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Research Paper

Investigation of blood-brain barrier disruption in an animal model of mania induced by D-amphetamine

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ABSTRACT

Background: High levels of inflammation and oxidative stress are observed in bipolar disorder (BD) being further associated with mood symptoms and cognitive dysfunction. Due to the crosstalk between the periphery and central nervous system, the blood-brain barrier (BBB) disruption has been considered a key mechanism of the BD pathophysiology. This study aimed to evaluate claudin-5 expression in the brain of a model of mania induced by D-amphetamine (AMPH).

Methods: Wistar rats were injected with AMPH (2 mg/kg i.p.) and treated with lithium (47.5 mg/kg i.p.). Locomotor behavior was assessed, followed by euthanasia, blood collection, and brain removal. Tumor necrosis factor (TNF) α and thiobarbituric acid reactive substances (TBARS) were quantified in the serum and brain tissue, and claudin-5 was quantified in the brain.

Results: AMPH-injected animals exhibited increased locomotor activity. In the serum, TBARS levels were augmented in lithium-treated groups, while TNFα was not detected. In the brain, TBARS and TNFα did not differ between groups but were positively andstrongly correlated in the striatum of AMPH-injected rats. Contrary to our hypothesis, AMPH and lithium injections did not affect claudin-5 levels in the brain.

Limitations: The main limitations include the lack of a dynamic marker of BBB integrity and limited number of biomarkers analyzed.

Conclusions: This is one of the first attempts to investigate the effects of AMPH on BBB integrity, and no disruption was observed. Still, we provide rationale for future research to elucidate the importance of BBB disruption in BD, recently proposed as a marker of illness progression.

1. Introduction

Bipolar disorder (BD) is a recurrent chronic and disabling disorder characterized by fluctuations in mood, energy, and functioning (American Psychiatric Association, 2013). Specifically, mood episodes include mania, hypomania, and alternating episodes of depression (Grande et al., 2016). Although no specific biomarker has been identified, individuals with BD present with increased peripheral levels of inflammatory and oxidative stress markers accompanied by altered levels of neurotrophic factors (Fernandes et al., 2011; Ghafouri-Fard et al., 2019; Kim et al., 2007; Petersen et al., 2021; van den Ameele et al., 2020, 2017). Hence, BD is associated with a chronic low-grade inflammatory state, which seems to be coordinated with mood symptoms and cognitive deficits during the course of the disorder (Kapczinski et al., 2010; Rosenblat and McIntyre, 2016).

The crosstalk between the periphery and the central nervous system (CNS) has implicated the blood-brain barrier (BBB) dysfunction in the pathophysiology of BD (Patel and Frey, 2015). The BBB consists of

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endothelial cells of the brain capillaries, astrocyte end-feet, and pericytes (Ballabh et al., 2004). The presence of tight and adherens junctions between the endothelial cells contributes to the tightness of the BBB and its selective function (Banks, 2015). As most blood-borne substances are not allowed to enter the brain under physiological conditions, BBB integrity is essential for CNS homeostasis (Segarra et al., 2021). Evidence indicates that inflammatory cytokines such as tumor necrosis factor (TNF) α and interferon (IFN) γ (Capaldo and Nusrat, 2009), and oxidative stress (Sajja et al., 2016) are detrimental and contribute to BBB disruption. Recently, extensive BBB leakage has been associated with a more severe and chronic course of BD (Kamintsky et al., 2019). Still, research on the potential involvement of the BBB in the pathophysiology of BD is very scant.

In this study, TNFα and TBARS were selected as relevant markers due to their role in BD and potential implication in BBB disruption. High peripheral levels of TNFα and TBARS have been described in BD and linked to acute episodes (i.e., mania) and/or early stages of the disorder (Ascoli et al., 2019; Brietzke and Kapczinski, 2008; Modabbernia et al., 2013; Siwek et al., 2016). Moreover, in vitro studies have shown that TNFα downregulates the expression of tight junctions (Stone et al., 2011) and induce the production of neurotoxic compounds (i.e., quinolinic acid; Guillemin et al., 2001), while, in vivo, this proinflammatory cytokine upregulates matrix metalloproteinases (MMP) – endopeptidases associated with the degradation of BBB tight junctions. Regarding TBARS, a byproduct of lipid peroxidation, increased levels have been found to precede BBB breakdown in a preclinical model (Barichello et al., 2011) and to be associated with traumatic brain injury, which is frequently followed by BBB disruption (Lin et al., 2014). Lastly, claudin-5 is the most enriched tight junction protein in the BBB, determining its permeability properties (Hashimoto et al., 2021).

In preclinical research, amphetamine (AMPH) has been used to resemble some aspects of the manic episode of BD in rodents (Sharma et al., 2016). Besides hyperlocomotion, AMPH-injected animals present with increased levels of inflammation (e.g., interleukin (IL)−6, TNFα) and oxidative stress (e.g., lipid peroxidation and protein carbonylation) in the periphery and CNS (Frey et al., 2006a; Gubert et al., 2016). Moreover, it is suggested that lithium can prevent and reverse most of these alterations induced by AMPH and its analogs (Frey et al., 2006b; Macêdo et al., 2013; Valvassori et al., 2015). Considering that both inflammation and oxidative stress increase BBB permeability, we aimed to investigate if BBB disruption is observed in an AMPH-induced animal model of mania. Also, we sought to evaluate whether treatment with lithium reverses any BBB damage induced by AMPH. To date, only a few studies have evaluated, to some extent, BBB disruption in an animal model of mania (Valvassori et al., 2015) or other psychostimulants-induced models (Northrop and Yamamoto, 2012), while none has investigated a specific marker, such as claudin-5.

2. Methods

2.1. Animals

Male Wistar rats ($n = 24$, 2-month-old, 220–310 g) were purchased from Charles Rivers Laboratories (Massachusetts, USA). Animals were housed ($n = 2$ per cage) at standard room temperature and 12-h inverse light/dark cycle, with free access to food and water. This study was approved by the institutional ethics committee from McMaster University (Animal Research Ethics Board, project #140828), and all experimental procedures were performed following national (Canadian Council on Animal Care, 2020) and international (National Research Council (US), 2011) ethical standards.

2.2. Treatment groups

The study was performed in a 2 (AMPH model) x 2 (treatment) design, including 4 groups. Animals were allocated into groups by stratified randomization based on body weight prior to the experiments. From day 1 to 14, rats were injected with AMPH (dextroamphetamine, 2 mg/kg i.p. diluted in saline solution 0.9%, at 1 mL/kg; SmithKline Beecham, Brentford, UK) or saline (SAL, 1 mL/kg i.p.) once a day. Then, from day 8 to 14, animals were treated lithium chloride (LI, 47.5 mg/kg i.p. diluted in saline solution 0.9%, at 1 mL/kg; Sigma-Aldrich Corp., St Louis, USA) or saline (1 mL/kg i.p.) twice a day. This protocol has been previously described as a reversal model of mania (Frey et al., 2006a, 2006b; Valvassori et al., 2015). Treatment order was also randomly determined within-animal. Overall, the animals were divided in four treatment groups including (1) SAL+SAL ($n = 6$), (2) AMPH+SAL ($n = 6$), (3) SAL+LI ($n = 6$) and (4) AMPH+LI ($n = 6$).

2.3. Open field

On day 14, locomotor behavior was evaluated 2 h after the last injection of AMPH using the open field. The apparatus consisted of a 60×40 cm open field with 50-cm-high walls divided into 12 equal rectangles (Frey et al., 2006a, 2006b). Each animal was placed in the center of the open field and allowed to explore the apparatus for 5 min. All sessions were recorded using a webcam (C270 HD, Logitech), and a video tracking system (ANY-maze 5.2; Stoelting Co.) was used to analyze behavior. Briefly, the analysis setup included drawing the apparatus (i.e., outline and rectangles) in the software. Then, for each video, the software automatically detected the presence of the animal in the recording and tracked the number of crossings, distance traveled (m), average speed (m/s), and time spent in the periphery (s).

2.4. Euthanasia and sample collection

After the behavioral task, rats were deeply anesthetized with isoflurane (4–5% with oxygen at 1–2 L/min) and immediately decapitated, and the brain was removed as quickly as possible. The right and left prefrontal cortex (PFC), hippocampus (HIP), and striatum (ST) were dissected and snap frozen in dry ice. The troncular blood was also collected and centrifuged (2057 g, 10 min, at room temperature). Finally, brain structures and serum were stored at −80 °C until sample preparation.

2.5. Sample preparation

Right- and left-brain structures were prepared differently for biochemical analyses and western blot. Brain tissue was homogenized using phosphate-buffered saline (PBS) for biochemical assays or RIPA buffer 1x (20–188, Merck Millipore) for western blot at 1:4 (w/v). Both lysis buffers were prepared with EDTA-free protease inhibitor cocktail (11836170001, Roche) according to the manufacturer's instructions. Then, samples were centrifuged (10,000 g, 5 min, 5 °C), and supernatants were collected and kept at −80 °C until further analysis.

2.6. Biochemical assays

2.6.1. Total protein

The total protein was determined in the brain structures using DC Protein Assay Reagent (5000116, BioRad) according to the manufacturer's instructions. Briefly, samples were diluted 1:21 (v/v) in PBS and, after the addition of proper reagents, the total protein content in each sample was quantified in a spectrophotometer at 750 nm.

2.6.2. TNFα

TNFα levels were quantified using a sandwich ELISA kit (KRC3011, Invitrogen™) following the manufacturer's instructions. Serum samples were not diluted, while all brain structures homogenates were diluted at a 1:10 (v/v) ratio.

2.6.3. Thiobarbituric acid reactive substances (TBARS)

TBARS levels were detected and quantified using a colorimetric assay kit (KGE013, R&D Systems) according to the manufacturer's instructions. Serum samples were diluted at 1:2 (v/v) ratio, PFC and ST homogenates were diluted at 1:20 (v/v) and HIP at 1:20 (v/v) ratios.

2.7. Western blot

Briefly, homogenates samples of PFC, HIP, and ST were further diluted 1:1 (v/v) in 2x Laemmli sample buffer (1610737, BioRad Laboratories, Inc). After that, 20 μg of each sample was loaded in 4–20% pre-cast and stain-free mini gels (4568094, BioRad Laboratories, Inc). A protein marker (1610373, BioRad Laboratories, Inc) was also loaded in the gels. Electrophoresis was performed at 100 V for 1 h 15 min. The protein transfer was performed using the Trans-Blot® Turbo™ Transfer System (BioRad Laboratories, Inc) to an LF-PVDF membrane (0.45 μm pore size, BioRad Laboratories, Inc) at 25 V for 7 min. The total protein and transfer were verified using the ChemiDoc™ Imaging Systems (BioRad Laboratories, Inc); no specific staining was used. Blocking was performed using skim milk powder 5% (diluted in TBS-T) for 1 h at room temperature. Primary (Claudin 5 Monoclonal Antibody, 35–2500, Thermo Fisher) and secondary (Peroxidase-AffiniPure Goat Anti-Mouse IgG ($H + L$), 115–035–003, Jackson Immuno Research) antibodies were diluted in TBS-T at 1:500 and 1:10,000, respectively. β-actin was the loading control (β-Actin Loading Control Monoclonal Antibody, MA5–15739, Thermo Fisher; same secondary antibody as previous), and antibodies were diluted in TBS-T at 1:10,000. Clarity Western ECL Substrate (1705060, BioRad Laboratories, Inc) was used for detection. Total protein was used for loading control as described previously (Taylor et al., 2013), and claudin-5 levels were normalized by the control group levels (i.e., SAL+SAL).

2.8. Statistical analysis

Shapiro-Wilk and Levene's tests were used to evaluate the normality of distribution and homogeneity of variance, respectively. Secondly, two-way ANOVA was performed, considering model (saline and AMPH) and treatment (saline and lithium) as independent factors, followed by Tukey posthoc analysis, if ANOVA is significant. Outliers were identified using the Grubbs' test (α=0.05) and excluded from the analysis ($n = 5$). All $p<0.05$ were considered statistically significant.

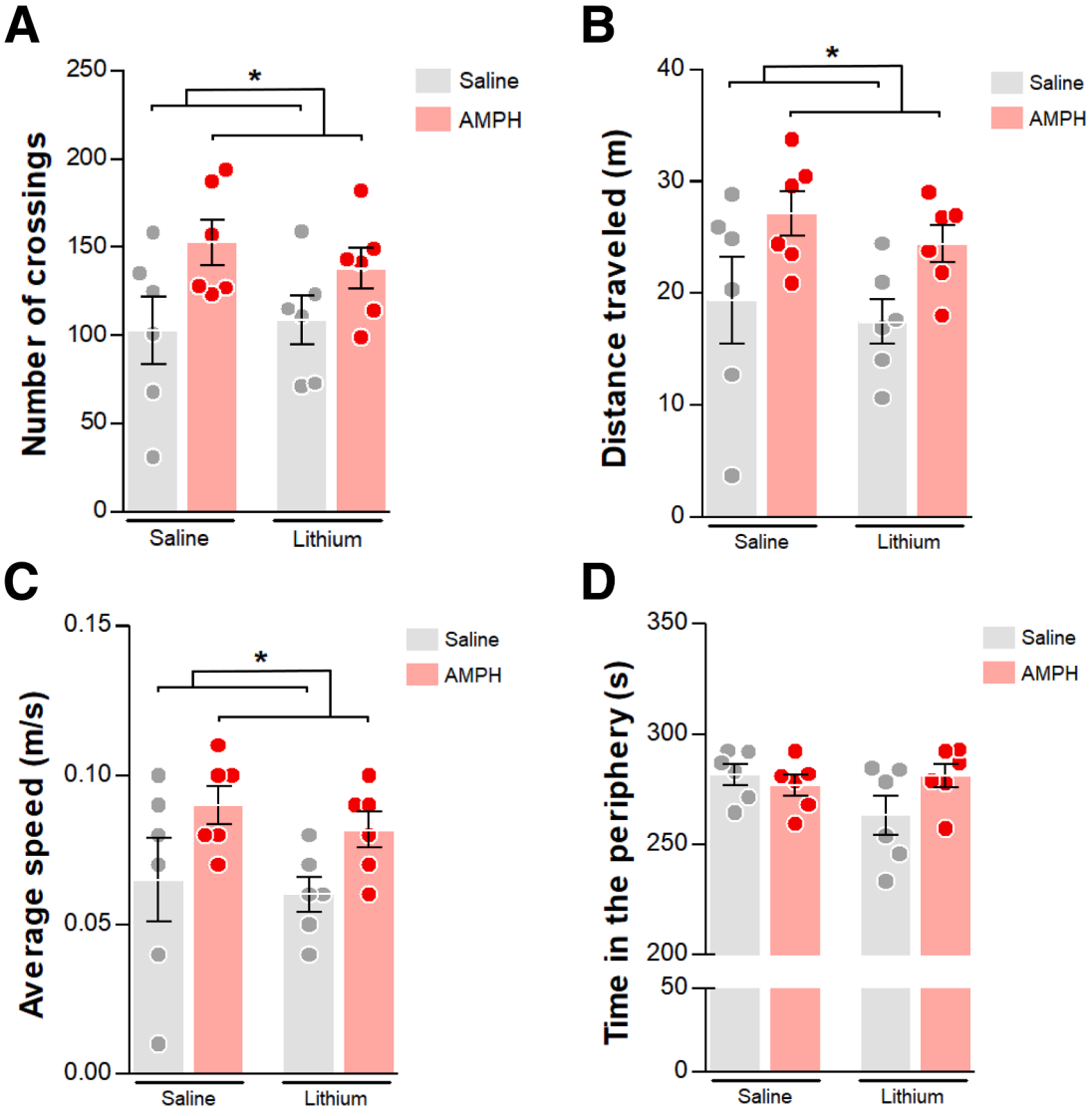

Fig. 1. Effects of AMPH and lithium in locomotor behavior. **A-C.** AMPH increased the frequency of crossing, average speed, and distance traveled in the open field (*main effect of AMPH model, $p<0.005$). **D.** Time spent in the periphery was similar among groups. Two-way ANOVA, data expressed by mean±SEM.

3. Results

3.1. AMPH induced hyperactivity

AMPH-injected rats presented overall hyperactivity in the open field, which was indicated by an increased number of crossings, average speed, and distance traveled (Fig. 1, A-C). Two-way ANOVA indicated a main effect of the AMPH model for all variables, with no main effects for treatment and interaction of factors (Table 1). Also, no differences were observed for time spent in the periphery (Fig. 1D, Table 1).

3.2. Effect of amph on serum and brain levels of TNFα and TBARS

In the serum, TNFα levels were below the limit of detection across all groups. In the brain regions analyzed, AMPH did not induce inflammation as evaluated through TNFα levels. Consequently, no effect of lithium was observed. Two-way ANOVA did not indicate main effects of AMPH model, treatment, and interaction for these variables (Fig. 2A-D, Table 2).

TBARS levels in the serum and brain structures remained unchanged following AMPH injection (Fig. 2E-H). However, lithium treatment increased lipid peroxidation in the serum, which was indicated by treatment effect, but no main effects for AMPH model and interaction of factors were observed following two-way ANOVA (Fig. 2E, Table 2). In the brain, there were no main effects of AMPH model, treatment, and interaction (Table 2).

Interestingly, a significant strong positive correlation was observed between TNFα and TBARS levels in the ST of the AMPH+SAL ($r = 0.87$, $p<0.001$) and AMPH+LI ($r = 0.96$, $p = 0.002$) groups. This correlation was weaker in the SAL+SAL group ($r = 0.80$, $p = 0.056$) and was negative in SAL+LI rats ($r=-0.89$, $p = 0.017$).

3.3. Claudin-5 protein levels in the PFC, HIP, and ST of AMPH-injected rats

Overall, the levels of claudin-5 in the brain remained unchanged following AMPH and lithium injection. Two-way ANOVA did not indicate main effects for the AMPH model, treatment, or interaction (Fig. 2I-L, Table 2).

4. Discussion

To our knowledge, this is the first study that investigated BBB disruption in an animal model of mania induced by AMPH. Corroborating with previous studies, AMPH-injected rats exhibited hyperactivity, which was determined by an increased frequency of crossings and distance traveled. Although our model showed face validity, no changes in peripheral and CNS levels of TNFα and TBARS were observed following AMPH and lithium injection. Consequently, protein levels of claudin-5, the most enriched tight junction protein in the BBB, also remained unchanged in the brain regions analyzed.

Psychostimulant-induced animal model of mania, such as the AMPH model, is frequently used to investigate biological mechanisms and alterations that have already been described in BD (Kara and Einat, 2013; Sharma et al., 2016). Clinical studies often report an increase in inflammatory and oxidative stress parameters in the disorder (Rowland et al., 2018), and some preclinical evidence is consistent with such findings. For instance, Valvassori et al. (2015) have shown that AMPH injections resulted in a pro-inflammatory effect. Injected rats presented with an augment of IL-4, IL-6, IL-10, and TNFα in the PFC, ST, and serum, which were restored to control levels following treatment with lithium. However, the authors did not find significant alterations in the HIP and cerebrospinal fluid (CSF). Other psychostimulant drugs, such as methylphenidate and methamphetamine, also seem to increase inflammatory markers in the HIP of rats (Beirami et al., 2017; Motaghinejad et al., 2017). However, no changes in TNFα levels were found in the serum and brain regions analyzed in our model. Still, no differences in inflammatory cytokines levels – such as TNFα, IL-1β, and IL-10 – have been previously described in rodents injected with AMPH or its derivates (Bristot et al., 2019; Gubert et al., 2016). Moreover, varying results might be related to differences in the protocol, such as type and regimen of psychostimulant and sensitivity of detection kits used for the markers of interest (e.g., inflammatory cytokines).

Classically, AMPH is responsible for enhancing dopamine (DA) release by inhibiting its reuptake, promoting reverse transport of DA into the synaptic cleft independent of stimulus and releasing DA from synaptic vesicles in the cytoplasm (Calipari and Ferris, 2013). These are the primary mechanisms involved in AMPH-induced hyperactivity and its neurotoxic effects (Valvassori et al., 2021). If not stored in synaptic vesicles, cytoplasmatic DA has a highly autoxidative capacity that can impair mitochondrial function and increase oxidative stress resulting in cell death (Brown and Yamamoto, 2003; Yamamoto and Bankson, 2005). However, in the present study, lipid peroxidation was not augmented following AMPH injection, given by TBARS levels. TBARS levels were higher in the ST, a brain region with many dopaminergic projections, but no statistical difference between groups was found. Still, a strong positive correlation between TBARS and TNFα levels was observed in this same brain region in AMPH-injected groups, regardless of treatment with lithium. Overall, findings regarding oxidative stress in AMPH models vary in the literature. More acute protocols or higher doses seem to be more likely to promote such alteration in the CNS (Frey et al., 2006a; Gomes et al., 2017; Gubert et al., 2016). Also, higher levels of TBARS were observed in the serum of lithium-treated animals, independent of AMPH injection. Lithium *per se* is known to be nephrotoxic (Carter et al., 2013), and increased levels of TBARS in the kidney have already been described in rats (Davis et al., 2018; Ossani et al., 2019).

Oxidative stress is an important promoter and product of the inflammatory response (Biswas, 2016), and it is supposed to underly AMPH-induced inflammation. As oxidative stress and inflammation are predictive of promoting BBB disruption and increasing its permeability (Patel and Frey, 2015), we hypothesized that AMPH would exert a deleterious effect in the BBB by downregulating claudin-5 expression. However, no significant changes were observed on claudin-5 levels in the PFC, ST, and HIP of rats after AMPH and lithium injections. A previous study showing increased levels of inflammatory cytokines in the brain parenchyma but no alteration in the CSF suggested that AMPH-injected rats might not present with disrupted BBB (Valvassori et al., 2015). However, in vivo and in vitro studies have already reported that methamphetamine and other psychostimulant drugs can disturb BBB integrity (Kousik et al., 2012; Northrop and Yamamoto, 2012).

Besides inflammation and oxidative stress, associated mechanisms have been proposed to underlie BBB disruption. For instance, the activation of inflammatory pathways, such as nuclear factor kappa B (NFκB), can result in the amplification of a large array of genes involved in inflammation, including MMPs (Hurtado-Alvarado et al., 2016). MMPs are enzymes that degrade tight junctions, such as claudin-5,

Table 1
Results of two-way ANOVA for behavioural assessment.

Dependent variable	Effects	*F*-value	df	*p*-value
Number of Crossing	AMPH model	7.30	1,20	**0.014**
	Treatment	0.10	1,20	0.761
	Interaction	0.48	1,20	0.495
Speed (m/s)	AMPH model	7.23	1,20	**0.014**
	Treatment	0.59	1,20	0.451
	Interaction	0.04	1,20	0.850
Distance (m)	AMPH model	8.35	1,20	**0.009**
	Treatment	0.84	1,20	0.371
	Interaction	0.02	1,20	0.885
Time in the periphery (s)	AMPH model	1.09	1,20	0.309
	Treatment	1.31	1,20	0.266
	Interaction	3.31	1,20	0.084

Abbreviations: AMPH, amphetamine; df, degrees of freedom.

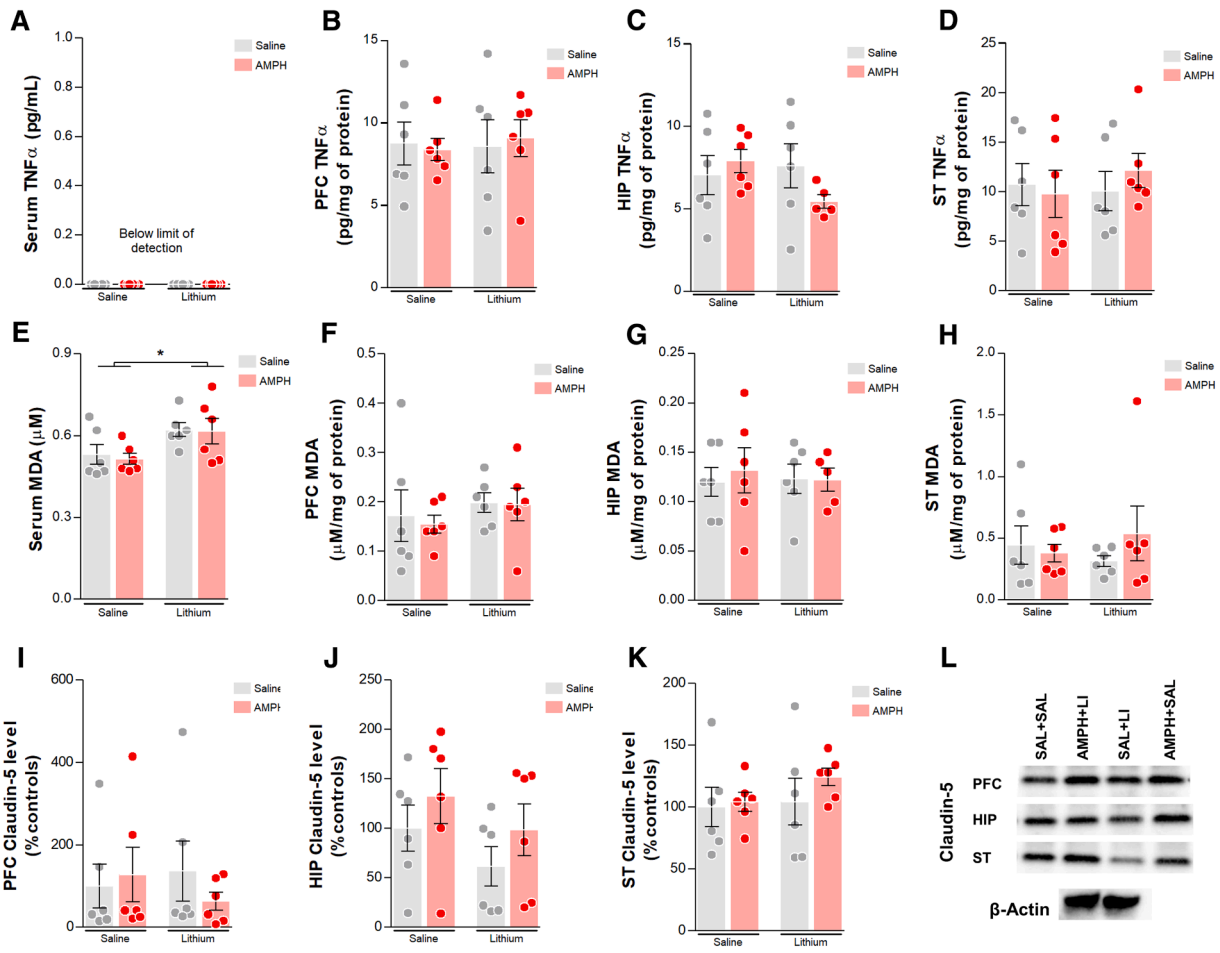

Fig. 2. TNFα, TBARS, and claudin-5 levels in the serum and brain. A. TNFα was not detected in the serum of the animals. **B-D.** AMPH and lithium injections did not change TNFα levels in the PFC, HIP, and ST. **E.** AMPH did not change lipid peroxidation levels, and an increase of serum TBARS was observed in lithium-treated rats (*main effect of treatment, $p = 0.009$). **F-H.** TBARS levels in the PFC, HIP, and ST were similar across groups. **I-L.** Claudin-5 protein levels in the PFC, HIP, and ST did not differ following AMPH and lithium injections. A band corresponding to claudin-5 was observed at 15–20 kDa, and β-actin was found at 37–50 kDa. Two-way ANOVA, data expressed by mean±SEM.

which are essential for maintaining BBB properties (Rempe et al., 2016). Methamphetamine has already been described to upregulate MMPs expression (Mizoguchi et al., 2007). Additionally, NFκB pathway activation following TNFα and IL-15 signaling may also be responsible for the downregulating the expression of tight junction proteins (e.g., claudin-2) in vitro (Stone et al., 2011). Therefore, it is relevant to investigate other pathways in BBB disruption and their potential association with BD and other psychiatric disorders.

Although BBB disruption has been implicated in the pathophysiology of psychiatric disorders (including BD; Greene et al., 2020), clinical and preclinical evidence is still scarce. A recent imaging study has shown that, among individuals with BD, only a sub-group exhibited an extensive BBB leakage that significantly differed from controls (Kamintsky et al., 2019). Interestingly, this sub-group of patients have a more chronic course of BD, with more severe symptoms of depression and anxiety. Other studies have evaluated biomarkers in the CSF and described higher levels of catecholamine and serotonin metabolites and inflammatory markers, such as IL-8, in individuals with BD (Isgren et al., 2015; Knorr et al., 2018). Furthermore, increased IL-8 levels in the CSF were associated with lithium treatment. Since its discovery, lithium has remained the first-line therapeutic choice for BD treatment (Yatham et al., 2018), but its effects on inflammation have yet to be fully elucidated. During euthymia, individuals with BD treated with lithium exhibited increased levels of TNFα and IL-4 compared to unmedicated patients (Guloksuz et al., 2010), which has also been described in vitro (Liu et al., 2011); but there is also evidence to show otherwise (Fernandes et al., 2011; Knijff et al., 2007). It is worth mentioning that augmented peripheral TNFα levels were further associated with poor response to lithium treatment in BD (Guloksuz et al., 2012).

Despite its novelty, some limitations should be addressed in our study. First, we did not explore more dynamic markers of the interface integrity between the blood, brain, and CSF, such as Evans Blue, which could be helpful as a first screening. Second, plasmatic levels of lithium were not assessed, but it has been described that therapeutic levels are reached following this protocol (Frey et al., 2006b). Third, lithium treatment did not decrease hyperlocomotion in AMPH-injected rats. While the attenuating effects of lithium on stimulant-induced hyperlocomotion are mostly consistent in the literature, some studies have reported a lack of effect (O'Donnell and Gould, 2007). Here, unaffected phenotypic response following lithium treatment may be attributed to inter-individual variability, but molecular changes (i.e., TBARS serum levels) were still observed in treated groups. Fourth, the use of volatile anesthetics, such as isoflurane, has been associated with anti-inflammatory and antioxidant effects in rodent models; thus, it might also be a potential experimental bias. Still, current evidence for animal models of AMPH is limited, and studies showing these protective effects primarily aimed to evaluate the use of volatile anesthetics as a pre-treatment or shortly after the model induction and for longer periods (Lee et al., 2015). Here, animals were briefly exposed to isoflurane (±5 min) at the end of the experiment until anesthetized. Lastly, while only a

Table 2
Results of two-way ANOVA for biochemical analyses.

Dependent variable	Effects	*F*-value	df	*p*-value
PFC TNFα	AMPH model	0.01	1,20	0.960
	Treatment	0.04	1,20	0.837
	Interaction	0.13	1,20	0.727
HIP TNFα[#]	AMPH model	0.42	1,19	0.523
	Treatment	0.88	1,19	0.361
	Interaction	2.23	1,19	0.152
ST TNFα	AMPH model	0.08	1,20	0.786
	Treatment	0.17	1,20	0.683
	Interaction	0.53	1,20	0.474
Serum TBARS	AMPH model	0.10	1,20	0.753
	Treatment	7.97	1,20	0.010
	Interaction	0.03	1,20	0.865
PFC TBARS	AMPH model	0.09	1,20	0.770
	Treatment	0.97	1,20	0.335
	Interaction	0.04	1,20	0.846
HIP TBARS[#]	AMPH model	0.09	1,19	0.763
	Treatment	0.04	1,19	0.854
	Interaction	0.15	1,19	0.705
ST TBARS	AMPH model	0.32	1,20	0.579
	Treatment	0.01	1,20	0.917
	Interaction	1.02	1,20	0.325
PFC Claudin-5	AMPH model	0.16	1,20	0.694
	Treatment	0.06	1,20	0.807
	Interaction	0.79	1,20	0.385
HIP Claudin-5	AMPH model	2.02	1,20	0.171
	Treatment	2.21	1,20	0.153
	Interaction	0.01	1,20	0.929
ST Claudin-5	AMPH model	0.81	1,20	0.379
	Treatment	0.81	1,20	0.379
	Interaction	0.34	1,20	0.568

Abbreviations: AMPH, amphetamine; df, degrees of freedom; HIP, hippocampus; PFC, prefrontal cortex; ST, striatum; TBARS, thiobarbituric acid reactive substances; TNFα, tumor necrosis factor α. [#]AMPH+LI, $n = 5$ for these variables.

few parameters were analyzed, their relevance to BD and the rationale for their role in the BBB disruption have been discussed.

Although an ideal animal model for BD has not been developed, AMPH injection in rodents remains an established animal model of mania with good construct, face, and predictive validity (Sharma et al., 2016). It should be noted that the latter has been questioned (Lan and Einat, 2019). Although this model may not mimic the vast complexity of BD pathophysiology, it would be advantageous to identify novel animal models that allow the evaluation of BBB disruption.

5. Conclusion

It is only recently that clinical research has provided BBB disruption as a marker of progression in BD (Kamintsky et al., 2019). Here, in one of the first attempts to investigate the effects of AMPH on BBB integrity, we did not find evidence that AMPH or lithium impact brain levels of claudin-5. Still, our results provide evidence and rationale for future research to establish the best approach to model and better understand this relatively novel pathophysiological mechanism implicated in BD.

Author statement

LPG, FK, RM, and BNF designed and wrote the protocol. LPG, BWA, DW, and WM performed the treatment, behavioral assessment, sample collection, and biochemical analyses. LPG, ARR, and BNF performed the statistical analysis. LPG wrote the manuscript draft. All authors contributed and approved the final article.

Declarations of Competing interest

None.

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References

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). American Psychiatric Pub.

Ascoli, B.M., Parisi, M.M., Bristot, G., Antqueviezc, B., Géa, L.P., Colombo, R., Kapczinski, F., Guma, F.T.C.R., Brietzke, E., Barbé-Tuana, F.M., Rosa, A.R., 2019. Attenuated inflammatory response of monocyte-derived macrophage from patients with BD: a preliminary report. Int. J. Bipolar Disord. 7, 13. https://doi.org/10.1186/s40345-019-0148-x.

Ballabh, P., Braun, A., Nedergaard, M., 2004. The blood-brain barrier: an overview: structure, regulation, and clinical implications. Neurobiol. Dis. 16, 1–13. https://doi.org/10.1016/j.nbd.2003.12.016.

Banks, W.A., 2015. Peptides and the blood–brain barrier. Peptides 72, 16–19. https://doi.org/10.1016/j.peptides.2015.03.010.

Barichello, T., Lemos, J.C., Generoso, J.S., Cipriano, A.L., Milioli, G.L., Marcelino, D.M., Vuolo, F., Petronilho, F., Dal-Pizzol, F., Vilela, M.C., Teixeira, A.L., 2011. Oxidative stress, cytokine/chemokine and disruption of blood-brain barrier in neonate rats after meningitis by Streptococcus agalactiae. Neurochem. Res. 36, 1922–1930. https://doi.org/10.1007/s11064-011-0514-2.

Beirami, E., Oryan, S., Seyedhosseini Tamijani, S.M., Ahmadiani, A., Dargahi, L., 2017. Intranasal insulin treatment alleviates methamphetamine induced anxiety-like behavior and neuroinflammation. Neurosci. Lett. 660, 122–129. https://doi.org/10.1016/j.neulet.2017.09.026.

Biswas, S.K., 2016. Does the Interdependence between Oxidative Stress and Inflammation Explain the Antioxidant Paradox? Oxid Med. Cell Longev., e5698931 https://doi.org/10.1155/2016/5698931, 2016.

Brietzke, E., Kapczinski, F., 2008. TNF-alpha as a molecular target in bipolar disorder. Prog. Neuropsychopharmacol. Biol. Psychiatry 32, 1355–1361. https://doi.org/10.1016/j.pnpbp.2008.01.006.

Bristot, G., Ascoli, B.M., Scotton, E., Géa, L.P., Pfaffenseller, B., Kauer-Sant'Anna, M., 2019. Effects of lithium on inflammatory and neurotrophic factors after an immune challenge in a lisdexamfetamine animal model of mania. Braz J Psychiatry e1. https://doi.org/10.1590/1516-4446-2017-0001.

Brown, J.M., Yamamoto, B.K., 2003. Effects of amphetamines on mitochondrial function: role of free radicals and oxidative stress. Pharmacol. Ther. 99, 45–53.

Calipari, E.S., Ferris, M.J., 2013. Amphetamine Mechanisms and Actions at the Dopamine Terminal Revisited. J Neurosci 33, 8923–8925. https://doi.org/10.1523/JNEUROSCI.1033-13.2013.

Canadian Council on Animal Care, 2020. Guide to the Care and Use of Experimental Animals, 2nd ed. Canadian Council on Animal Care, Ottawa, Ont.

Capaldo, C.T., Nusrat, A., 2009. Cytokine regulation of tight junctions. Biochimica et Biophysica Acta (BBA) - Biomembranes. Apical. Junction. Complexes Part II 1788, 864–871. https://doi.org/10.1016/j.bbamem.2008.08.027.

Carter, L., Zolezzi, M., Lewczyk, A., 2013. An updated review of the optimal lithium dosage regimen for renal protection. Can. J. Psychiatry 58, 595–600. https://doi.org/10.1177/070674371305801009.

Davis, J., Desmond, M., Berk, M., 2018. Lithium and nephrotoxicity: a literature review of approaches to clinical management and risk stratification. BMC Nephrol. 19, 305. https://doi.org/10.1186/s12882-018-1101-4.

Fernandes, B.S., Gama, C.S., Ceresér, K.M., Yatham, L.N., Fries, G.R., Colpo, G., de Lucena, D., Kunz, M., Gomes, F.A., Kapczinski, F., 2011. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis. J. Psychiatr Res. 45, 995–1004. https://doi.org/10.1016/j.jpsychires.2011.03.002.

Frey, Benício Noronha, Martins, M.R., Petronilho, F.C., Dal-Pizzol, F., Quevedo, J., Kapczinski, F., 2006a. Increased oxidative stress after repeated amphetamine exposure: possible relevance as a model of mania. Bipolar Disord. 8, 275–280. https://doi.org/10.1111/j.1399-5618.2006.00318.x.

Frey, Benicio N., Valvassori, S.S., Réus, G.Z., Martins, M.R., Petronilho, F.C., Bardini, K., Dal-Pizzol, F., Kapczinski, F., Quevedo, J., 2006b. Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. J. Psychiatry Neurosci. 31, 326–332.

Ghafouri-Fard, S., Oskooei, V.K., Omrani, M.D., Taheri, M., 2019. Dysregulation of cytokine coding genes in peripheral blood of bipolar patients. J. Affect. Disord. 256, 578–583. https://doi.org/10.1016/j.jad.2019.06.028.

Gomes, L.M., Carvalho-Silva, M., Teixeira, L.J., Rebelo, J., Mota, I.T., Bilesimo, R., Michels, M., Arent, C.O., Mariot, E., Dal-Pizzol, F., Scaini, G., Quevedo, J., Streck, E. L., 2017. Omega-3 fatty acids and mood stabilizers alter behavioral and oxidative stress parameters in animals subjected to fenproporex administration. Metab. Brain Dis. 32, 519–528. https://doi.org/10.1007/s11011-016-9942-7.

Grande, I., Berk, M., Birmaher, B., Vieta, E., 2016. Bipolar disorder. Lancet 387, 1561–1572 https://doi.org/10.1016/S0140-6736(15)00241-X.

Greene, C., Hanley, N., Campbell, M., 2020. Blood-brain barrier associated tight junction disruption is a hallmark feature of major psychiatric disorders. Transl. Psychiatry 10, 1–10. https://doi.org/10.1038/s41398-020-01054-3.

Gubert, C., Fries, G.R., Pfaffenseller, B., Ferrari, P., Coutinho-Silva, R., Morrone, F.B., Kapczinski, F., Battastini, A.M.O., 2016. Role of P2×7 Receptor in an Animal Model of Mania Induced by D-Amphetamine. Mol. Neurobiol. 53, 611–620. https://doi.org/10.1007/s12035-014-9031-z.

Guillemin, G.J., Kerr, S.J., Smythe, G.A., Smith, D.G., Kapoor, V., Armati, P.J., Croitoru, J., Brew, B.J., 2001. Kynurenine pathway metabolism in human astrocytes: a paradox for neuronal protection. J. Neurochem. 78, 842–853. https://doi.org/10.1046/j.1471-4159.2001.00498.x.

Guloksuz, S., Altinbas, K., Aktas Cetin, E., Kenis, G., Bilgic Gazioglu, S., Deniz, G., Oral, E.T., van Os, J., 2012. Evidence for an association between tumor necrosis factor-alpha levels and lithium response. J. Affect. Disord. 143, 148–152. https://doi.org/10.1016/j.jad.2012.04.044.

Guloksuz, S., Cetin, E.A., Cetin, T., Deniz, G., Oral, E.T., Nutt, D.J., 2010. Cytokine levels in euthymic bipolar patients. J. Affect. Disord. 126, 458–462. https://doi.org/10.1016/j.jad.2010.04.027.

Hashimoto, Y., Campbell, M., Tachibana, K., Okada, Y., Kondoh, M., 2021. Claudin-5: a Pharmacological Target to Modify the Permeability of the Blood-Brain Barrier. Biol Pharm Bull 44, 1380–1390. https://doi.org/10.1248/bpb.b21-00408.

Hurtado-Alvarado, G., Domínguez-Salazar, E., Pavon, L., Velázquez-Moctezuma, J., Gómez-González, B., 2016. Blood-brain barrier disruption induced by chronic sleep loss: low-grade inflammation may be the link. J. Immunol. Res., 4576012 https://doi.org/10.1155/2016/4576012, 2016.

Isgren, A., Jakobsson, J., Pålsson, E., Ekman, C.J., Johansson, A.G.M., Sellgren, C., Blennow, K., Zetterberg, H., Landén, M., 2015. Increased cerebrospinal fluid interleukin-8 in bipolar disorder patients associated with lithium and antipsychotic treatment. Brain Behav. Immun. 43, 198–204. https://doi.org/10.1016/j.bbi.2014.10.001.

Kamintsky, L., Cairns, K.A., Veksler, R., Bowen, C., Beyea, S.D., Friedman, A., Calkin, C., 2019. Blood-brain barrier imaging as a potential biomarker for bipolar disorder progression. Neuroimage Clin. 26 https://doi.org/10.1016/j.nicl.2019.102049.

Kapczinski, F., Dal-Pizzol, F., Teixeira, A.L., Magalhaes, P.V.S., Kauer-Sant'Anna, M., Klamt, F., Pasquali, M.A., de, B., Quevedo, J., Gama, C.S., Post, R., 2010. A systemic toxicity index developed to assess peripheral changes in mood episodes. Mol. Psychiatry 15, 784–786. https://doi.org/10.1038/mp.2009.112.

Kara, N.Z., Einat, H., 2013. Rodent models for mania: practical approaches. Cell Tissue Res. 354, 191–201. https://doi.org/10.1007/s00441-013-1594-x.

Kim, Y.-K., Jung, H.-G., Myint, A.-M., Kim, H., Park, S.-H., 2007. Imbalance between pro-inflammatory and anti-inflammatory cytokines in bipolar disorder. J. Affect. Disord. 104, 91–95. https://doi.org/10.1016/j.jad.2007.02.018.

Knijff, E.M., Breunis, M.N., Kupka, R.W., Wit, H.J.D., Ruwhof, C., Akkerhuis, G.W., Nolen, W.A., Drexhage, H.A., 2007. An imbalance in the production of IL-1β and IL-6 by monocytes of bipolar patients: restoration by lithium treatment. Bipolar Disord. 9, 743–753. https://doi.org/10.1111/j.1399-5618.2007.00444.x.

Knorr, U., Simonsen, A.H., Zetterberg, H., Blennow, K., Hasselbalch, S.G., Kessing, L.V., 2018. Biomarkers in cerebrospinal fluid of patients with bipolar disorder versus healthy individuals: a systematic review. Eur. Neuropsychopharmacol. 28, 783–794. https://doi.org/10.1016/j.euroneuro.2018.04.002.

Kousik, S.M., Napier, T.C., Carvey, P.M., 2012. The effects of psychostimulant drugs on blood brain barrier function and neuroinflammation. Front. Pharmacol. 3 https://doi.org/10.3389/fphar.2012.00121.

Lan, A., Einat, H., 2019. Questioning the predictive validity of the amphetamine-induced hyperactivity model for screening mood stabilizing drugs. Behav. Brain Res. 362, 109–113. https://doi.org/10.1016/j.bbr.2019.01.006.

Lee, Y.-M., Song, B.C., Yeum, K.-J., 2015. Impact of volatile anesthetics on oxidative stress and inflammation. Biomed. Res. Int., 242709 https://doi.org/10.1155/2015/242709, 2015.

Lin, W.-M., Chen, M.-H., Wang, H.-C., Lu, C.-H., Chen, P.-C., Chen, H.-L., Tsai, N.-W., Su, Y.-J., Li, S.-H., Kung, C.-T., Chiu, T.-M., Weng, H.-H., Lin, W.-C., 2014. Association between peripheral oxidative stress and white matter damage in acute traumatic brain injury. Biomed. Res. Int., 340936 https://doi.org/10.1155/2014/340936, 2014.

Liu, K.-J., Lee, Y.-L., Yang, Y.-Y., Shih, N.-Y., Ho, C.-C., Wu, Y.-C., Huang, T.-S., Huang, M.-C., Liu, H.-C., Shen, W.W., Leu, S.-J., 2011. Modulation of the development of human monocyte-derived dendritic cells by lithium chloride. J. Cell. Physiol. 226, 424–433. https://doi.org/10.1002/jcp.22348.

Macêdo, D.S., de Lucena, D.F., Queiroz, A.I.G., Cordeiro, R.C., Araújo, M.M., Sousa, F.C., Vasconcelos, S.M., Hyphantis, T.N., Quevedo, J., McIntyre, R.S., Carvalho, A.F., 2013. Effects of lithium on oxidative stress and behavioral alterations induced by lisdexamfetamine dimesylate: relevance as an animal model of mania. Prog. Neuropsychopharmacol. Biol. Psychiatry 43, 230–237. https://doi.org/10.1016/j.pnpbp.2013.01.007.

Mizoguchi, H., Yamada, K., Mouri, A., Niwa, M., Mizuno, T., Noda, Y., Nitta, A., Itohara, S., Banno, Y., Nabeshima, T., 2007. Role of matrix metalloproteinase and tissue inhibitor of MMP in methamphetamine-induced behavioral sensitization and reward: implications for dopamine receptor down-regulation and dopamine release. J. Neurochem. 102, 1548–1560. https://doi.org/10.1111/j.1471-4159.2007.04623.x.

Modabbernia, A., Taslimi, S., Brietzke, E., Ashrafi, M., 2013. Cytokine alterations in bipolar disorder: a meta-analysis of 30 studies. Biol. Psychiatry 74, 15–25. https://doi.org/10.1016/j.biopsych.2013.01.007.

Motaghinejad, M., Motevalian, M., Babalouei, F., Abdollahi, M., Heidari, M., Madjd, Z., 2017. Possible involvement of CREB/BDNF signaling pathway in neuroprotective effects of topiramate against methylphenidate induced apoptosis, oxidative stress and inflammation in isolated hippocampus of rats: molecular, biochemical and histological evidences. Brain Res. Bull. 132, 82–98. https://doi.org/10.1016/j.brainresbull.2017.05.011.

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011. Guide for the Care and Use of Laboratory Animals. The National Academies Collection: Reports funded By National Institutes of Health, 8th ed. National Academies Press, (US), Washington (DC).

Northrop, N.A., Yamamoto, B.K., 2012. Persistent neuroinflammatory effects of serial exposure to stress and methamphetamine on the blood-brain barrier. J. Neuroimmune Pharmacol. 7, 951–968. https://doi.org/10.1007/s11481-012-9391-y.

O'Donnell, K.C., Gould, T.D., 2007. The Behavioral Actions of Lithium in Rodent Models. Neurosci. Biobehav. Rev. 31, 932–962. https://doi.org/10.1016/j.neubiorev.2007.04.002.

Ossani, G.P., Uceda, A.M., Acosta, J.M., Lago, N.R., Repetto, M.G., Martino, D.J., Toblli, J.E., 2019. Role of oxidative stress in lithium-induced nephropathy. Biol. Trace Elem. Res. https://doi.org/10.1007/s12011-018-1617-2.

Patel, J.P., Frey, B.N., 2015. Disruption in the blood-brain barrier: the missing link between brain and body inflammation in bipolar disorder? Neural Plast., 708306 https://doi.org/10.1155/2015/708306, 2015.

Petersen, N.A., Nielsen, M.Ø., Coello, K., Stanislaus, S., Melbye, S., Kjærstad, H.L., Sletved, K.S.O., McIntyre, R.S., Frikke-Smith, R., Vinberg, M., Kessing, L.V., 2021. Brain-derived neurotrophic factor levels in newly diagnosed patients with bipolar disorder, their unaffected first-degree relatives and healthy controls. BJPsych Open 7, e55. https://doi.org/10.1192/bjo.2021.9.

Rempe, R.G., Hartz, A.M., Bauer, B., 2016. Matrix metalloproteinases in the brain and blood–brain barrier: versatile breakers and makers. J. Cereb. Blood Flow Metab. 36, 1481–1507. https://doi.org/10.1177/0271678×16655551.

Rosenblat, J.D., McIntyre, R.S., 2016. Bipolar Disorder and Inflammation. Psychiatr. Clin. North Am. 39, 125–137. https://doi.org/10.1016/j.psc.2015.09.006.

Rowland, T., Perry, B.I., Upthegrove, R., Barnes, N., Chatterjee, J., Gallacher, D., Marwaha, S., 2018. Neurotrophins, cytokines, oxidative stress mediators and mood state in bipolar disorder: systematic review and meta-analyses. Br. J. Psychiatry 213, 514–525. https://doi.org/10.1192/bjp.2018.144.

Sajja, R.K., Rahman, S., Cucullo, L., 2016. Drugs of abuse and blood-brain barrier endothelial dysfunction: a focus on the role of oxidative stress. J. Cereb. Blood Flow Metab. 36, 539–554. https://doi.org/10.1177/0271678×15616978.

Segarra, M., Aburto, M.R., Acker-Palmer, A., 2021. Blood-brain barrier dynamics to maintain brain homeostasis. Trends Neurosci. https://doi.org/10.1016/j.tins.2020.12.002.

Sharma, A.N., Fries, G.R., Galvez, J.F., Valvassori, S.S., Soares, J.C., Carvalho, A.F., Quevedo, J., 2016. Modeling mania in preclinical settings: a comprehensive review. Prog. Neuropsychopharmacol. Biol. Psychiatry 66, 22–34. https://doi.org/10.1016/j.pnpbp.2015.11.001.

Siwek, M., Sowa-Kucma, M., Styczen, K., Misztak, P., Szewczyk, B., Topor-Madry, R., Nowak, G., Dudek, D., Rybakowski, J.K., 2016. Thiobarbituric acid-reactive substances: markers of an acute episode and a late stage of bipolar disorder. Neuropsychobiology 73, 116–122. https://doi.org/10.1159/000444491.

Stone, K.P., Kastin, A.J., Pan, W., 2011. NFκB is an unexpected major mediator of interleukin-15 signaling in cerebral endothelia. Cell. Physiol. Biochem. 28, 115–124. https://doi.org/10.1159/000331720.

Valvassori, S.S., Gava, F.F., Cararo, J.H., Quevedo, J., 2021. Chapter 9 - The evolution of animal models for bipolar disorder. Eds.. In: Quevedo, J., Carvalho, A.F., Vieta, E. (Eds.), Neurobiology of Bipolar Disorder. Academic Press, pp. 109–115. https://doi.org/10.1016/B978-0-12-819182-8.00009-0.

Valvassori, S.S., Tonin, P.T., Varela, R.B., Carvalho, A.F., Mariot, E., Amboni, R.T., Bianchini, G., Andersen, M.L., Quevedo, J., 2015. Lithium modulates the production of peripheral and cerebral cytokines in an animal model of mania induced by dextroamphetamine. Bipolar Disord. 17, 507–517. https://doi.org/10.1111/bdi.12299.

van den Ameele, S., Coppens, V., Schuermans, J., De Boer, P., Timmers, M., Fransen, E., Sabbe, B., Morrens, M., 2017. Neurotrophic and inflammatory markers in bipolar disorder: a prospective study. Psychoneuroendocrinology 84, 143–150. https://doi.org/10.1016/j.psyneuen.2017.07.003.

van den Ameele, S., van Nuijs, A.L., Lai, F.Y., Schuermans, J., Verkerk, R., van Diermen, L., Coppens, V., Fransen, E., de Boer, P., Timmers, M., Sabbe, B., Morrens, M., 2020. A mood state-specific interaction between kynurenine metabolism and inflammation is present in bipolar disorder. Bipolar Disord. 22, 59–69. https://doi.org/10.1111/bdi.12814.

Yamamoto, B.K., Bankson, M.G., 2005. Amphetamine neurotoxicity: cause and consequence of oxidative stress. Crit. Rev. Neurobiol. 17, 87–117. https://doi.org/10.1615/CritRevNeurobiol.v17.i2.30.

Yatham, L.N., Kennedy, S.H., Parikh, S.V., Schaffer, A., Bond, D.J., Frey, B.N., Sharma, V., Goldstein, B.I., Rej, S., Beaulieu, S., Alda, M., MacQueen, G., Milev, R.V., Ravindran, A., O'Donovan, C., McIntosh, D., Lam, R.W., Vazquez, G., Kapczinski, F., McIntyre, R.S., Kozicky, J., Kanba, S., Lafer, B., Suppes, T., Calabrese, J.R., Vieta, E., Malhi, G., Post, R.M., Berk, M., 2018. Canadian network for mood and anxiety treatments (CANMAT) and international society for bipolar disorders (ISBD) 2018 guidelines for the management of patients with bipolar disorder. Bipolar Disord. 20, 97–170. https://doi.org/10.1111/bdi.12609.