Brain, Behavior, and Immunity 79 (2019) 186–194

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Lipid dynamics in LPS-induced neuroinflammation by DESI-MS imaging

Onésia Cristina Oliveira-Lima[a], Juliana Carvalho-Tavares[b], Marcella F. Rodrigues[c], Marcus V. Gomez[d], A.C.P. Oliveira[e], Rodrigo R. Resende[f,g], Renato Santiago Gomez[h], Boniek Gontijo Vaz[c], M.C.X. Pinto[a,*]

[a] Laboratório de Neuroquímica e Neurofarmacologia, Departamento de Farmacologia, Instituto de Ciência Biológicas, Universidade Federal de Goiás, Goiânia-GO, Brazil
[b] Departamento de Fisiologia e Biofísica, Instituto de Ciência Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte-MG, Brazil
[c] Instituto de Química, Universidade Federal de Goiás, Goiânia-GO, Brazil
[d] Instituto de Ensino e Pesquisa Santa Casa de Misericórdia, Belo Horizonte-MG, Brazil
[e] Departamento de Farmacologia, Universidade Federal de Minas Gerais, Belo Horizonte-MG, Brazil
[f] Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte-MG, Brazil
[g] Instituto Nanocell, Divinópolis-MG, Brazil
[h] Departamento de Cirurgia, Universidade Federal de Minas Gerais, Belo Horizonte-MG, Brazil

ARTICLE INFO

Keywords:
Lipopolysaccharide
Neuroinflammation
Sickness behavior
DESI-MS
Cerebral lipid dynamics

ABSTRACT

It is well-established that bacterial lipopolysaccharides (LPS) can promote neuroinflammation through receptor Toll-like 4 activation and induces sickness behavior in mice. This phenomenon triggers changes in membranes lipid dynamics to promote the intracellular cell signaling. Desorption electrospray ionization mass spectrometry (DESI-MS) is a powerful technique that can be used to image the distribution of lipids in the brain tissue directly. In this work, we characterize the LPS-induced neuroinflammation and the lipid dynamics in C57BL/6 mice at 3 and 24 h after LPS injection. We have observed that intraperitoneal administration of LPS (5 mg/kg body weight) induces sickness behavior and triggers a peripheral and cerebral increase of pro- and anti-inflammatory cytokine levels after 3 h, but only IL-10 was upregulated after 24 h. Morphological analysis of hypothalamus, cortex and hippocampus demonstrated that microglial activation was present after 24 h of LPS injection, but not at 3 h. DESI-MS revealed a total of 14 lipids significantly altered after 3 and 24 h and as well as their neuroanatomical distribution. Multivariate statistical analyzes have shown that ions associated with phosphatidylethanolamine [PE(38:4)] and docosatetraenoic acid [FA (22:4)] could be used as biomarkers to distinguish samples from the control or LPS treated groups. Finally, our data demonstrated that monitoring cerebral lipids dynamics and its neuroanatomical distribution can be helpful to understand sickness behavior and microglial activation after LPS administration.

1. Introduction

Numerous scientific works have demonstrated that systemic administration of bacterial lipopolysaccharides (LPS) is a potent immune system activator and causes sickness behavior in mice (Biesmans et al., 2013; Dantzer, 2009). LPS activates the Toll-like 4 (TLR4) receptor in cells triggering innate immune response. In the brain, TLR4 receptors are widely expressed and their direct activation can promote glial activation, brain inflammation, and consequently, sickness behavior (Hines et al., 2013).

Sickness behavior is an evolutionary strategy to fight infections, which present with psychological and behavioral components associated with neuroendocrine changes (Dantzer, 2009). This condition is characterized by locomotor activity decrease, lethargy, appetite loss, anhedonia and increased sensitivity to pain (Biesmans et al., 2013; Dantzer, 2009). There are strong evidences indicating the relationship between the brain and immune system showing that these systems can influence each other and modulating the behavior (Biesmans et al., 2013; Dantzer, 2009). Sickness behavior is triggered by proinflammatory cytokines, such as interleukin 1 (IL-1α and IL-1β), interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α), which are produced by activated immune cells (Biesmans et al., 2013; Dantzer, 2009; Inui, 2001). In the brain, astrocytes and microglia activation are associated with behavioral changes, being responsible to modulating

* Corresponding author at: Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Farmacologia, Laboratório de Neuroquímica e Neurofarmacologia. Av. Esperança, S/N, UFG, Prédio ICB II, Sala 114, Goiânia-GO 74690-900, Brazil.
E-mail address: pintomcx@ufg.br (M.C.X. Pinto).

https://doi.org/10.1016/j.bbi.2019.01.029
Received 28 September 2018; Received in revised form 20 December 2018; Accepted 31 January 2019
Available online 01 February 2019
0889-1591/

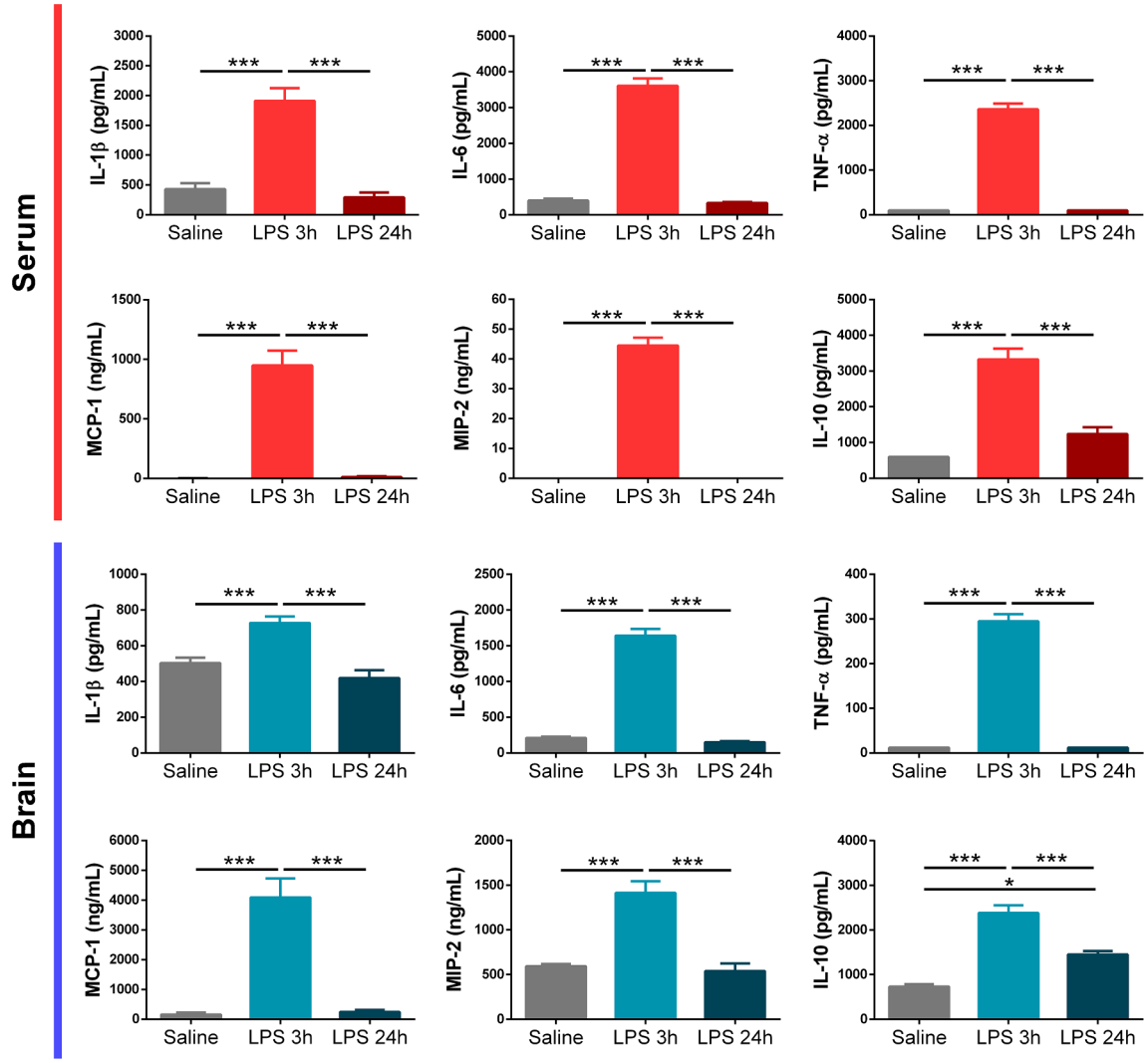

Fig. 1. Chemokines and cytokines levels in serum and brain after systemic LPS injection. ELISA measured cytokine and chemokine levels in serum and brain from C57BL/6 mice. Data are expressed as mean ± SEM, n = 6 animals/group, $^{*}P < 0.05$, $^{***}P < 0.001$, One-way ANOVA followed by Dunnett's post-test.

several neuronal functions (Biesmans et al., 2013; Dantzer, 2009). Despite the plethora of neuroinflammatory studies, the dynamic relationship between cytokines release, microglia activation, and sickness behavior still present some unanswered questions (Biesmans et al., 2013; Dantzer, 2009; Hines et al., 2013). During the inflammatory process, changes in brain lipid dynamics from neurons, astrocytes, and microglia are responsible to trigger and control of the inflammatory process. In this context, lipidomics approaches, which consist on a large-scale study of cellular lipids in biological systems, can provide information to fulfill the gaps of our knowledge in this field (Guo et al., 2017; Li et al., 2016).

To broaden our understanding about the changes in lipid dynamics in the neuroinflammation process, we have evaluated the neuroinflammation induced by LPS in C57BL/6 mice using different techniques to quantify cytokines production, microglial activation, lipid dynamics and sickness behavior at different time points. Firstly, we performed behavioral tests after LPS administration in C57BL/6 mice to understand this phenomenon at different time points. Thereafter, we analyzed the expression of pro and anti-inflammatory cytokines in serum and brain. Then, we evaluated whether the expression of the microglial marker ionized calcium-binding adapter molecule 1 (Iba1) follows the sickness behavior. At last, to check if there are also changes in lipid dynamics, we used the Desorption electrospray ionization mass spectrometry (DESI-MS) technique for imaging of fresh brain slices of mice exposed to LPS.

2. Methods

2.1. Animals and LPS injection

We obtained 8-week-old male (20–25 g) C57BL/6 wild-type mice from the animal facility of the Federal University of Minas Gerais (UFMG). Animals were maintained under temperature-controlled conditions with an artificial 12-h light/dark cycle and were allowed standard chow and water ad libitum. Every effort was made to avoid any unnecessary animal distress, and all the experiments were conductedby NIH guidelines for the care and use of laboratory animals. The animal protocols were approved by the Animal Ethics Committee of UFMG (Protocol: 259/2012).

The animals were injected intraperitoneally (i.p.) with LPS (5 mg/Kg body weight), (Escherichia coli O111:B4, Sigma–Aldrich, St Louis, MO) prepared in nonpyrogenic 0.9% sterile saline, the volume of LPS injection was 10 μL/g body weight (Qin et al., 2007; Masocha, 2009). On the control group we injected (i.p.) 10 μL/g body weight of saline solution. The experiments were performed 3 and 24 h after LPS injection.

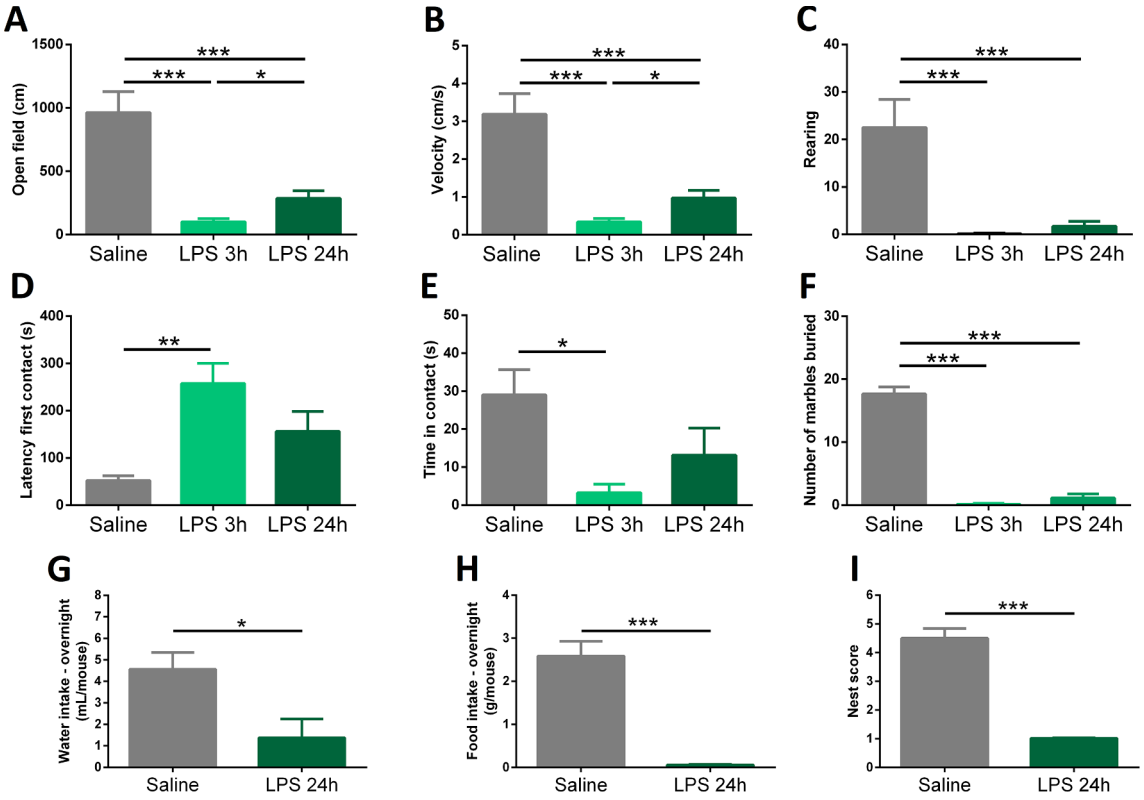

Fig. 2. Effect of LPS injection in C57BL/6 mice behavior. The C57BL/6 mice were treated with LPS (5 mg/Kg body weight) and the behavior test were performed 3 and 24 h after LPS injection. A) Total distance moved of mice treated with LPS in the open field task. B) The average speed of mice treated with LPS in open field. C) Rearing activity in mice treated with LPS. D) Number of marbles buried by mice treated with LPS in marble burying task. E) The latency of the first contact of mice treated with LPS in the social recognition task. F) Time in contact of mice treated with LPS in the social recognition task. G) Food intake in mice after 24 h of LPS injection. H) Water intake in mice after 24 h. I) Nest score of mice after 24 h of LPS injection. Data are expressed as the mean ± SEM, n = 7 animals/group. $^{*}P < 0.05$; $^{**}P < 0.005$, $^{***}P < 0.001$, One-way ANOVA followed by Dunnett's post-test.

2.2. Elisa

To analyze the production of cytokines, we harvested plasma and brain extracts from C57BL/6 mice and used different ELISA kit as described by the manufacturer. Briefly, the mice were anesthetized and cardiac puncture collected the blood in tubes containing EDTA. Whole blood was centrifuged at 1500 × g for 10 min at 4 °C, and plasma was harvested and stored at −20 °C until the use. Brain tissue was homogenized in an extraction solution (containing 0.4 mol/L NaCl, 0.05% Tween-20, 0.5% BSA, 0.1 mmol/L phenylmethyl sulfonyl fluoride, 0.1 mmol/L benzethoniumchloride, 10 mmol/L EDTA, and 20 KI aprotinin) using Ultra-Turrax (Fisher Scientific, Pittsburgh, PA) at the rate of 1 mL per 100 mg of brain tissue. The brain homogenate was centrifuged at 3000 × g for 10 min at 4 °C, and the supernatant was collected and stored at −20 °C. Samples were diluted in PBS containing 0.1% BSA and then analyzed for brain expression levels of MCP-1 (CCL2), MIP-2 (CXCL2), IL-1β, IL-6, TNF-α and IL-10 using Duo set ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. The sensitivity of the kits was: MCP-1, 3.9 pg/mL; MIP-2, 15.62 pg/mL; IL-1β, 15.62 pg/mL and IL-10, 31.25 pg/mL.

2.3. Evaluation of the sickness behavior

2.3.1. Physiological parameters

After LPS injection, food and water intake were measured in mice with ad libitum access to feeding for 24 h. The body weight and temperature were evaluated at baseline, 3 and 24 h after LPS challenge. A rectal probe (Fine Science Tools, USA) was used to measure body temperature. To standardize stress effects, the mice were pre-adapted to the measurement of rectal temperature for two days prior to the experiment.

2.3.2. Open field

In order to evaluate the exploratory activity, we submitted C57BL/6 mice to open field task as previously described by Schaafsma et al. (2017). We used a 30 cm × 30 cm circular apparatus surrounded by 30 cm-high walls made of transparent plexiglass (Insight®, São Paulo, Brazil). The floor of the open field is divided into 12 rectangles by black lines. The animals were gently placed on the left rear quadrant, and then they could explore the arena for five min. The number of crossings (the number of times that animals crossed the black lines) and rearing (the exploration behavior observed in rats subjected to a new environment) were recorded. The tests were performed by the same investigator who was blinded to the animal group (control or infected).

2.3.3. Social recognition task

To assess social memory, we submitted the animals to social recognition task as previously described by Schaafsma et al. (2017). Initially, the adult mouse (experimental subject) were placed insight the clean cage containing an empty and transparent cylinder (10 cm diameter) for 20 min. After that, a transparent cylinder containing a male juvenile mouse (intruder) was presented to the experimental subjects during 5 min for social investigation. After 30 min, the transparent cylinder containing male juvenile mouse was introduced again in the cage for another round of social investigation. The time of contact between the adult mouse and the male juvenile mouse was quantified. The test was performed by the same investigator who was blinded to the animal groups.

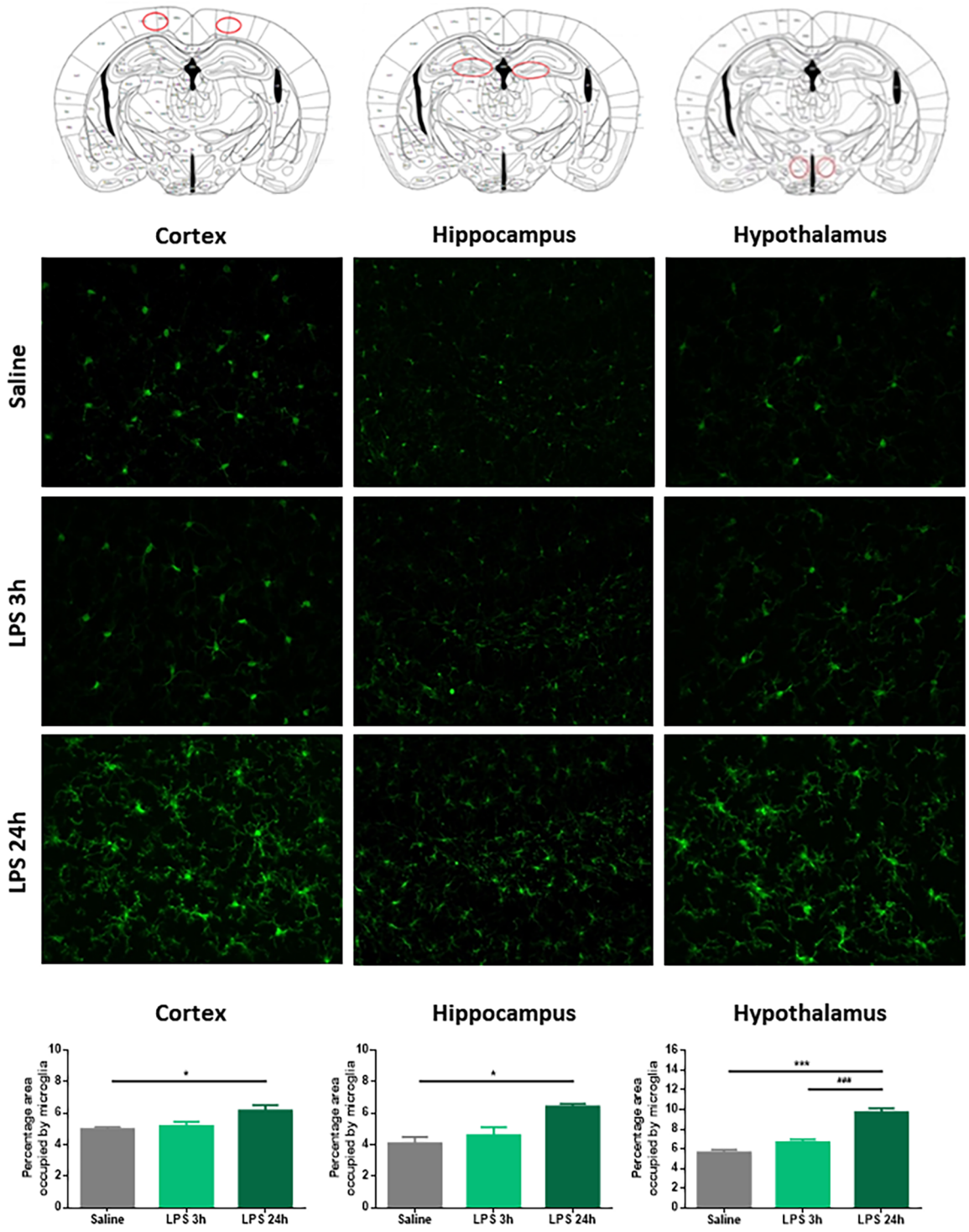

Fig. 3. Microglial activation in C57BL/6 mice after LPS injection. The C57BL/6 mice were treated with LPS (5 mg/Kg body weight), and the microglial activation were analyzed 3 and 24 h after LPS injection. A) Demonstrative image of chosen areas in mice brain. B) Demonstrative images of cortex, hippocampus, and hypothalamus of mice treated with LPS or saline. C) Quantitative analysis of microglial activation in different brain areas. Data are expressed as the mean ± SEM, n = 5 animals/group, $^{*}P < 0.05$; $^{***}P < 0.001$, One-way ANOVA followed by Dunnett's post-test.

2.3.4. Marble burying task

To analyze motivation, we conduct the Marble burying task, as described by Jirkof (2014). Briefly, we introduce the mouse into a clean cage (27 × 16.5 × 12.5 cm) with 4.5-cm corncob bedding for 30 min. Thereafter, we remove the animal and then we placed 20 glass marbles (10 mm diameter) in an equidistant 4 × 5 arrangement for 20 min. The number of marbles buried was recorded (> 50% marble covered by bedding material). The test was performed by the same investigator who was blinded to the animal groups.

2.3.5. Nest building task

The sickness behavior was also assessed by Nest building task as described by Jirkof (2014). Briefly, the animals were placed in the test room for 30 min of acclimation. The animals received LPS or saline by intraperitoneal injection. After pretreatment, each mouse was briefly transferred to a new home cage with one cotton square and kept

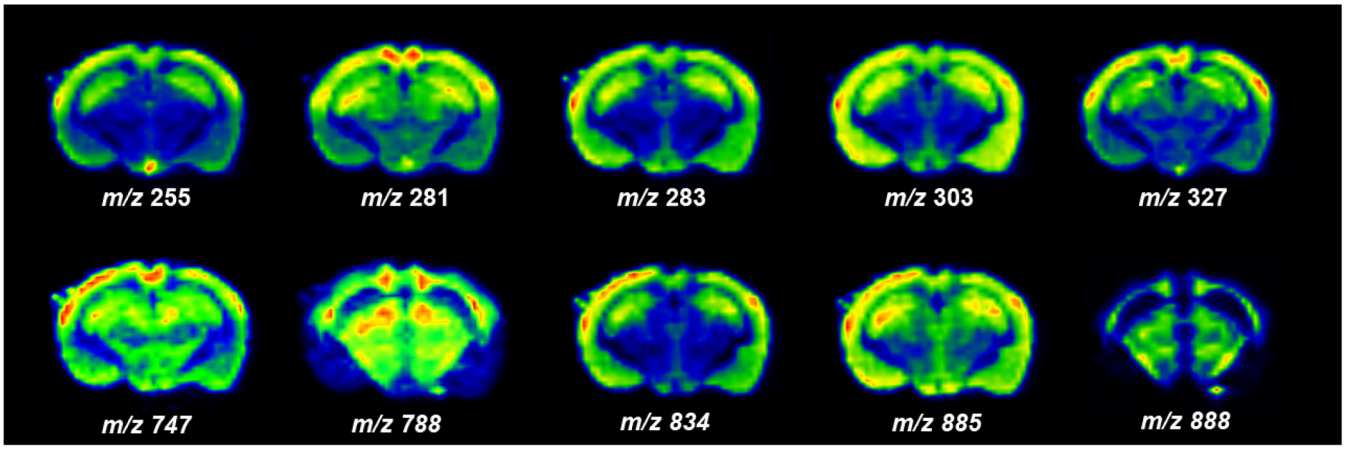

Fig. 4. Representative images of more intense lipids ions in the mouse brain by DESI-MS. The most intense ions were *m/z* 255.2 (Palmitic acid), *m/z* 281.2 (Oleic acid), *m/z* 283.2 (Stearic acid), *m/z* 303.1 (Arachidonic acid), *m/z* 327.3 (Docosahexaenoic acid), *m/z 747.5 (Glycerophosphoglycerol); m/z 788* (phosphatidylcholine)*; m/z* 834.4 (phosphatidylserine), *m/z* 885.5 (phosphatidylinositol) and *m/z* 888.6 (Sulfatide).

Table 1
Lipids detected in negative ion mode by DESI-MS.

m/z	Ion	Possible molecule	Control (A)	LPS − 3 h (B)	LPS − 24 h (C)	B/A	C/A
253.1	FA(16:1)	Palmitoleic acid	10.2 ± 0.7	13.1 ± 1.7*	15.3 ± 2.4**	Up	Up
279.2	FA(18:2)	Linoleic acid	12.4 ± 1.2	22.4 ± 5.3*	36.4 ± 5.8***	Up	Up
281.2	FA(18:1)	Oleic acid	143.9 ± 12.83	200.6 ± 48.38*	197.4 ± 19.12*	Up	Up
303.1	FA(20:4)	Arachidonic acid	317.3 ± 20.2	377.8 ± 25.9*	326.3 ± 17.4	Up	None
305.3	FA(20:3)	Dihomo-γ-Linolenic acid	13.6 ± 1.2	17.3 ± 2.1*	20.8 ± 1.8***	Up	Up
327.3	FA(22:6)	Docosahexaenoic acid	53.4 ± 3.7	70.8 ± 9.3*	66.7 ± 6.2**	Up	Up
328.3	FA(22:5)	Docosapentaenoic acid	12.5 ± 0.9	16.6 ± 2.2*	15.7 ± 1.4**	Up	Up
747.5	PG(36:1)	Glycerophosphoglycerol	25.3 ± 0.9	22.8 ± 1.5*	21.8 ± 0.7***	Down	Down
750.4	pls-PE(38:4)	Plasmenylethanolamine	17.6 ± 1.3	15.0 ± 1.1*	15.8 ± 0.8	Down	None
762.4	PS(34:0)	Phosphatidylserine	11.6 ± 0.7	10.4 ± 0.7	10.0 ± 0.5**	None	Down
767.5	PE(38:4)	Phosphatidylethanolamine	10.4 ± 0.9	19.9 ± 1.0	19.0 ± 0.7*	None	Down
774.6	PE(P-18:0/22:6)	Phosphatidylethanolamine	24.3 ± 1.6	21.5 ± 1.3*	21.2 ± 0.8**	Down	Down
790.5	PE(40:6)	Phosphatidylethanolamine	32.2 ± 2.2	29.0 ± 1.8	25.8 ± 0.9**	None	Down
806.6	PC(16:0–22:6)	Phosphatidylcholine	13.6 ± 1.1	12.9 ± 1.1	15.3 ± 0.7*	None	Up
834.4	PS(40:6)	Phosphatidylserine	276.4 ± 7.9	250.2 ± 24.8	255.1 ± 4.9***	None	Down
836.6	PS(40:5)	Phosphatidylserine	49.7 ± 1.1	44.7 ± 4.5	45.9 ± 0.7***	None	Down
858.6	GPI(36:4)	Glycerophosphatidylinositol	15.7 ± 0.5	14.4 ± 0.7*	13.8 ± 0.3***	Down	Down
883.5	PI(38:5)	Phosphatidylinositol	25.73 ± 1.32	24.11 ± 2.12	29.99 ± 0.83	None	Up
885.5	PI(38:4)	Phosphatidylinositol	202.6 ± 10.8	183.4 ± 6.8*	172.8 ± 5.8***	Down	Down
887.7	PI(38:3)	Phosphatidylinositol	34.6 ± 1.5	31.3 ± 1.0**	30.9 ± 1.1**	Down	Down

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared to control group, n = 6 animals/group, One-way ANOVA, followed by Dunnett's post-test. B/A is the ratio between LPS 3 h and control. C/A is the ratio between LPS 24 h and control.

overnight. The Nest building task was evaluated on the next day by the same investigator who was blinded to the animal groups.

2.4. Immunofluorescence and confocal microscopy

To analyze microglial activation in brain sections, we used the protocol previously described by Oliveira-Lima et al. (2015). Brain sections of 25 µm (free-floating) were incubated with primary antibodies specific for Iba-1 (1:500) during2 days at 4 °C. After that, we washed the sections and stained with species-specific secondary antibodies conjugated to Alexa Fluor 488 (Invitrogen) for 2 h at 25 °C. Sections were rinsed, mounted with Vectashield (Vector Labs) and examined on a Leica TCS confocal microscope (Leica Microsystems).

2.5. Desorption electrospray ionization mass spectrometry and imaging mass spectrometry analyses (DESI-IMS)

To analyze the lipid dynamic in brain sections, we used the protocol previously described by Fernandes et al. (2016). Briefly, coronal brain sections of 15 µm of thickness were obtained using a cryostatmicrotome (Leica Biosystems, Nussloch, Germany). The sections were mounted onto microscope glass slides and were stored at −80 °C.

The DESI-IMS analyses were performed in a Q-Exactive mass spectrometer (Thermo Scientific, Bremen, Germany) with a resolution of 100,000 at *m/z* 400 coupled with an Omni Spray Ion Source 2-D (Prosolia Inc.) for data acquisition. We have used the solvent mix dimethylformamide (DMF): acetonitrile (1: 1), the nebulizing gas pressure was 160 psi, the flow rate was 1.5 µL/min, and the surface scan rate was 600 µm/s. The distance from the capillary tip to the sample surface was 2 mm, the distance from the capillary tip to the MS inlet was 4 mm, and the angle of the delivery capillary in ion source was 54°. Analyses were performed in the negative ion mode. The S-Lens RF level was set to 20 to increase the transmission of low *m/z* ions, the capillary temperature was 280 °C and the spray voltage was 3.4 kV. For DESI imaging, the sprayer motion controls were set such that data was recorded in 200 µm × 200 µm pixel sizes over a series of horizontal lines scans. The signals attributed to the lipids were accurately assigned with errors < 2 ppm. The software Firefly v.2.2.00 was used to generate the images, which were treated in BIOMAP 3.8.0.4. The lipids identification was performed using the Thermo Xcalibur Software and the LIPID MAPS® Tools, a free resource sponsored by the Wellcome Trust (http://www.lipidmaps.org/). The numerical data of the chromatograms were used to perform chemometrics.

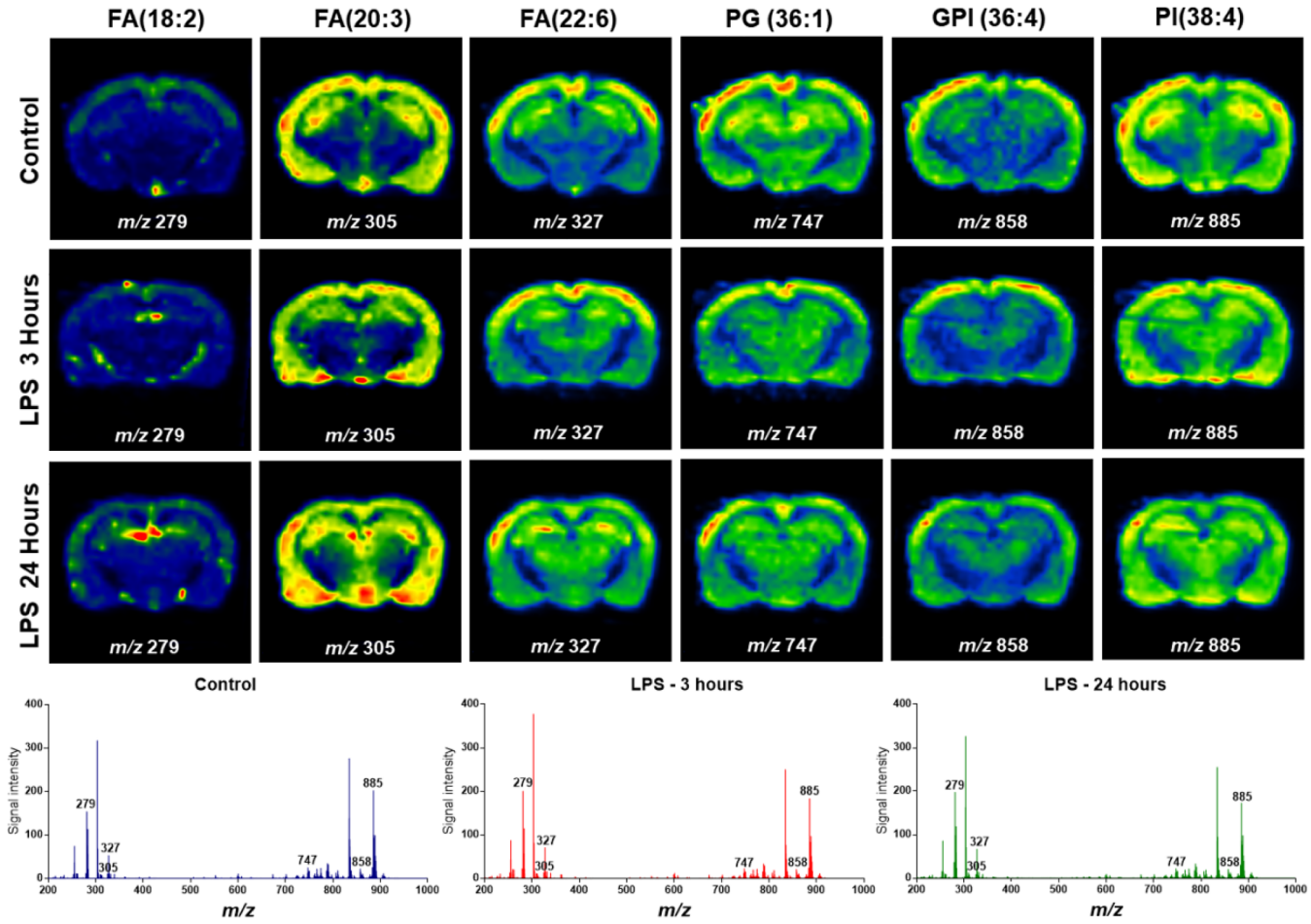

Fig. 5. Monitoring changes of lipid dynamics during the LPS-induced neuroinflammation in mice using DESI-MS imaging. The C57BL/6 mice were treated with LPS (5 mg/Kg body weight) and the lipid dynamics in the mouse brain were analyzed 3 and 24 h after LPS injection. The selected ions were *m/z* 279.2, Linoleic acid (up); *m/z* 305.3, Dihomo-γ-Linolenic acid (up); *m/z* 327.3, Docosahexaenoic acid (up), *m/z* 747.5, Glycerophosphoglycerol (down); *m/z* 858.6, Glycerophosphatidylinositol (down); and *m/z* 885.5, Phosphatidylinositol (down).

2.6. Statistical analyses

To compare data, we used One-way ANOVA followed by Dunnett's post-test. Statistical significance was established at $P < 0.05$. Data sets are presented as the mean ± standard error of the mean (SEM). We analyzed the data using Graph Pad Prism, version 6.0 (GraphPad Software Inc., San Diego, CA, USA).

To perform chemometrics analysis, original MS data were acquired using BIOMAP 3.8.0.4 and the whole dataset was separated into individual mass spectrum corresponding to a global scan of the image. The MS data was normalized by the mean of the signal intensity of each spectrum and partial least squares discriminant analysis (PLS-DA) was performed using MetaboAnalyst tool (http://www.metaboanalyst.ca/).

3. Results

3.1. LPS injection promoted brain and systemic inflammation

It is well known that high doses of LPS are capable of inducing brain and systemic inflammation. In order to evaluate the inflammation induced by the injection of LPS (5 mg/Kg body weight), we analyzed the plasma levels of chemoattractants for monocytes (MCP-1) and neutrophils (MIP-2), as well as, the plasma levels of an anti-inflammatory cytokine, IL-10, and pro-inflammatory cytokines, IL-1β, IL-6 and TNF-α.

The LPS induced an increase in plasma levels of chemokines and pro-inflammatory cytokines 3 h after the injection, but these levels were close to the control after 24 h (Fig. 1). Plasma levels of the anti-inflammatory cytokine IL-10 were increased at 3 h (3328 ± 297,7 pg/mL) and 24 h (1237 ± 184,3 pg/mL) after LPS injection when compared to the control (592,0 ± 0,0 pg/mL) ($^{*}p < 0.05$).

We also evaluated the brain levels of chemoattractants and chemokines (Fig. 1). We found the same pattern previously observed in plasma, where the brain levels of inflammatory cytokines and chemokines were higher in mice 3 h after LPS injection, followed by a reduction at 24 hrs. The brain levels of IL-10 were also increased 3 h (2382 ± 170.1 pg/mL) and 24 h (1451 ± 77.24 pg/mL) after LPS injection compared to the control group (732,1 ± 51,83 pg/mL) ($^{*}p < 0.05$).

3.2. LPS injection induced sickness behavior in C57BL/6 mouse

Once observed the brain and systemic inflammation induced by LPS injection, we analyze how this process could alter mouse behavior. Firstly, we analyzed the physiological parameters of mice that received LPS injection (Fig. 2). The food and water intake were reduced 24 h after the injection of LPS (5 mg/Kg body weight). The mice also presented a reduction on body temperature after 3 h (34.65 ± 0.46 °C) and 24 h (34.01 ± 0.14 °C) after LPS injection when compared with the control (36.19 ± 0.16 °C). The nest score presented a reduction after 24 h of LPS injection ($^{*}p < 0.05$).

We submitted the mice to the open field in order to analyze motor activity and willingness (Fig. 2a). The LPS injection reduced the total

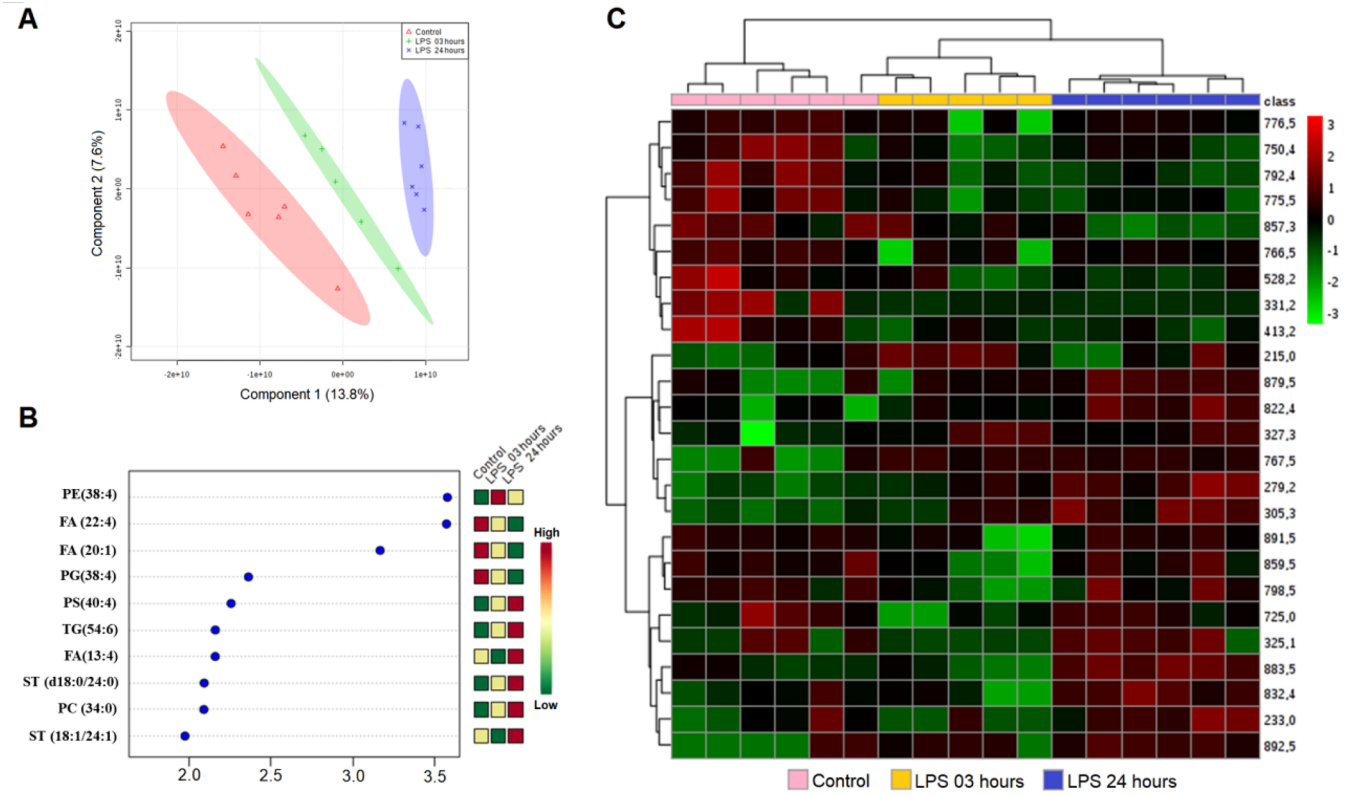

Fig. 6. Partial least squares Discriminant Analysis (PLS-DA) of brain inflammation induced by LPS. A) PLS-DA score plot shows the statistical discrimination between MS profile of the control group, LPS 3 h and LPS 24 h groups. B) VIP scores by PLS-DA. C) Clustering result is shown as a heatmap (distance measure using euclidean, and clustering algorithm using ward).

mouse distance moved insight the open field apparatus after 3 h (100.9 ± 24.70 cm) and 24 h (286.8 ± 59.67 cm) when compared with the saline group (963.4 ± 164.7 cm). The LPS injection also reduced the average speed of mouse in the open field apparatus after 3 h (0.34 ± 0.09 cm/s) and 24 h (0.97 ± 0.20 cm/s) when compared with the saline group (3.18 ± 0.55 cm/s). The LPS injection also reduced the rearing activity in the open field apparatus for both groups in comparison to the control group. Finally, we also observed the difference between animals on average speed and rearing after 3and 24 h of LPS injection. These data indicate that there is a slight behavioral improvement over the time.

We also analyze social interaction and motivation in mice (Fig. 2e). The LPS injection increase the latency of social interaction after 3 h (257.4 ± 42.60 s) and 24 h (156.1 ± 41.91 s) when compared with the control (52.00 ± 10.31 s). The time expended in social contact reduced after 3 h (3.29 ± 2.23 s) and 24 h (13.14 ± 7.17 s) of LPS injection when compared to control (29.00 ± 6.65 s).The mouse motivation in Marble Burying task was also reduced after 3 (0.14 ± 0.14) and 24 h (1.14 ± 0.63) of LPS injection when compared to saline control (17.67 ± 1.12). Taken together, these results demonstrate that the injection of LPS (5 mg/Kg body weight) induces sickness behavior in C57BL/6 mice.

3.3. LPS injection induced microglial activation after 24 h in C57BL/6 mouse

Based on the sickness behavior observed in the mice after LPS injection, we analyzed the microglial activation in different parts of the brain. The results for microglial activation in the cortex, hippocampus, and hypothalamus are shown in Fig. 3.

We observed an increase on microglial activation in cortex, hippocampus and hypothalamus 24 h after LPS injection ($^{*}p < 0.05$). Interestingly, we did not observe a significant difference in microglial activation after 3 h of LPS injection in comparison with control group. Taken together, our results indicate that sickness behavior is not related to microglial activation.

3.4. LPS injection alters lipid dynamics after 24 h of LPS injection

DESI-MS analysis of lipids species typically involves monitoring deprotonated molecules in negative ion mode through loss of hydrogen from the carboxylic acid moiety. Here, we can observe the neuroanatomical distribution of lipids in LPS model of neuroinflammation. Fig. 4 presents the most intensity ions of lipids observed in mouse brain.

LPS injection in the mouse can induce a rapid and deep change in brain biochemistry. On Table 1, we selected 20 ions associated with lipids that were changed in mouse brain after LPS injection. After 3 h of LPS injection, we observed an increase on Palmitoleic acid, Arachidonic acid, Linoleic acid, Dihomo-γ-Linolenic Acid, Docosahexaenoic acid and Docosapentaenoic acid, as well as a reduction on Glycerophosphoglycerol PG(36:1), Plasmenylethanolamine pls-PE(38:4), Phosphatidylethanolamine PE(P-18:0/22:6), Phosphatidylinositol PI(38:4), Phosphatidylinositol PI(38:3), Glycerophosphatidylinositol GPI(36:4).

On the other hand, after 24 h of LPS injection, we observed an increase on Palmitoleic acid, Linoleic acid, Arachidonic acid, Dihomo-γ-Linolenic Acid, Docosahexaenoic acid, Docosapentaenoic acid, Phosphatidylcholine PC(16:0–22:6) and Phosphatidylinositol PI(38:5), as well as a reduction on Glycerophosphoglycerol PG (36:1), Phosphatidylserine PS(34:0), Phosphatidylethanolamine PE(38:4), Phosphatidylethanolamine PE(P-18:0/22:6), Phosphatidylethanolamine PE(40:6), Phosphatidylserine PS(40:6), Phosphatidylserine PS(40:5), Glycerophosphatidylinositol GPI (36:4), Phosphatidylinositol PI(38:4), Phosphatidylinositol PI(38:3). Fig. 5 shows the ions that were significantly altered in mouse brain after 3 and 24 h after LPS injection.

3.5. Changes in lipid dynamics can distinguish health brain from LPS induced neuroinflammation

The absolute intensities of 148 detected lipids were used to understand the changes in LPS-induced neuroinflammation. Changes in the levels of these lipids after 3 or 24 h after LPS injection, as well as normal control, were analyzed using multivariate statistics. As shown in Fig. 6, partial least squares discriminant analysis (PLS-DA) indicated that the LPS injection in 3- and 24-hours groups could be differentiated by the component 1 (13.8%), and the component 2 (7.6%) could further differentiate the normal control from animal with neuroinflammation. The ion associated to phosphatidylethanolamine [PE(38:4)] and docosatetraenoic acid [FA (22:4)] has a potential ability to discriminate between neuroinflammation and control at the different time points. The ion associated with proinflammatory $PGF_{2\alpha}$ (2,3-dinor-11b-PGF2α) is important to discriminate LPS 3 h group from other groups. The model was evaluated using the goodness-of-fit parameter (R2) and the predictive ability parameter (Q2). R2 represents the proportion of variance explained by a given component in the model, whereas Q2 is defined as the proportion of variance in the predictable data model under cross-validation. Model validation with the number of permutations equaling 100 generated intercepts of R2 = 0.822; Q2 = 0.343, with an accuracy of 0.588 which mean that the PLS-DA may indicate the separation of brain neuroinflammation and healthy brains.

4. Discussion

This study is the first report to detect *in situ* changes on lipids levels in the brain of LPS-challenged mice. Here, we observed that LPS injection increased cytokines and chemokines levels in the blood and brain, promoted changes in lipid dynamics and induced sickness behavioral, which can be observed 3 and 24 h after the injection. However, microglia activation was observed only at 24 h after the injection, being absent at 3 h after LPS. For this reason, understand the relationship of LPS-induced neuroinflammation and changes in lipids dynamics may clarify how and when glial activation occurs.

LPS activates the innate immune receptor Toll-like 4 (TLR4) present in immune cells and promotes the production and release of cytokine by these cells. Here, we observed that LPS injection increased the level of IL-1β, IL-6, TNF-α, IL-10, MCP-1, and MIP-2 at 3 h in the blood and brain, but only IL-10 increased from these tissues after 24 h. It is well known that peripheral actions of LPS promote cytokine production and release, which is transported into the brain causing sickness behavior (Inui, 2001; Dantzer, 2009). The sickness behavior was observed at the two-time points, indicating an association with cytokines levels. A recent study demonstrated that animal pre-treatment with a TLR4 inhibitor 30 min after LPS injection can prevent TNF-α production in the brain at the time of 45 min and also reduced the sickness behavior (Hines et al., 2013). It is also known that central injection of IL-10 antagonizes the behavioral effects of lipopolysaccharide in rats (Bluthé et al., 1999). Recent studies indicate that LPS can directly promote microglia activation and cytokine release when it reaches the circumventricular organs like the hypothalamus which lack a functional blood–brain barrier (BBB) (Jin et al., 2016).

In our work, the increased IL10 levels after 24 h were associated with microglial activation profile, which could be related to an anti-inflammatory response triggered by M2 microglia. We observed microglial activation in the hippocampus, cortex, and hypothalamus 24 h after LPS injection, but it was absent after 3 h in these tissues. Intracerebroventricular injection of LPS also triggers sickness behavior, neuroinflammation, astrocyte and microglial activation (Aid et al., 2008; Jin et al., 2016). It was demonstrated that systemic inflammation coincided with astrocyte activation and sickness behavior in the period from two to 48 h after LPS injection (Biesmans et al., 2013). These authors also observed an increase in microglial activation in the hippocampus after 24 h of 0.63 mg/kg LPS injection but did not present data after a short time period (Biesmans et al., 2013). Taken together, these studies indicate that the peripheral LPS-induced sickness behavioral is associated with systemic and cerebral levels of cytokines and astrocyte activation, but it is not associated with microglial activation.

In our study, we have brought more information associated with the neuroinflammation process through the lipid dynamics monitoring in the mouse brain. This monitoring can provide information about cell signaling during neuroinflammation and inflammation resolution, which can help us to understand the relationship between cytokine release and sickness behavior. The follow lipids were altered after 3 h and 24 h of LPS injections: Palmitoleic acid [FA(16:1)], Linoleic acid [FA(18:2)], Dihomo-γ-Linolenic Acid [FA(20:3)], Docosahexaenoic acid [FA(22:6)], Docosapentaenoic acid [FA(22:5)], Glycerophosphoglycerol [PG (36:1)], Phosphatidylethanolamine [PE(38:4)], Glycerophosphatidylinositol [GPI (38:4)], Phosphatidylinositol [PI(38:4)], Phosphatidylinositol [PI(38:3)]. The lipids Linoleic acid [FA(18:2)], Dihomo-γ-Linolenic Acid [FA(20:3)] are associated to the arachidonic acid pathway and pro-inflammatory lipids signaling (Sergeant et al., 2016). Arachidonic acid signal was increased in the brain tissue after 3 h of LPS injection, but the signal returned to normal levels after 24 h, a pattern that was similar to those observed for pro-inflammatory cytokines and chemokines (IL-1β, IL-6, TNF-α, MCP-1, MIP-2). On the other hand, Palmitoleic acid [FA(16:1)], docosahexaenoic acid [FA(22:6)], docosapentaenoic acid [FA(22:5)], phosphatidylethanolamine [PE(38:4)] play important role on decreasing inflammatory reaction (Tian et al., 2017; Souza et al., 2017), being its pattern similar to that observed with IL-10. For instance, new DESI-MS study has point out that docosahexaenoic acid-containing lipids may participate in the recovery process of brain injury (Guo et al., 2017).

The ion associated to phosphatidylethanolamine [PE(38:4)] presented the higher VIP score in PLS-DA analysis and it is the most important ion to discriminate between LPS-induced neuroinflammation and control condition. The relative concentrations of phosphatidylethanolamine [PE(38:4)] increased at the acute phase of neuroinflammation (3 h) and gradually decrease at the 24 h after LPS injection. This profile is in opposition with that observed in traumatic brain injury in rat (Guo et al., 2017). On the other hand, the ion attributed to docosatetraenoic acid, belonging to the omega-6 family, also presented a high VIP score in PLS-DA analysis, being used to discriminate control group from LPS 3 and LPS 24 h. The relative concentrations of docosatetraenoic acid decrease after 3 h, and then, decrease more after 24 h after LPS injection. The ion that better discriminate acute inflammation (LPS 3 h) itself was the proinflammatory prostaglandin $PGF_{2\alpha}$ (2,3-dinor-11b-PGF2α). These features are interesting because they could be used as biomarkers for LPS-induced neuroinflammation.

In conclusion, our data demonstrated that intraperitoneal administration of LPS triggers a peripheral and cerebral increase of cytokine levels, changes in cerebral lipid dynamics and sickness behavior without microglial activation after 3 h. After 24 h, the presence of pro-inflammatory cytokines is reduced in blood and brain, but it still observed changes in lipids dynamics, microglial activation and sickness behavior. This data indicates that the neuroanatomical monitoring of lipid dynamics could be better way to understand sickness behavior than microglial activation and cytokines levels after LPS administration.

5. Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by Minas Gerais Research Support Foundation - FAPEMIG, Brazil (№ APQ-00820-17); Goias Research Support Foundation - FAPEG, Brazil (№ 88887.305550/2018-00), (№ 314878/2018-1); Coordination for the Improvement of Higher Education Personnel - CAPES, Brazil; and National Council for Scientific and Technological Development - CNPq, Brazil (№ 400593/2016-5), (№ 407075/2018-6).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2019.01.029.

References

Aid, S., Langenbach, R., Bosetti, F., 2008. Neuroinflammatory response to lipopolysaccharide is exacerbated in mice genetically deficient in cyclooxygenase-2. J. Neuroinflammat. 5, 17.

Biesmans, S., Meert, T.F., Bouwknecht, J.A., Acton, P.D., Davoodi, N., De Haes, P., Kuijlaars, J., Langlois, X., Matthews, L.J.R., Ver Donck, L., Hellings, N., Nuydens, R., 2013. Systemic immune activation leads to neuroinflammation and sickness behavior in mice. Mediat. Inflamm. 2013 271359.

Bluthé, R.-M., Castanon, N., Pousset, F., Bristow, A., Ball, C., Lestage, J., Michaud, B., Kelley, K.W., Dantzer, R., 1999. Central injection of IL-10 antagonizes the behavioural effects of lipopolysaccharide in rats. Psychoneuroendocrinology 24, 301–311.

Dantzer, R., 2009. Cytokine, sickness behavior, and depression. Immunol. Allergy Clin. North Am. 29, 247–264.

Fernandes, A.M.A.P., Vendramini, P.H., Galaverna, R., Schwab, N.V., Alberici, L.C., Augusti, R., Castilho, R.F., Eberlin, M.N., 2016. Direct visualization of neurotransmitters in rat brain slices by desorption electrospray ionization mass spectrometry imaging (DESI - MS). J. Am. Soc. Mass Spectrom. 27, 1944–1951.

Guo, S., Zhou, D., Zhang, M., Li, T., Liu, Y., Xu, Y., Chen, T., Li, Z., 2017. Monitoring changes of docosahexaenoic acid-containing lipids during the recovery process of traumatic brain injury in rat using mass spectrometry imaging. Sci. Rep. 7, 5054.

Hines, D.J., Choi, H.B., Hines, R.M., Phillips, A.G., MacVicar, B.A., 2013. Prevention of LPS-Induced Microglia Activation, Cytokine Production and Sickness Behavior with TLR4 Receptor Interfering Peptides. PLoS ONE 8 e60388.

Inui, A., 2001. Cytokines and sickness behavior: implications from knockout animal models. Trends Immunol. 22, 469–473.

Jin, S., Kim, J.G., Park, J.W., Koch, M., Horvath, T.L., Lee, B.J., 2016. Hypothalamic TLR2 triggers sickness behavior via a microglia-neuronal axis. Sci. Rep. 6, 29424.

Jirkof, P., 2014. Burrowing and nest building behavior as indicators of well-being in mice. J. Neurosci. Methods 234, 139–146.

Li, J., Ren, S., Piao, H.-L., Wang, F., Yin, P., Xu, C., Lu, X., Ye, G., Shao, Y., Yan, M., Zhao, X., Sun, Y., Xu, G., 2016. Integration of lipidomics and transcriptomics unravels aberrant lipid metabolism and defines cholesteryl oleate as potential biomarker of prostate cancer. Sci. Rep. 6, 20984.

Masocha, W., 2009. Systemic lipopolysaccharide (LPS)-induced microglial activation results in different temporal reduction of CD200 and CD200 receptor gene expression in the brain. J. Neuroimmunol. 214, 78–82.

Oliveira-Lima, O.C., Pinto, M.C.X., Duchene, J., Qadri, F., Souza, L.L., Alenina, N., Bader, M., Santos, R.A.S., Carvalho-Tavares, J., 2015. Mas receptor deficiency exacerbates lipopolysaccharide-induced cerebral and systemic inflammation in mice. Immunobiology 220, 1311–1321.

Qin, L., Wu, X., Block, M.L., Liu, Y., Breese, G.R., Hong, J.-S., Knapp, D.J., Crews, F.T., 2007. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 55, 453–462.

Schaafsma, S.M., Gagnidze, K., Reyes, A., Norstedt, N., Månsson, K., Francis, K., Pfaff, D.W., 2017. Sex-specific gene–environment interactions underlying ASD-like behaviors. PNAS 114, 1383–1388.

Sergeant, S., Rahbar, E., Chilton, F.H., 2016. Gamma-linolenic acid, dihommo-gamma linolenic, eicosanoids and inflammatory processes. Eur. J. Pharmacol. 785, 77–86.

Souza, C.O., Teixeira, A.A.S., Biondo, L.A., Silveira, L.S., Calder, P.C., Rosa Neto, J.C., 2017. Palmitoleic acid reduces the inflammation in LPS-stimulated macrophages by inhibition of NFκB, independently of PPARs. Clin. Exp. Pharmacol. Physiol. 44, 566–575.

Tian, Y., Katsuki, A., Romanazzi, D., Miller, M.R., Adams, S.L., Miyashita, K., Hosokawa, M., 2017. Docosapentaenoic acid (22:5n–3) downregulates mRNA expression of pro-inflammatory factors in LPS-activated murine macrophage like RAW264.7 cells. J. Oleo Sci. 66, 1149–1156.