JNK 54 kDa [$F_{(3, 28)} = 0.97$, $p = 0.97$], ERK1 [$F_{(3, 16)} = 1.76$, $p = 0.20$], or ERK2 [$F_{(3, 16)} = 1.29$, $p = 0.31$]. Moreover, phosphorylation of Akt [$F_{(3, 12)} = 1.23$, $p = 0.33$] and GSK-3β [$F_{(3,16)} = 1.16$, $p = 0.35$] was not altered by VML (Fig. 5 panels d, e).

Discussion

Lectins are the foremost molecules studied in glycobiology, and plant lectins can be an important tool to study the role of carbohydrate/protein interaction in the modulation of cell function. In the context of the CNS, little information is available concerning the possible effects of lectins on neural activity. Therefore, in the present study, we aimed to contribute to the present body of knowledge by

studying the possible actions of VML, a galactose-binding lectin, on neural function. Our results show that central administration of VML produced an increase in the immobility time in the FST, which is indicative of depressive-like behavior. The FST is a behavioral despair model classically used to study compounds with antidepressant properties, and its value is based on the observation that immobility time is reduced by many different classes of antidepressant compounds [53]. However, the utility of the FST can go beyond the verification of antidepressant activity of certain compounds. The increase in the immobility time in the FST has been associated with depressive-like behavior [49, 58–60]. From this perspective, it was demonstrated that i.c.v administration of TNF-α increased the immobility time in the FST when administered 60 min before the test [49], suggesting a depressive-like effect of this cytokine, very similar to the effect we observed with VML administration in the present study.

Vatairea macrocarpa lectin is a very stable protein that binds to galactose/N-acetylgalactosamine in a large range of pH values, and it can bind strongly to multiantennary glycans of the N-acetyllactosamine type [29]. The effect of VML in the FST appears to be dependent from its native tridimensional structure and from the carbohydrate-binding domain of the lectin, since VML action was abrogated, respectively, by denaturation or blockage of its CRD by galactose. Therefore, VML interaction with glycans is a key step in anchoring the lectin on its target and promoting its effects. It is noteworthy that the cerebral endogenous lectin gal-1 displays its neuroprotective response in a manner that is dependent on its CRD interaction with β-galactosides [14]. Thus, VML induces a depressive-like behavior in mice, suggesting a possible deleterious activity on neural function.

Several studies have reported that VML produces peripheral proinflammatory responses in rodents, with induction of neutrophil migration to peritoneal cavity and activation of macrophages [30–32]. The peripheral proinflammatory activity of VML appears to be associated with the release of certain cytokines, mostly TNF-α, although it was apparently independent on COX [31, 32]. COX is a rate-limiting enzyme in the metabolism of arachidonic acid to prostaglandins that exists in two distinct isoforms, COX-1 and COX-2. COX-1 is constitutively expressed and is related to physiological functions, whereas COX-2 is inducible and plays a more important role in inflammation [39, 40]. The present study indicates that VML induced an increase COX-2 levels in the mouse hippocampus (Fig. 3). Increased COX-2 expression and activity is associated with depressive disorders in animal models [61–65], and it has been reported that COX-2 inhibitors may be useful in the treatment of depression [41, 65]. Thus, early inflammatory responses induced by VML, including COX-2 activation, might be
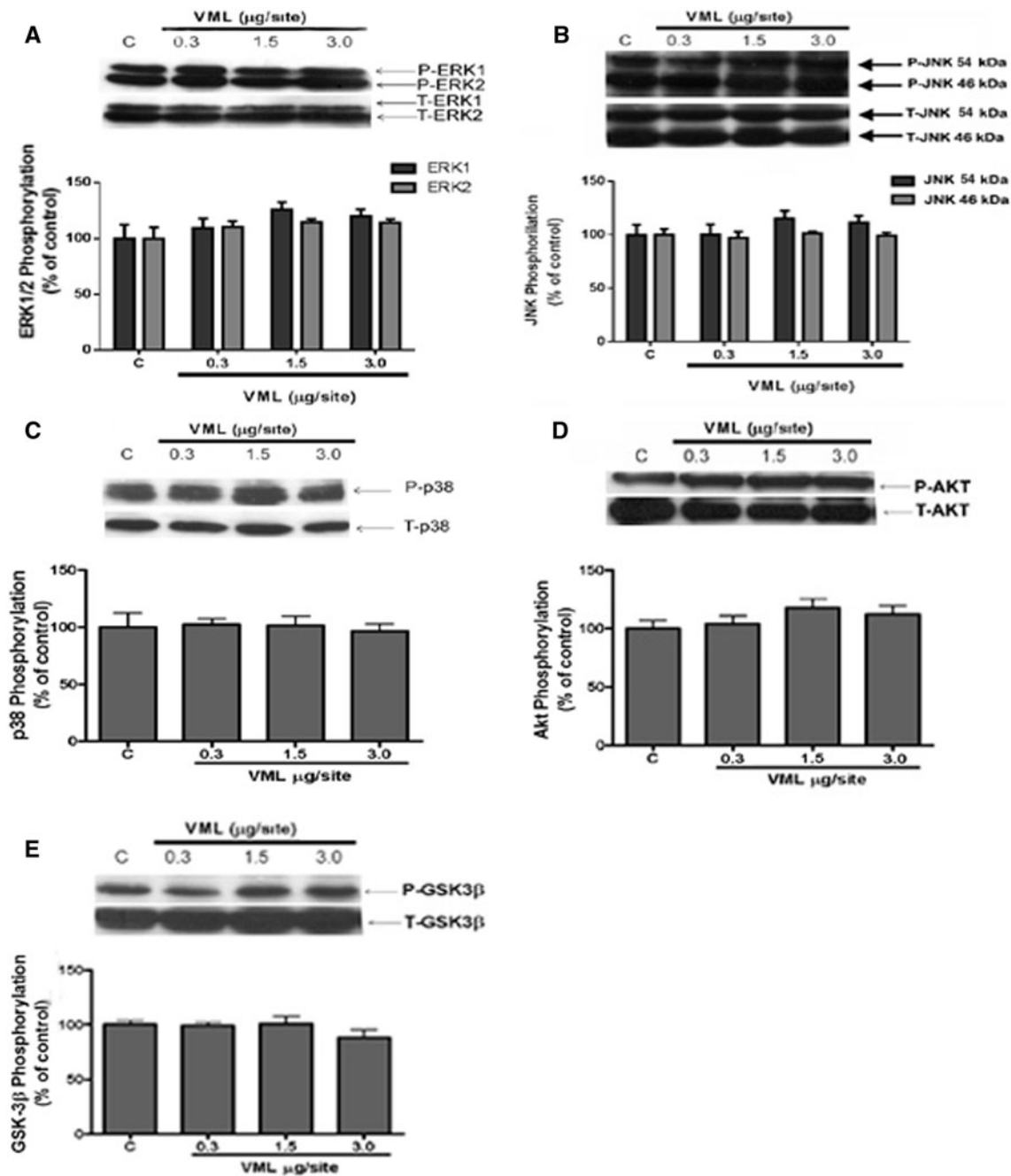
Fig. 5 *Western blot* analysis of phosphorylated ERK 1/2 (**a**), JNK 1/2/3 (**b**), p38MAPK (**c**), Akt (**d**) and GSK-3β (**e**) in the mouse hippocampus 60 min after VML i.c.v administration. The phosphorylation level of each protein was determined by computer-assisted densitometry as a ratio of the OD of the phosphorylated band over the OD of the total band, and the data are expressed as a percentage of the control. The values are presented as mean ± SEM (n = 5–6)

involved in the observed behavioral alterations. However, this possibility needs to be addressed in future studies.

The proinflammatory action of VML, both in peripheral tissues and on CNS, is an important finding because some lectins may exert different effects according to the administration route. ConBr, for example, was apparently not able to induce neuroinflammatory response and produced a neuroprotective activity in the hippocampus when administered via i.c.v [26]. Moreover, ConBr can induce pro-inflammatory response when administered intraperitoneally [44]. Our study shows that VML stimulates the expression of COX-2 in the hippocampus, an action that differs from the effect of VML observed in response to intraperitoneal administration, which occurs independent on COX-2 activity [31, 32]. Furthermore, VML administered intraperitoneally at a dose that produces

proinflammatory activity [31] did not alter the immobility time in the FST, the number of crossings in the open-field task, and the immunocontent of COX-2 in mice hippocampus 60 min after its administration (data not shown). Thus, the mechanisms involved in the inflammatory response promoted by VML can be different for the brain tissue compared with its effects on peripheral tissues.

Our study further indicates that VML (3.0 µg/site) induces a significant increase in GFAP content in mouse hippocampus. It is well documented that astrocyte activation with further astrogliosis causes cell hypertrophy and increased GFAP expression that may play a key role in neuroinflammation [36, 66]. Therefore, one possible explanation for the effect of VML on GFAP expression might be a neuroinflammatory response induced by the lectin. However, this possibility deserves to be tested further.

Vatairea macrocarpa lectin also induces an increase in S100B levels in mouse hippocampus 60 min after i.c.v administration. Similarly, Ye et al. [67] reported that the depressive-like behavior induced by chronic mild stress, an animal model of depression, is accompanied by increased S100B expression in rat hippocampus. Additionally, a high serum level of S100B has been reported in depressive patients [68, 69].

The MAPK and Akt/GSK cascades are associated with various cellular effects, such as cell death or cell survival responses, neuroplasticity or inflammatory processes [70, 71]. MAPKs (ERK1/2, p38^{MAPK} and JNKs) may also be associated with the behavioral modification observed in the FST [72]. Moreover, modulation of GSK-3β may be related to the development of depressive disorders [73, 74] and may modulate COX-2 expression in the hippocampus [43]. However, our study shows that the phosphorylation state of all these enzymes was not altered 60 min after VML injection.

In conclusion, the results reported here demonstrate, for the first time, that the lectin from *V. macrocarpa* seeds, (VML), was able to change the neural function and cause a depressive-like effect as well as activate neuroinflammatory markers. In spite of having carbohydrate affinity similar to gal-1, these results suggest that VML may exert neurotoxic effects in mouse hippocampus, rather than neuroprotective action, as already reported for gal-1. Therefore, our findings suggest a possible dual role of galactose binding lectins in the modulation of CNS function.

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