

Translational Research in Bipolar Disorders

Guest Editors: Rodrigo Machado-Vieira, Benicio N. Frey, Ana C. Andreazza, and João Quevedo





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Neural Plasticity

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Editorial

Translational Research in Bipolar Disorders

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Translational research is at the vanguard of contemporary psychiatry. Its bidirectional continuum (from bench to bedside and vice versa) has become a primary target in the search of personalized treatments in psychiatry. Although the term “translational” has various definitions, its common ground has focused on a better understanding of the pathophysiology of psychiatric disorders as well as on the identification of new diagnostic tests and development of new, improved treatments. In psychiatry, these aims seem more challenging than in other medical areas due to the complexity of brain mechanisms and behaviors.

Several other areas in medicine (e.g., oncology and endocrinology) have successfully decreased the gap between initial drug discovery steps and the subsequent approval of successful therapies for human diseases using translational approaches. In mental health, the use of tools and technologies that are able to positively impact health parameters in psychiatric conditions has been put forward as the ultimate objective of translational neuroscience research, which may tailor clinical practice [1]. Even though several advances have been obtained, this is a key but complex step to pursue, since psychiatric disorders have potential heterogeneous symptomatology, cognitive functioning, and comorbidities as well as having a wide range of genetic and environmental risk

factors [2]. Furthermore, recent genome-wide association studies have identified potential candidate genes, but lack of replication by subsequent larger studies and low power have been major limitations in this field.

One example that fits well in this paradigm is bipolar disorder (BD). This is a major mental illness with high prevalence (especially when considering its spectrum), which still has low recovery rates and high rates of treatment-resistant cases, with consequent high morbidity and mortality rates. Despite this major public health issue, existing pharmacological treatments for BD are mostly modifications of older versions and have not been demonstrated to be superior to agents from the same class in terms of efficacy and effectiveness. Except for lithium, all first- and second-line treatments for BD phases and maintenance were first developed and approved for other disorders (e.g., anticonvulsants and atypical antipsychotics). It is noteworthy that the use of lithium has been progressively declining despite the strong evidence base in favor of its use [3, 4].

Another degree of complexity is based on its pathophysiology. This involves dysfunctions at multiple levels and systems with a convergent impact at subcellular downstream cascades that directly regulate cellular resilience and neural plasticity. Some of these molecular targets include central

and peripheral stress pathways, inflammation, intracellular signaling cascades, and organelles (mitochondria and endoplasmic reticulum) dysfunction [5]. In this context, some of these newly identified potential treatment targets may represent an advance in the therapeutics of BD, going beyond the so-called “me too” agents. These may include modulators at the glutamate and purinergic neurotransmitter systems, intracellular signaling, neuropeptide systems, and others recently tested [6, 7]. These recent developments may also provide new avenues to help neuroscientists explore in more detail the underlying mechanisms involved in the neurobiology of BD using convergent translational approaches [5] and identify new targets for future proof of concept studies.

The use of biomarkers may also play a critical role in this strategy to overcome issues that have limited drug discovery in BD [8, 9]. This includes the use of a new generation of “omics” technologies and the identification of novel mechanisms at genetic, molecular, cellular, cognitive, circuits, and behavioral levels [5]. This approach using biological dimensions at different levels may also help to decipher brain network functioning and validate targets as probes of disease mechanisms. For instance, research on certain candidate genes from genome-wide association studies, such as *ANKK3*, a gene involved in the function of voltage gated sodium channels, and *CACNA1C*, a gene encoding a protein which is part of a voltage dependent calcium channel, may represent relevant targets for future functional studies [10] integrated in multiple levels and psychiatric disorders. For instance, genes associated with personality traits and behaviors, such as impulsivity, anhedonia, or suicidality, and involved in circadian rhythm disruption in mood disorders are promising areas to perform studies from molecular to circuit-level.

Even though the previously so expected “holy grail” in psychiatric research seems hard to achieve based on the heterogeneous clinical picture and variable treatment response and polygenic basis of psychiatric disorders, the identification of neural and metabolic (brain/periphery) circuits related to treatment response through longitudinal studies is lacking. Brain imaging studies have also shown cortical and subcortical abnormalities in regions associated with emotional regulation, especially in frontolimbic circuitry; however studies exploring target engagement (e.g., PET receptor occupancy studies) are still scarce and essential to give more consistency when evaluating potential modulators and moderators associated with clinical efficacy in proof of concept trials. Other areas deserving further studies include the neurobiology of cycling, sleep, psychomotor activity studies, and early intervention. Similarly, studies on the role of early stressors/trauma and the role of epigenetics as well as intermediate phenotypes and illness subtypes can benefit from translational approaches in BD research.

Animal and preclinical models also represent valuable tools to further studies underlying neurobiological underpinnings of BD, helping to develop the next generation of strategies using circuit-centered psychiatric dimensions [11]. Findings from clinical neuroscience may be integrated into the next generation of animal models. The main challenge of

animal modeling in BD is the inability to develop a model that includes the cycling nature of the illness.

There has been a recent shift in paradigm in psychiatric research. Despite promising perspectives and the appealing search for the “wow factor” when developing new and sophisticated tools and technological machinery with respective technical expertise, these have shown little impact in public health parameters in psychiatric disorders. There is an urgent need for the new generation of clinicians to take a step ahead and be trained to develop skills in neuroscience research, with the same approach for neuroscientists in regard to the study of BD neurobiology and therapeutics, since valuable translational research arises from “clinically relevant” hypotheses. This seems the critical (if not the only) path able to fill the gap between discoveries in basic research and its potential utility and impact in drug development and potential benefit to individuals with mental disorders. Also, a better understanding on the biological basis of the observed variability in treatment response, cognitive dysfunction, neuroprogression, role of comorbidities, and investigation on the treatment-resistant cases are critical aspects to be addressed by the next generation of “clinician-scientists.”

New interesting conceptual frameworks have been recently developed to fill this gap using different units of analysis, such as the NIMH RDoC (Research Domain Criteria) project. This is based on concepts originated from basic behavioral neuroscience. Importantly, this new research framework focuses on the behavior-brain interaction and systems rather than the standard diagnostic classifications [12]. Also, new initiatives such as the NIMH BRAIN initiative [13], which emphasizes the need for sharing large-scale datasets, may help to build the foundation on understanding how the brain works and apply this to the identification of better diagnostic tools and treatments. The development of new neuroscience-based approaches for mental disorders targeted at circuits- and systems-level measurements (e.g., modeling positive and negative affect and anhedonia for depression) may lead to the identification of new targets and more effective treatments.

Disclosure

This work was written as part of Rodrigo Machado-Vieira’s official duties as a government employee.

Disclaimer

The views expressed in this article do not necessarily represent the views of the NIMH, NIH, HHS, or the United States Government.

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References

- [1] T. R. Insel, "Translating scientific opportunity into public health impact: a strategic plan for research on mental illness," *Archives of General Psychiatry*, vol. 66, no. 2, pp. 128–133, 2009.
- [2] R. Machado-Vieira, "Tracking the impact of translational research in psychiatry: state of the art and perspectives," *Journal of Translational Medicine*, vol. 10, no. 1, p. 175, 2012.
- [3] F. K. Goodwin, "Rationale for long-term treatment of bipolar disorder and evidence for long-term lithium treatment," *The Journal of Clinical Psychiatry*, vol. 63, supplement 10, pp. 5–12, 2002.
- [4] R. Machado-Vieira, H. K. Manji, and C. A. Zarate Jr., "The role of lithium in the treatment of bipolar disorder: convergent evidence for neurotrophic effects as a unifying hypothesis," *Bipolar Disorders*, vol. 11, no. 2, pp. 92–109, 2009.
- [5] R. Machado-Vieira, M. G. Soeiro-De-Souza, E. M. Richards, A. L. Teixeira, and C. A. Zarate Jr., "Multiple levels of impaired neural plasticity and cellular resilience in bipolar disorder: developing treatments using an integrated translational approach," *World Journal of Biological Psychiatry*, vol. 15, no. 2, pp. 84–95, 2014.
- [6] R. Machado-Vieira, G. Salvadore, N. DiazGranados et al., "New therapeutic targets for mood disorders," *TheScientificWorldJournal*, vol. 10, pp. 713–726, 2010.
- [7] C. Zarate Jr., R. Machado-Vieira, I. Henter, L. Ibrahim, N. Diazgranados, and G. Salvadore, "Glutamatergic modulators: the future of treating mood disorders," *Harvard Review of Psychiatry*, vol. 18, no. 5, pp. 293–303, 2010.
- [8] B. N. Frey, A. C. Andreazza, J. Houenou, S. Jamain, B. I. Goldstein, and M. A. Frye, "Biomarkers in bipolar disorder: a positional paper from the International Society for Bipolar Disorders Biomarkers Task Force," *Australian & New Zealand Journal of Psychiatry*, vol. 47, no. 4, pp. 321–332, 2013.
- [9] G. Scola and A. C. Andreazza, "Current state of biomarkers in bipolar disorder," *Current Psychiatry Reports*, vol. 16, no. 12, p. 514, 2014.
- [10] F. S. Price and J. A. Morris, "The genetics of bipolar disorder," *British Medical Journal*, vol. 346, article no. f530, 2013.
- [11] R. Machado-Vieira and C. A. Zarate Jr., "Next-generation strategies in animal model research to translate preclinical discoveries into better treatments for circuit-centered psychiatric dimensions," *Revista Brasileira de Psiquiatria*, vol. 35, supplement 2, pp. S75–S76, 2013.
- [12] B. N. Cuthbert and T. R. Insel, "Toward the future of psychiatric diagnosis: the seven pillars of RDoC," *BMC Medicine*, vol. 11, article 126, 2013.
- [13] C. I. Bargmann and J. A. Lieberman, "What the BRAIN initiative means for psychiatry," *The American Journal of Psychiatry*, vol. 171, no. 10, pp. 1038–1040, 2014.

Research Article

Preclinical Evidences for an Antimanic Effect of Carvedilol

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Oxidative imbalance, alterations in brain-derived neurotrophic factor (BDNF), and mitochondrial dysfunction are implicated in bipolar disorder (BD) pathophysiology and comorbidities, for example, cardiovascular conditions. Carvedilol (CVD), a nonselective beta-blocker widely used for the treatment of hypertension, presents antioxidant and mitochondrial stabilizing properties. Thus, we hypothesized that CVD would prevent and/or reverse mania-like behavioral and neurochemical alterations induced by lisdexamfetamine dimesylate (LDX). To do this, male Wistar rats were submitted to two different protocols, namely, prevention and reversal. In the prevention treatment the rats received daily oral administration (mg/kg) of CVD (2.5, 5 or 7.5), saline, valproate (VAL200), or the combination of CVD5 + VAL100 for 7 days. From the 8th to 14th day LDX was added. In the reversal protocol LDX was administered for 7 days with the drugs being added from the 8th to 14th day of treatment. Two hours after the last administration the behavioral (open field and social interaction) and neurochemical (reduced glutathione, lipid peroxidation, and BDNF) determinations were performed. The results showed that CVD prevented and reversed the behavioral and neurochemical alterations induced by LDX. The administration of CVD5 + VAL100 potentiated the effect of VAL200 alone. Taken together these results demonstrate a possible antimanic effect of CVD in this preclinical model.

1. Introduction

Bipolar disorder (BD) is one of the most serious mental illnesses characterized by depressive and manic episodes with spontaneous cycling. The pathophysiology of this mental disorder remains unclear; however, evidences point towards the involvement of genetics, signal transmission deregulation, deleterious inflammatory profile, dysregulation in oxidative stress, and neurotrophins [1, 2].

The disorder presents a chronic course associated with functional decline, elevated mortality, and significant disease burden [3, 4]. Importantly, BD patients present an excess

burden of cardiovascular risk and higher rate of hypertension compared to the general population [4]. Additionally, this risk is increased by modern treatments for BD, such as antipsychotics [5].

The number of affective episodes in BD patients is directly related to social and cognitive deficits as well as risk of suicide among others [6]. Still in relation to affective episodes, the number of manic episodes was related to impairment of verbal memory implicating, thus, frontal structures [3]. Besides frontal structures, such as prefrontal cortex, striatum and hippocampus are putative brain areas related to mania as observed in clinical [7] and preclinical studies [8, 9].

Based on the importance of manic episodes for BD outcome [10], the preclinical model most widely used to study BD is based on the induction of mania-like episodes by the administration of amphetamine-related compounds, such as d-amphetamine (d-AMPH) [11, 12]. Due to the restrictions on d-AMPH acquisition and use, recently our research group proposed an animal model of mania in rats based on the administration of lisdexamfetamine dimesylate (LDX) [9], a prodrug metabolically converted to d-AMPH [13]. In our previous study [9], LDX caused behavioral and neurochemical alterations similar to those of d-AMPH [11, 14] with the alterations prevented and reversed by the mood stabilizer lithium.

Indeed, the administration of amphetamine-related compounds to rodents resembles some alterations related to mania [1] such as increase in dopamine (DA) neurotransmission with consequent hyperlocomotion, oxidative imbalance, and mitochondrial dysfunction, and decrease in brain derived neurotrophic factor (BDNF) in putative brain areas related to BD pathophysiology, namely, prefrontal cortex (PFC), hippocampus (HC), and striatum (ST) [9, 14, 15].

Thus, based on the prooxidant alterations and mitochondrial dysfunctions observed in BD patients [1, 5] and in the animal models of mania [9, 15] as well as on the cardiovascular risk presented by BD patients, we decided to study the effects of carvedilol (CVD, {1-(carbazolyl)-(4-oxy)-3-(2-methoxyphenoxyethyl amino)-propanol-(2)}) against mania induced by LDX.

Carvedilol is prescribed for the treatment of congestive heart failure, mild to moderate hypertension, and myocardial infarction. The drug competitively blocks β_1 , β_2 , and α_1 -adrenergic receptors while displaying vasodilating properties. A distinctive characteristic of CVD in comparison to other β -adrenergic receptor antagonists is its potent antioxidant properties [16]. This antioxidant activity of CVD is attributed to its ability to chelate free iron [17]. The drug also presents mitochondria protective [18] and antiapoptotic/anti-inflammatory properties [19].

Therefore, herein we aimed to determine the effects of CVD alone and associated with the mood stabilizer drug, valproate, in the prevention and/or reversal of behavioral (hyperlocomotion and social interaction) and neurochemical (reduced glutathione (GSH) and lipid peroxidation) alterations in the PFC and ST, as well as hippocampal BDNF levels of animals submitted to the model of mania induced by LDX [9].

2. Materials and Methods

2.1. Drugs. Carvedilol (CVD; Coreg, Roche, Brazil), lisdexamfetamine dimesylate (LDX; Vyvanse, Shire, USA), and sodium valproate (VAL; Life Pharmaceutical Company) were used. The drugs were made up freshly within 1-2 h of dosing. All other chemicals used were of analytical grade.

2.2. Animals. The experiments were performed in adult male Wistar rats (weighting: 180–250 g) obtained from the Animal House of Federal University of Ceará. The animals were housed 6 per cage in standard polycarbonate rat cages (42 ×

20.5 × 20 cm) and standard environmental conditions (22 ± 1°C; humidity 60 ± 5%; 12 h light/dark cycle with lights on at 7:00 am) with access to food (FRI-LAB Rat II, FRI-Ribe) and water *ad libitum*. All experimental procedures were conducted between 8:00 and 14:00 h and were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals [20] and the Brazilian College of Animal Experimentation (COBEA). The raters were blind to the experimental groups. This research protocol was approved by the local ethical committee of Federal University of Ceará.

2.3. Study Design. The rats were randomly divided into experimental groups (8–10 animals/group) distributed in two protocols, namely, prevention and reversal treatments (Figure 1), as described below. The use of LDX 10 mg/kg, p.o., to induce a mania-like behavior was based on a previous study from our research group [9]. The doses of CVD used here were calculated based on previous studies showing the neuroprotective effects of this drug [21] and human doses used for hypertension treatment as described elsewhere [22].

2.3.1. Prevention Treatment. In the prevention model, we simulated the maintenance phase of BD treatment, as previously proposed [23]. Briefly, different groups of animals were treated with CVD (2.5, 5, or 7.5 mg/kg, p.o.) once a day, VAL (200 mg/kg i.p.) twice a day, the association of VAL 100 mg/kg, i.p. (twice a day) + CVD 5 mg/kg, with a 15 min interval between drugs in the first daily administration of VAL, or saline for 14 days. The reason for choosing the association CVD 5 mg/kg + VAL 100 mg/kg was based, in the case of CVD, on the first behavioral results obtained where it seemed for us that this was the best dose to conduct this protocol; in the case of VAL the dose was reduced by half to determine a possible potentiation of its effect by CVD. It is important to mention that the dose of VAL usually used in models of mania is 200 mg/kg twice a day [11]. Between the 8th and 14th day, the experimental groups additionally received one oral dose of LDX daily. The time interval between the administration of the drugs and LDX was 30 min.

Locomotor activity using the open field test (as described in Section 2.4) and social interaction (as described in Section 2.5) were measured on the 14th day of drug administration, 2 hours after the last drug administration. Following behavioral determinations, the rats were sacrificed by decapitation and the prefrontal cortex (PFC), hippocampus (HC), and striatum (ST) were dissected, rapidly frozen, and stored at -70°C until assayed.

2.3.2. Reversal Treatment. In the reversal model, we reproduced the treatment of acute manic episodes as previously proposed [9]. In brief, each group of animals received one oral daily dose of LDX (10 mg/kg) or saline for 14 days. On the 8th day of treatment, the animals in the saline and LDX group additionally received oral administration of CVD (2.5, 5, and 7.5 mg/kg) once a day, VAL (200 mg/kg) twice a day, the association of VAL 100 mg/kg i.p. (twice a day) + CVD (with a 15 min interval), or saline, with a 1-hour interval

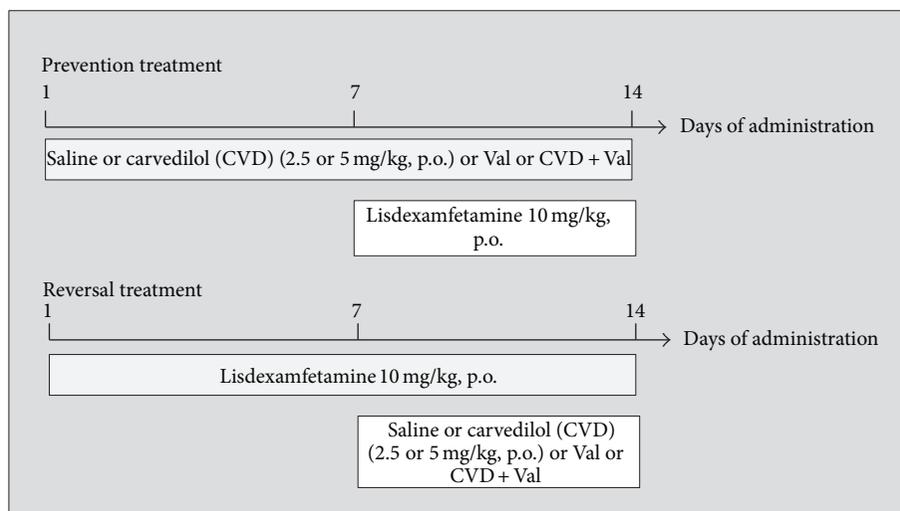


FIGURE 1: Schematic representation of the experimental design.

between treatments. Locomotor activity and social interaction were measured on the 14th day of treatment, 2 hours after the last drug administration. After behavioral determinations the rats were sacrificed and the brain areas dissected for the neurochemical determinations.

In the present study our primary outcome was to determine the behavioral changes induced by CVD alone and associated with VAL in the model of mania induced by LDX. The secondary outcome was to determine the neurochemical alterations underlying these alterations.

2.4. Open Field Test. The locomotor activity was assessed using the open field test [24]. This test was performed in a 50×50 cm open field surrounded by 50 cm high walls made of acrylic. The open field floor was divided into four equal parts by black lines. The apparatus was placed in a red light room. The animals were gently placed on the center of the field and allowed to freely explore the arena for 5 min. Crossings of the black lines (used to determine horizontal activity) and rearing behavior (used to determine vertical activity) were counted, during the 5 min period, by experienced raters who were blinded to treatment.

2.5. Social Interaction Test. The testing apparatus consisted of a 60 × 40 cm Plexiglas box divided into three chambers. Rats were able to move between chambers through a small opening (6 × 6 cm) in the dividers. Iron cages in each of the two side chambers contained, in one side, the probe rat, whereas in the other side, the cage was empty. Test animals were placed in the center chamber. Rats were allowed 5 min of exploration time in the box, after which an unfamiliar, same-sex probe rat from the same experimental group was placed in one of two restraining cages [25]. The time spent in each of the three chambers was measured, and social preference was defined as follows: (% time spent in the social chamber) – (% time spent in the opposite chamber).

2.6. Neurochemical Determinations

2.6.1. Tissue Preparation. Brain tissue samples were homogenized (10 times (w/v) with ice-cold 0.1M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 rpm for 15 minutes, and aliquots of supernatants were separated and used for determination of oxidative stress parameters.

For enzyme immunoassay determinations (ELISA) 20 times (w/v) homogenates prepared in cold phosphate-buffered saline (PBS, pH 7.4) were used. A protease inhibitor cocktail (Sigma-Aldrich, St. Louis, USA) was added to the buffer and the homogenate was centrifuged at 14,000 rpm for 30 min.

2.6.2. Determination of Reduced Glutathione (GSH) Levels. Reduced glutathione levels were evaluated to estimate endogenous defenses against oxidative stress. The method was based on Ellman's reagent (DTNB) reaction with free thiol groups [26]. The brain areas were diluted in EDTA 0.02 M buffer (10% w/v) and added to a 50% trichloroacetic acid solution. After centrifugation (3,000 rpm/15 min), the supernatant of the homogenate was collected and mixed with 0.4 M tris-HCl buffer, pH 8.9, and 0.01 M 5,5-dithiobis 2-nitrobenzoic acid (DTNB). The yellow color product was read immediately at 412 nm using a spectrophotometer (Beckman coulter UV/Visible). Results were calculated based on a standard glutathione curve and are expressed as ng of GSH/g wet tissue.

2.6.3. Thiobarbituric Acid Reactive Species (TBARS) Levels. Lipid peroxides formation was analyzed by measuring the thiobarbituric-acid reacting substances (TBARS) in the homogenates [27] as an index of reactive oxygen species (ROS) production. The samples were mixed with 1 mL of trichloroacetic acid 10% (TCA) and 1 mL of thiobarbituric acid 0.67% (TBA), then heated in a boiling water bath for 15 min, and immediately kept cold in a bath of ice. Lipid peroxidation was determined by the absorbance at 532 nm.

Results are expressed as μmol of malonaldehyde (MDA)/g tissue.

2.6.4. Determination of Hippocampal BDNF Levels. The levels of BDNF (ELISA; Millipore, USA) were determined in each sample by enzyme immunoassays according to the specific manufacturers' directions. Results are expressed as pg/g tissue.

2.7. Statistical Analysis. Statistical analyses were performed with GraphPad Prism 6.0 for Windows, GraphPad Software (San Diego, CA, USA). The results of the behavioral and neurochemical studies are expressed as means \pm SEM (standard errors of the mean). Regular two-way ANOVA with "treatment protocol" and "experimental groups" as factors was performed. Tukey's test was used as post hoc test. Before ANOVA, D'Agostino-Pearson omnibus test was conducted to verify the normal distribution of the data. For all analyses, the significance level was set at $\alpha = 0.05$.

3. Results

3.1. Carvedilol Alone and Associated with VAL Prevented and Reversed Hyperlocomotion and Alterations in Social Interaction Induced by LDX. Locomotor agitation and increased sociability are common features of mania [28]. In the present study two-way ANOVA of the number of crossings revealed a significant interaction between "treatment protocol" and "experimental group" [$F_{9,88} = 2.287, P = 0.0425$] with significant main effect of "treatment protocol" [$F_{1,88} = 4.210, P = 0.0431$] and "experimental group" [$F_{9,88} = 21.02, P < 0.001$]. Regarding rearing behavior there was no significant interaction between factors [$F_{9,85} = 1.57, P = 0.1671$], but significant main effects of "treatment protocol" [$F_{1,85} = 10.02, P = 0.0022$] and "experimental groups" [$F_{9,85} = 9.77, P < 0.0001$] were observed. Post hoc analysis showed that in both prevention and reversal protocols LDX caused hyperlocomotion (Figures 2(a) and 2(b)) and increased rearing behavior (Figures 2(c) and 2(d)) as compared to control (Sal + Sal) rats. In the prevention treatment the administration of CVD 5, the combination of CVD5 + VAL100, and VAL200 significantly prevented the hyperlocomotion induced by LDX ($P < 0.001$). Regarding rearing behavior CVD 2.5, CVD5, CVD7.5, and VAL200 prevented the increase in this parameter ($P < 0.001$). In the reversal treatment CVD5, CVD7.5, CVD5 + VAL100, and VAL200 significantly reversed the hyperlocomotion induced by LDX ($P < 0.001$). The increase in rearing behavior was reversed by CVD 2.5, CVD5, CVD7.5, CVD5 + VAL100, and VAL200 ($P < 0.001$). After the administration of CVD alone we could observe that only CVD 2.5 significantly decreased the number of crossings in both treatments.

In the evaluation of social interaction two-way ANOVA revealed a significant interaction between factors [$F_{6,72} = 2.24, P = 0.0490$] with significant main effect of "treatment protocol" [$F_{1,72} = 19.48, P < 0.0001$] and "experimental group" [$F_{6,72} = 5.28, P = 0.0001$]. Post hoc analysis

showed that the administration of LDX in both prevention and reversal treatments significantly increased the percent of social interaction as compared to control (Sal + Sal) animals. In the prevention paradigm CVD2.5, CVD5, CVD5 + VAL100, and VAL200 significantly prevented the alteration induced by LDX ($P < 0.01$) (Figure 3(a)). On the other hand in the reversal paradigm CVD2.5, CVD5, and CVD5 + VAL100 significantly reversed the alterations induced by LDX ($P < 0.05$) (Figure 3(b)).

3.2. Carvedilol Alone and Associated with VAL Prevented and Reversed the Alterations in GSH and Lipid Peroxidation Induced by LDX in the Prefrontal Cortex and Striatum of Rats. Two-way ANOVA of GSH levels revealed in the PFC a significant interaction between "treatment protocol" and "experimental group" [$F_{6,83} = 5.95, P < 0.0001$] with significant main effect of "treatment protocol" [$F_{1,83} = 13.97, P = 0.0003$] and "experimental group" [$F_{6,83} = 4.99, P = 0.0002$]. In the ST a significant interaction between factors [$F_{6,98} = 2.68, P = 0.0188$] with significant main effect of "treatment protocol" [$F_{1,98} = 13.73, P = 0.0003$] and "experimental group" [$F_{6,98} = 8.34, P < 0.0001$] was also observed. In relation to MDA levels in the PFC a significant interaction between "treatment protocol" and "experimental group" [$F_{6,70} = 3.58, P = 0.0038$] with significant main effect of "treatment protocol" [$F_{1,70} = 19.21, P < 0.0001$] and "experimental group" [$F_{6,70} = 18.67, P < 0.0001$] was observed. In the ST there was no significant interaction between factors [$F_{6,92} = 1.26, P = 0.2841$], but significant main effects of "treatment protocol" [$F_{1,92} = 10.69, P = 0.0015$] and "experimental group" [$F_{6,92} = 18.64, P < 0.0001$] were observed.

Post hoc test showed that the administration of LDX in both prevention and reversal treatments significantly decreased the levels of GSH (Figure 4) as well as increased MDA levels (Figure 5) in the PFC and ST when compared to control (Sal + Sal) rats, as expected for an animal model of mania.

Regarding GSH levels, in the prevention treatment, the administration of CVD 2.5, CVD 5, CVD5 + VAL100, and VAL200 not only significantly prevented the decrement in GSH levels induced by LDX ($P < 0.001$) in the PFC (Figure 4(a)) but also significantly increased GSH levels in these experimental groups in relation to control animals ($P < 0.001$). In the ST all treatments prevented the decrease in GSH induced by LDX ($P < 0.001$). In the reversal treatment only VAL200 prevented the decrease in GSH levels induced by LDX in the PFC. In the ST CVD2.5, CVD5, CVD5 + VAL100, and VAL200 reversed the GSH decrement induced by LDX ($P < 0.001$) (Figure 4(b)).

The evaluation of lipid peroxidation revealed that in the PFC and ST of the animals subjected to the prevention (Figure 5(a)) and reversal (Figure 5(b)) treatments both doses of CVD and VAL200 and the association of CVD + VAL100 prevented and reversed the alterations induced by LDX ($P < 0.001$). The exception was the PFC of the rats administered

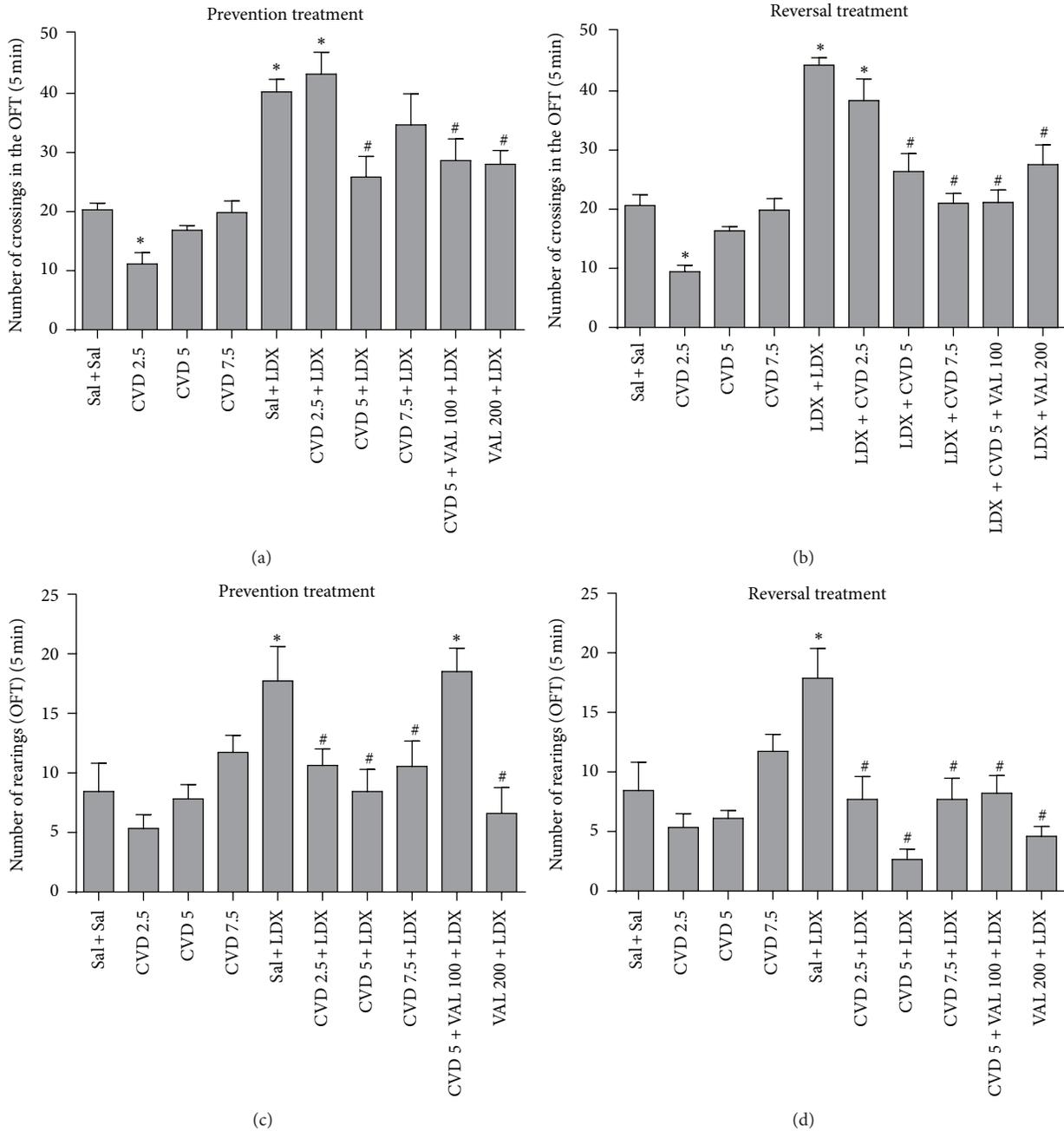


FIGURE 2: Number of crossings and rearings in the open field test in animals submitted to the prevention ((a), (c)) and reversal ((b), (d)) treatments. Data were analyzed by one-way ANOVA followed by Student-Newman-Keuls post hoc test. Values represent mean \pm SEM (6–8 animals/group). * $P < 0.05$ versus Sal + Sal; # $P < 0.05$ versus Sal + LDX or LDX + LDX. CVD: carvedilol; LDX: lisdexamfetamine; OFT: open field test; Sal: saline; VAL: valproate.

CVD5 + VAL100 in which the alterations in MDA levels were not prevented by this treatment.

3.3. Carvedilol Alone and Associated with VAL Prevents and Reverses the Alterations in the Hippocampal Levels of BDNF Induced by LDX. Two-way ANOVA of hippocampal BDNF levels revealed no significant interaction between factors [$F_{6,62} = 1.93, P = 0.0905$], but main effect of “experimental

group” [$F_{6,62} = 7.48, P < 0.0001$] was observed. BDNF is being considered a potential candidate as a biomarker for bipolar disorder [1]. In this context, as observed in Figure 6, post hoc analysis showed that the administration of LDX decreased BDNF levels in the hippocampus of rats that underwent the prevention and reversal treatments. In the prevention treatment CVD5, CVD5 + VAL100, and VAL200 significantly prevented the decrement in BDNF

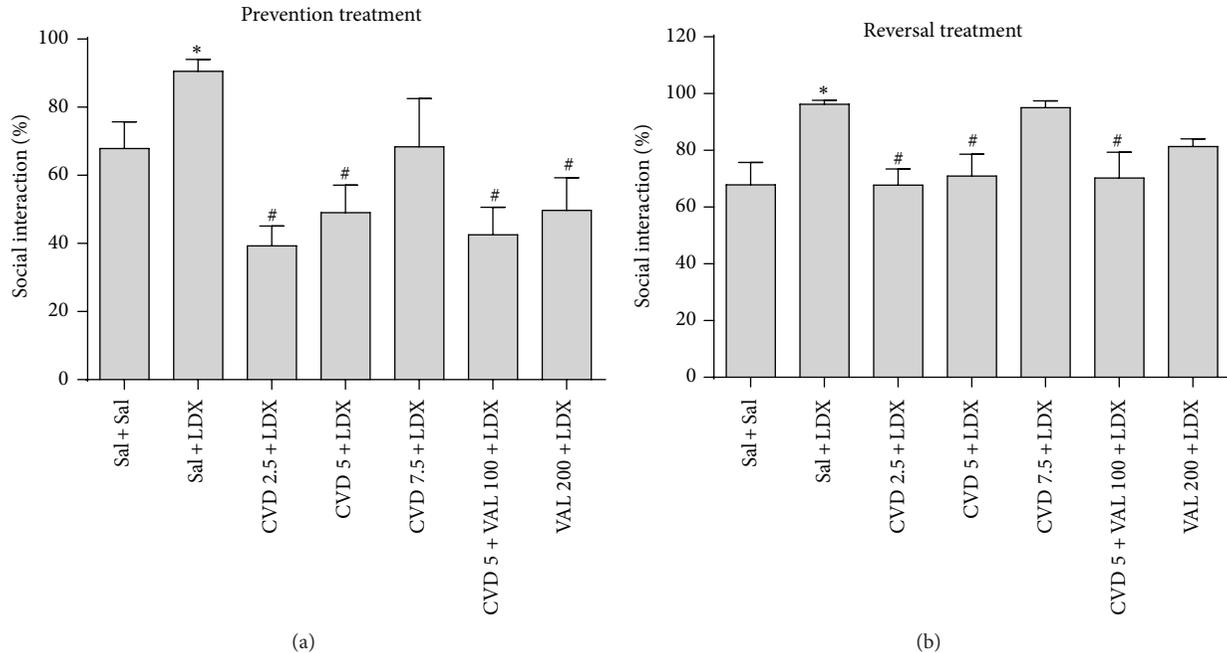


FIGURE 3: Percent of social interaction in animals submitted to the prevention (a) and reversal (b) treatments. Data were analyzed by one-way ANOVA followed by Student-Newman-Keuls post hoc test. Values are expressed as mean \pm SEM (6–8 animals/group). * $P < 0.05$ versus Sal + Sal; # $P < 0.05$ versus Sal + LDX or LDX + LDX. CVD: carvedilol; LDX: lisdexamfetamine; Sal: saline; VAL: valproate.

levels induced by LDX ($P < 0.05$). In the reversal treatment both doses of CVD as well as CVD5 + VAL100 and VAL200 reversed the decrease in these neurotrophin levels induced by LDX ($P < 0.01$).

4. Discussion

The results of the present work demonstrated, to the best of our knowledge, for the first time that CVD is effective in the prevention and reversal of LDX-induced mania-like behavioral and neurochemical alterations. Additionally, CVD potentiated the effects of the mood-stabilizing VAL.

Manic states include complex and multifaceted symptoms such as overactivity, hypersexuality, irritability, and reduced need for sleep [29]. To date, the evaluation of these symptoms in preclinical models of mania is almost restricted to the observation of hyperlocomotion; nevertheless this was recently criticized [12]. Thus, in our study we decided to evaluate the social interaction of the animals since this feature is increased in mania [28].

Based on our behavioral results we observed that the administration of CVD 5 mg/kg, VAL 200 mg/kg, and the combination of VAL 100 mg/kg + CVD 5 mg/kg prevented and reversed hyperlocomotion and the increased sociability induced by LDX, with the exception of VAL alone that was not able to reverse the LDX-induced alteration in social interaction. Regarding the mood-stabilizing drugs, when we first proposed this LDX-induced model of mania [9] lithium was the only drug used to assess the predictive validity of the model. Based on a previous preclinical study that demonstrated a potentiation of anticonvulsant effects by

the administration of VAL 100 mg/kg and CVD 5 mg/kg, we decided to use, in the present study, VAL as mood-stabilizing drug [30].

The behavioral alterations observed in BD patients are linked to central mechanisms, such as the following [7, 31]: (i) alterations in monoamine levels, for example, the dopamine dysregulation syndrome; (ii) mitochondrial dysregulation; (iii) alterations in calcium homeostasis; (iv) neuroinflammation; (v) oxidative imbalance; and (vi) dysregulation in neurotrophin's levels. These mechanisms are also involved in the neuroprogression of this mental disorder [32], emerging, mainly the last five, as important targets for BD treatment [1, 33].

Focusing on the aforementioned targets for BD treatment, CVD is a drug that presents antioxidant and mitochondrial stabilizing properties as well as regulating intracellular calcium that may be considered as an important option for the treatment of BD. Additionally, in the last ten years the neuroprotective properties of this drug began to be studied although with incipient findings to date [34, 35].

As observed in our results CVD in addition to regulating oxidative imbalance also increased the levels of BDNF when administered alone and when combined with VAL. In fact when GSH levels are reduced there is an increased potential for cellular oxidative stress, characterized by an increase and accumulation of ROS. Furthermore, the decreased levels of antioxidants or increased levels of free radicals are related to chain reactions, such as lipid peroxidation, leading to cellular dysfunction [36]. Overall glutathione antioxidant system and lipid peroxidation are altered in animal models of mania [8, 9] as well as in BD patients [37, 38]. Oxidative imbalance leads

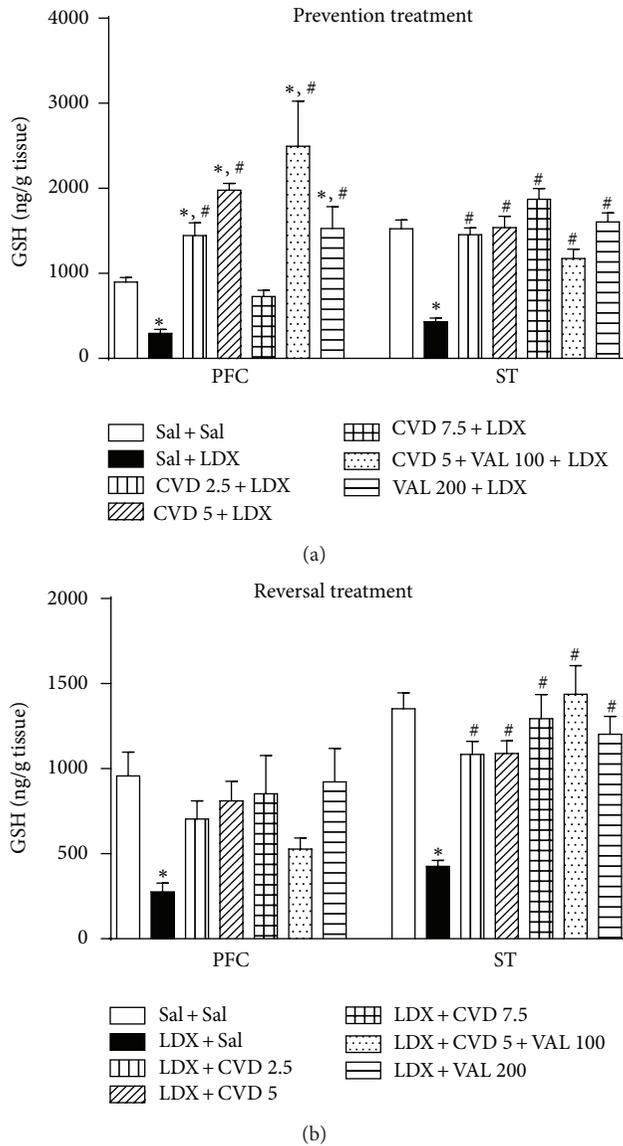


FIGURE 4: Levels of reduced glutathione (GSH) in the prefrontal cortex (PFC) and striatum (ST) of mice submitted to the prevention (a) and reversal (b) treatments. Data were analyzed by one-way ANOVA followed by Student-Newman-Keuls post hoc test. Values are expressed as mean ± SEM (6–8 animals/group). * $P < 0.05$ versus Sal + Sal; # $P < 0.05$ versus Sal + LDX or LDX + LDX. CVD: carvedilol; LDX: lisdexamfetamine; Sal: saline; VAL: valproate.

to reduced expression of BDNF. The mechanisms linking oxidative stress to decreased BDNF levels involve several factors such as the following: (i) decrease of DNA-binding activities of activator protein-1 and cAMP response element-binding protein (CREB), BDNF transcription factor, which is associated with reduction of BDNF gene expression [39], and (ii) energy imbalance which causes a dysfunction in the N-methyl-D-aspartate (NMDA) channel resulting in decreases in BDNF gene expression [40].

Regarding BDNF, the serum levels of this neurotrophin in BD patients are decreased in depressive and manic episodes,

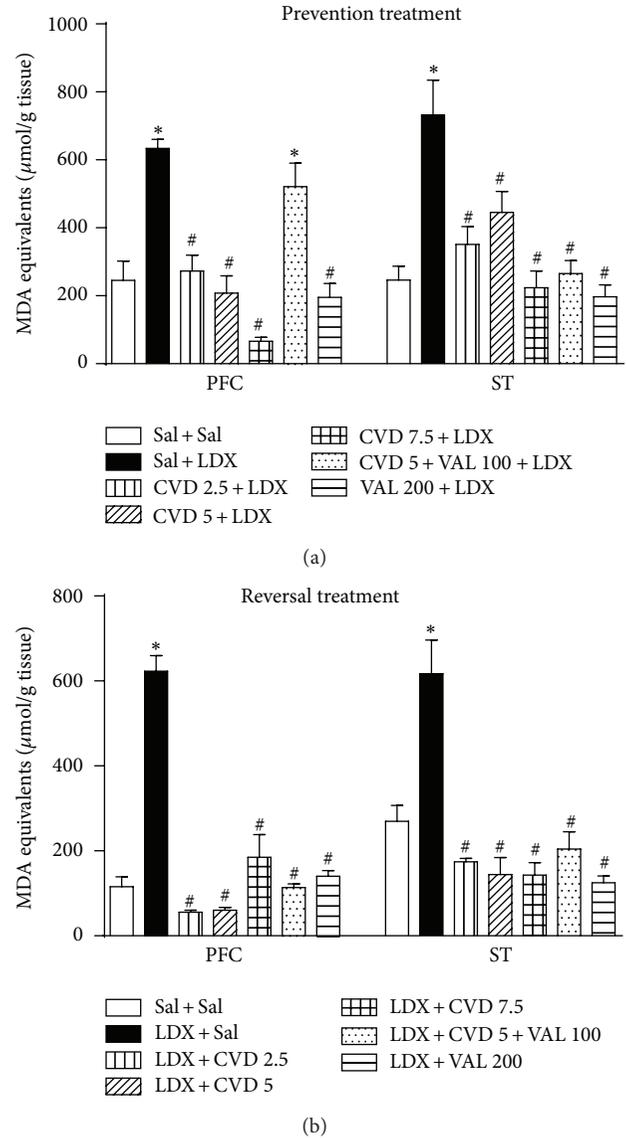


FIGURE 5: Levels of malondialdehyde (MDA), a lipid peroxidation marker, in the prefrontal cortex (PFC) and striatum (ST) of mice submitted to the prevention (a) and reversal (b) treatments. Data were analyzed by one-way ANOVA followed by Student-Newman-Keuls post hoc test. Values are expressed as mean ± SEM (6–8 animals/group). * $P < 0.05$ versus Sal + Sal; # $P < 0.05$ versus Sal + LDX or LDX + LDX. CVD: carvedilol; LDX: lisdexamfetamine; Sal: saline; VAL: valproate.

returning to normal levels in euthymia [41] with a similar pattern of alteration in animal models of mania [9, 14]. Together, these mechanisms have been extensively implicated in the pathophysiology of schizophrenia and BD [37, 42].

Thus, based on the importance of oxidative imbalance in the pathophysiology of BD, an increasing number of preclinical [8] and clinical [43] studies are proposing antioxidants as adjunct therapies for BD. Interestingly, it was recently proposed in BD patients with medical comorbidities, such as cardiovascular and endocrine conditions, that the use

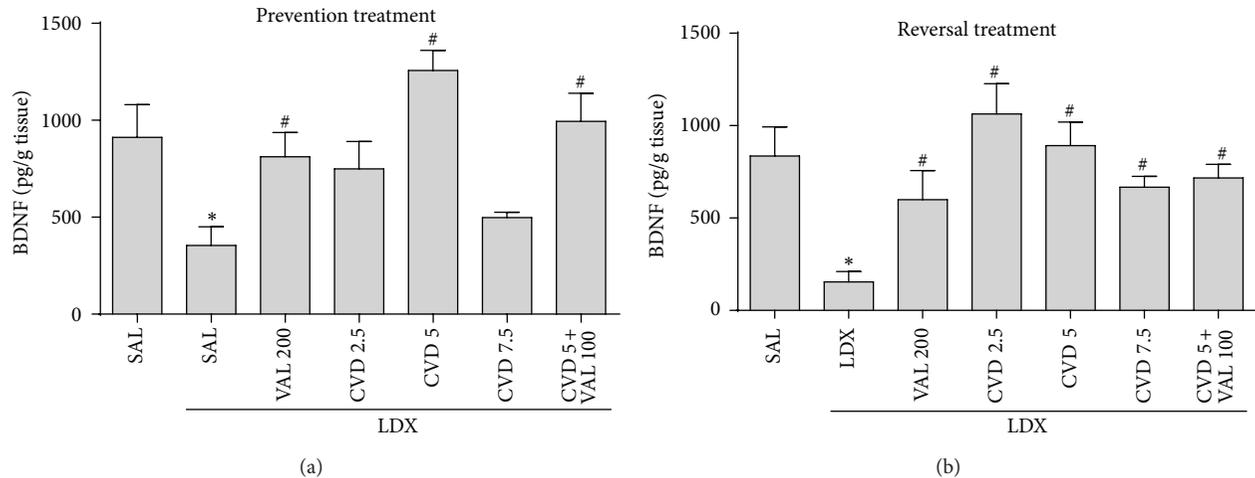


FIGURE 6: Levels of brain derived neurotrophic factor (BDNF) in the hippocampus of rats submitted to the prevention (a) and reversal (b) treatments. Data were analyzed by one-way ANOVA followed by Student-Newman-Keuls post hoc test. Values are expressed as mean \pm SEM (6–8 animals/group). * $P < 0.05$ versus Sal + Sal; # $P < 0.05$ versus Sal + LDX or LDX + LDX. CVD: carvedilol; LDX: lisdexamfetamine; Sal: saline; VAL: valproate.

of the antioxidant N-acetyl cysteine improved functional outcomes [43]. Therefore, this adds further evidences for the importance of CVD, a drug that treats cardiovascular conditions besides being antioxidant, in BD treatment.

Of note, the use, in the present study, of the association of half the dose of VAL (100 mg/Kg, twice a day) with CVD 5 maintained the effect of VAL 100 in a similar pattern to the one presented by VAL 200 mg/kg twice a day. One exception was noticed in the social interaction test where in the reversal protocol the association reversed the alteration induced by LDX whereas VAL alone did not. This is an important finding because VAL presents important side effects, such as tremor, weight gain, alopecia, more frequent sedation, and infection [44] that could be alleviated by the use of lower doses. Indeed a previous preclinical study suggested that CVD potentiates the anticonvulsant activity of VAL possibly by a pharmacodynamics interaction [30]. In this previous study the association of CVD 5 + VAL 100 was the best for the increase in seizure threshold induced by pentylentetrazole, an effect that was accompanied by increase in GSH levels and decrease in lipid peroxidation [30].

The combination CVD 5 + VAL 100 did not prevent LDX-induced rearing alteration. This result does not compromise the antimanic-like effect of this combination observed in this study, since the parameter number of crossings is the most reliable [45, 46]. On the contrary, CVD 7.5 did not prevent LDX-induced alterations in the number of crossings, social interaction, GSH levels in the PFC, and BDNF. One possible explanation is that when compared to humans on an mg/m² basis, 7.5 mg/kg CVD in rats corresponds approximately to 75 mg/daily CVD in humans. Of note, the total daily doses of 6.25 to 50 mg CVD are the most prescribed for cardiovascular conditions [47]. Thus, based on this evidence possibly 7.5 mg/kg CVD in rats was an excessive dose.

Our study has some limitations: first, animal models in psychiatry are fair representations of real complexity of

the disorders. That is to say, clinical studies are necessary before we can conclude that CVD could bring tangible benefits to BD patients. Furthermore, our study evaluated a correlation between intervention and outcome and was not equipped to parse possible mechanistic pathways. Therefore, we could not establish the real mechanisms that are necessary and adequate to explain the behavioral effects of CVD; for example, mitochondrial protection by CVD was not evaluated here.

In conclusion, CVD was able to prevent and reverse oxidative imbalance and BDNF levels in the model of mania induced by LDX. Overall, the results presented here give the first preclinical evidences for the future design of clinical trials investigating the use of CVD in mania.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

- [1] B. N. Frey, A. C. Andreazza, J. Houenou et al., "Biomarkers in bipolar disorder: a positional paper from the International Society for Bipolar Disorders Biomarkers Task Force," *Australian and New Zealand Journal of Psychiatry*, vol. 47, no. 4, pp. 321–332, 2013.
- [2] R. F. Bachmann, R. J. Schloesser, T. D. Gould, and H. K. Manji, "Mood stabilizers target cellular plasticity and resilience cascades: implications for the development of novel therapeutics," *Molecular Neurobiology*, vol. 32, no. 2, pp. 173–202, 2005.
- [3] A. Martínez-Arán, E. Vieta, M. Reinares et al., "Cognitive function across manic or hypomanic, depressed, and euthymic states in bipolar disorder," *The American Journal of Psychiatry*, vol. 161, no. 2, pp. 262–270, 2004.
- [4] A. M. Kilbourne, J. R. Cornelius, X. Han et al., "Burden of general medical conditions among individuals with bipolar disorder," *Bipolar Disorders*, vol. 6, no. 5, pp. 368–373, 2004.
- [5] M. Weiner, L. Warren, and J. G. Fiedorowicz, "Cardiovascular morbidity and mortality in bipolar disorder," *Annals of Clinical Psychiatry*, vol. 23, no. 1, pp. 40–47, 2011.
- [6] M. Berk, "Neuroprogression: pathways to progressive brain changes in bipolar disorder," *International Journal of Neuropsychopharmacology*, vol. 12, no. 4, pp. 441–445, 2009.
- [7] H. K. Manji, J. A. Quiroz, J. L. Payne et al., "The underlying neurobiology of bipolar disorder," *World Psychiatry*, vol. 2, pp. 136–146, 2003.
- [8] D. S. Macêdo, C. D. Medeiros, R. C. Cordeiro et al., "Effects of alpha-lipoic acid in an animal model of mania induced by d-amphetamine," *Bipolar Disorders*, vol. 14, no. 7, pp. 707–718, 2012.
- [9] D. S. Macêdo, D. F. de Lucena, A. I. G. Queiroz et al., "Effects of lithium on oxidative stress and behavioral alterations induced by lisdexamphetamine dimesylate: relevance as an animal model of mania," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 43, pp. 230–237, 2013.
- [10] R. H. Perlis, M. J. Ostacher, J. K. Patel et al., "Predictors of recurrence in bipolar disorder: primary outcomes from the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD)," *American Journal of Psychiatry*, vol. 163, no. 2, pp. 217–224, 2006.
- [11] B. N. Frey, S. S. Valvassori, G. Z. Réus et al., "Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania," *Journal of Psychiatry & Neuroscience*, vol. 31, no. 5, pp. 326–332, 2006.
- [12] J. W. Young, B. L. Henry, and M. A. Geyer, "Predictive animal models of mania: hits, misses and future directions," *British Journal of Pharmacology*, vol. 164, no. 4, pp. 1263–1284, 2011.
- [13] M. Pennick, "Absorption of lisdexamphetamine dimesylate and its enzymatic conversion to d-amphetamine," *Neuropsychiatric Disease and Treatment*, vol. 6, pp. 317–327, 2010.
- [14] B. N. Frey, A. C. Andreazza, K. M. M. Ceresér et al., "Effects of mood stabilizers on hippocampus BDNF levels in an animal model of mania," *Life Sciences*, vol. 79, no. 3, pp. 281–286, 2006.
- [15] S. S. Valvassori, G. T. Rezin, C. L. Ferreira et al., "Effects of mood stabilizers on mitochondrial respiratory chain activity in brain of rats treated with d-amphetamine," *Journal of Psychiatric Research*, vol. 44, no. 14, pp. 903–909, 2010.
- [16] T.-L. Yue, H.-Y. Cheng, P. G. Lysko et al., "Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger," *Journal of Pharmacology and Experimental Therapeutics*, vol. 263, no. 1, pp. 92–98, 1992.
- [17] N. Noguchi, K. Nishino, and E. Niki, "Antioxidant action of the antihypertensive drug, carvedilol, against lipid peroxidation," *Biochemical Pharmacology*, vol. 59, no. 9, pp. 1069–1076, 2000.
- [18] A. Kumar, S. Dogra, and A. Prakash, "Effect of carvedilol on behavioral, mitochondrial dysfunction, and oxidative damage against D-galactose induced senescence in mice," *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 380, no. 5, pp. 431–441, 2009.
- [19] R. F. D. A. Júnior, T. O. Souza, C. A. X. de Medeiros et al., "Carvedilol decrease IL-1 β and TNF- α , inhibits MMP-2, MMP-9, COX-2, and RANKL expression, and up-regulates OPG in a rat model of periodontitis," *PLoS ONE*, vol. 8, no. 7, Article ID e66391, 2013.
- [20] NIH, *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Research, National Research Council, National Academies Press, 1996.
- [21] S. I. Savitz, J. A. Erhardt, J. V. Anthony et al., "The novel β -blocker, carvedilol, provides neuroprotection in transient focal stroke," *Journal of Cerebral Blood Flow & Metabolism*, vol. 20, no. 8, pp. 1197–1204, 2000.
- [22] S. Reagan-Shaw, M. Nihal, and N. Ahmad, "Dose translation from animal to human studies revisited," *The FASEB Journal*, vol. 22, no. 3, pp. 659–661, 2008.
- [23] B. N. Frey, M. R. Martins, F. C. Petronilho, F. Dal-Pizzol, J. Quevedo, and F. Kapczinski, "Increased oxidative stress after repeated amphetamine exposure: possible relevance as a model of mania," *Bipolar Disorders*, vol. 8, no. 3, pp. 275–280, 2006.
- [24] J. Archer, "Tests for emotionality in rats and mice: a review," *Animal Behaviour*, vol. 21, no. 2, pp. 205–235, 1973.
- [25] K. Radyushkin, K. Hammerschmidt, S. Boretius et al., "Neurologin-3-deficient mice: model of a monogenic heritable form of autism with an olfactory deficit," *Genes, Brain and Behavior*, vol. 8, no. 4, pp. 416–425, 2009.
- [26] G. L. Ellman, "Tissue sulfhydryl groups," *Archives of Biochemistry and Biophysics*, vol. 82, no. 1, pp. 70–77, 1959.
- [27] H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," *Analytical Biochemistry*, vol. 95, no. 2, pp. 351–358, 1979.
- [28] L. G. Sylvia, L. B. Alloy, J. A. Hafner, M. C. Gauger, K. Verdon, and L. Y. Abramson, "Life events and social rhythms in bipolar spectrum disorders: a prospective study," *Behavior Therapy*, vol. 40, no. 2, pp. 131–141, 2009.
- [29] R. A. Kowatch, E. A. Youngstrom, A. Danielyan, and R. L. Findling, "Review and meta-analysis of the phenomenology and clinical characteristics of mania in children and adolescents," *Bipolar Disorders*, vol. 7, no. 6, pp. 483–496, 2005.
- [30] R. Goel and A. Goel, "Interactions between carvedilol and sodium valproate along with neurobehavioural co-morbidities in various epilepsy models," *Drug Invention Today*, vol. 5, no. 2, pp. 87–91, 2013.
- [31] A. R. Newberg, L. A. Catapano, C. A. Zarate, and H. K. Manji, "Neurobiology of bipolar disorder," *Expert Review of Neurotherapeutics*, vol. 8, no. 1, pp. 93–110, 2008.
- [32] M. Berk, F. Kapczinski, A. C. Andreazza et al., "Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors," *Neuroscience and Biobehavioral Reviews*, vol. 35, no. 3, pp. 804–817, 2011.

- [33] C. A. Zarate Jr. and H. K. Manji, "Bipolar disorder: candidate drug targets," *Mount Sinai Journal of Medicine*, vol. 75, no. 3, pp. 226–247, 2008.
- [34] K. Yamagata, S. Ichinose, and M. Tagami, "Amlodipine and carvedilol prevent cytotoxicity in cortical neurons isolated from stroke-prone spontaneously hypertensive rats," *Hypertension Research*, vol. 27, no. 4, pp. 271–282, 2004.
- [35] Y. Ouyang, Z. Chen, M. Tan et al., "Carvedilol, a third-generation β -blocker prevents oxidative stress-induced neuronal death and activates Nrf2/ARE pathway in HT22 cells," *Biochemical and Biophysical Research Communications*, vol. 441, no. 4, pp. 917–922, 2013.
- [36] J. N. Keller and M. P. Mattson, "Roles of lipid peroxidation in modulation of cellular signaling pathways, cell dysfunction, and death in the nervous system," *Reviews in the Neurosciences*, vol. 9, no. 2, pp. 105–116, 1998.
- [37] O. M. Dean, M. van den Buuse, A. I. Bush et al., "Role for glutathione in the pathophysiology of bipolar disorder and schizophrenia? Animal models and relevance to clinical practice," *Current Medicinal Chemistry*, vol. 16, no. 23, pp. 2965–2976, 2009.
- [38] A. C. Andreazza, C. Cassini, A. R. Rosa et al., "Serum S100B and antioxidant enzymes in bipolar patients," *Journal of Psychiatric Research*, vol. 41, no. 6, pp. 523–529, 2007.
- [39] E. Iwata, M. Asanuma, S. Nishibayashi, Y. Kondo, and N. Ogawa, "Different effects of oxidative stress on activation of transcription factors in primary cultured rat neuronal and glial cells," *Molecular Brain Research*, vol. 50, no. 1-2, pp. 213–220, 1997.
- [40] M. Roceri, W. Hendriks, G. Racagni, B. A. Ellenbroek, and M. A. Riva, "Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus," *Molecular Psychiatry*, vol. 7, no. 6, pp. 609–616, 2002.
- [41] I. Grande, G. R. Fries, M. Kunz, and F. Kapczinski, "The role of BDNF as a mediator of neuroplasticity in bipolar disorder," *Psychiatry Investigation*, vol. 7, no. 4, pp. 243–250, 2010.
- [42] R. Machado-Vieira, A. C. Andreazza, C. I. Viale et al., "Oxidative stress parameters in unmedicated and treated bipolar subjects during initial manic episode: a possible role for lithium antioxidant effects," *Neuroscience Letters*, vol. 421, no. 1, pp. 33–36, 2007.
- [43] P. V. Magalhães, O. M. Dean, A. I. Bush et al., "Systemic illness moderates the impact of N-acetyl cysteine in bipolar disorder," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 37, no. 1, pp. 132–135, 2012.
- [44] K. A. Macritchie, J. R. Geddes, J. Scott, D. R. Haslam, and G. M. Goodwin, "Valproic acid, valproate and divalproex in the maintenance treatment of bipolar disorder," *The Cochrane Database of Systematic Reviews*, vol. 10, Article ID CD003196, 2001.
- [45] R. E. Riegel, S. S. Valvassori, G. Elias et al., "Animal model of mania induced by ouabain: evidence of oxidative stress in submitochondrial particles of the rat brain," *Neurochemistry International*, vol. 55, no. 7, pp. 491–495, 2009.
- [46] R. Lien, S. Flaisher-Grinberg, C. Cleary, M. Hejny, and H. Einat, "Behavioral effects of Bcl-2 deficiency: implications for affective disorders," *Pharmacological Reports*, vol. 60, no. 4, pp. 490–498, 2008.
- [47] P. Information and C. Pharmacology, "Prescribing Information".

Review Article

Magnetic Seizure Therapy for Unipolar and Bipolar Depression: A Systematic Review

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Objective. Magnetic seizure therapy (MST) is a novel, experimental therapeutic intervention, which combines therapeutic aspects of electroconvulsive therapy (ECT) and transcranial magnetic stimulation, in order to achieve the efficacy of the former with the safety of the latter. MST might prove to be a valuable tool in the treatment of mood disorders, such as major depressive disorder (MDD) and bipolar disorder. Our aim is to review current literature on MST. **Methods.** OVID and MEDLINE databases were used to systematically search for clinical studies on MST. The terms “magnetic seizure therapy,” “depression,” and “bipolar” were employed. **Results.** Out of 74 studies, 8 met eligibility criteria. There was considerable variability in the methods employed and samples sizes were small, limiting the generalization of the results. All studies focused on depressive episodes, but few included patients with bipolar disorder. The studies found reported significant antidepressant effects, with remission rates ranging from 30% to 40%. No significant cognitive side effects related to MST were found, with a better cognitive profile when compared to ECT. **Conclusion.** MST was effective in reducing depressive symptoms in mood disorders, with generally less side effects than ECT. No study focused on comparing MST to ECT on bipolar depression specifically.

1. Introduction

1.1. Mood Disorders and ECT. Mood disorders, such as major depressive disorder (MDD) and bipolar disorder (BD), are highly prevalent and debilitating conditions, associated with high suicidality rates, elevated treatment costs, and heavy social and economic burden [1]. The treatment of depressive episodes is associated with a 30–40% rate of nonresponse [2]. Few effective treatments are currently used and approved for resistant unipolar and bipolar depression.

Electroconvulsive therapy (ECT) is regarded as the most effective treatment for depression [3], in both BD and MDD, with remission rates ranging from 50% to 75% [4–6]. ECT is also effective for manic episodes, with reported remission rates of up to 85% [7–11].

Despite its high effectiveness, the use of ECT is limited by its side effects, especially the development of cognitive deficits and, in particular, memory impairment [12–16]. Such side effects can range from mild or nonexistent in some patients up to severe and distressing in others [14, 17–19]. Of those, memory loss is the single most often cognitive complaint reported by patients undergoing ECT [12]. The cognitive effects of ECT have been shown to be dependent upon numerous ECT parameters, namely, electrode placement, frequency of sessions, electric dose, and pulse width [14, 17, 20].

1.2. Magnetic Seizure Therapy. The search for improved ECT techniques, which could be able to reduce or minimize memory-related side effects whilst maintaining efficacy, has

been the subject of a considerable amount of investigation [21]. However, the use of an electrical stimulus to induce seizures is a fundamental limitation in refining convulsive therapy and limiting its side effects profile [22]. Control over the spatial distribution and magnitude of intracerebral current density is limited by high skull impedance, which shunts most of the electrical stimulus through the scalp and cerebrospinal fluid (CSF) and away from the brain. The substantial impedance of the scalp and skull means that the bulk of the electrical stimulus is shunted away from the brain, resulting in widespread stimulation of cortical and subcortical regions. There are also individual differences in skull anatomy that result in uncontrolled variation in intracerebral current density [23]. Measurements in humans of shunting across the scalp and skull range from 80% up to 97%. Recent studies with computer models further support the notion that current dissipation in ECT is considerable and that the electrode placement associated with more severe cognitive side effects, bitemporal (BL-ECT), is also the placement with higher shunting and with deeper brain stimulation [24].

The possibility of noninvasive stimulation of specific areas in the cerebral cortex through magnetic stimulation was first demonstrated in 1985 [25]. The use of transcranial magnetic stimulation (TMS) has since been studied as a treatment for depression, and a number of meta-analyses and Sham-controlled trials have confirmed that it is associated with statistically significant antidepressant effect [26]. TMS employs a rapidly changing magnetic field to induce an electrical current in a targeted brain region, most often the left dorsolateral prefrontal cortex (DLPFC) [27]. Magnetic stimulation is more focal than electrical stimulation because it avoids the impedance of the scalp and skull and results in an induced electric field confined to superficial cortex. Thus, magnetic stimulation has allowed more control over current paths and current density within cortical tissue [22, 28] and grants TMS a safer, well-tolerated side effects profile [27]. However, TMS efficacy in treatment-resistant patients is limited. A recent meta-analysis [26] has shown that repetitive transcranial magnetic stimulation (rTMS), despite significantly reducing depressive symptoms, is not as effective as ECT for the treatment of refractory depression, leading to a mean reduction of 9.3 points on the Hamilton Depression Rating Scale (HDRS), while ECT leads to a reduction of 15.42 points. Also, ECT is reported to be more effective in reducing suicidality than rTMS [26].

It has long been known that TMS could induce seizures. Such phenomenon was initially considered to be accidental and regarded as a complication of method [29–31]. However, the possibility of intentionally inducing seizures by magnetic pulses has been formally proposed as early as 1994 by Sackeim [32]. Once rTMS had been found to have significant antidepressant effects at subconvulsive levels, and considering the superior antidepressant potency of ECT, it has been hypothesized that, under controlled conditions in a patient under anesthesia, increasing the magnetic stimulus into the convulsive range the resultant seizure could confer robust antidepressant properties as seen with ECT [33]. It was also hypothesized that the more accurate and focal seizures triggered by magnetic stimulation could lead to less adverse

effects than seizures triggered by ECT, while retaining its therapeutic capacity [22].

The first published report of seizures successfully and deliberately induced by rTMS dates back to 2001, described by Lisanby et al. [23]. Two exemplars of *Macaca mulatta* were chosen for the experiments because of brain-to-coil size ratio that was closer to humans when pediatric sized coils were used and for their ability to perform cognitive tasks with which to access the potential cognitive side effects of the method [34]. The animals were subjected to rTMS sessions under general anesthesia, using both a commercially available TMS device (MAGSTIM Super Rapid, Magstim Company Ltd., Whitland, Wales) and a customized MAGSTIM device, with eight booster modules instead of the usual four modules, capable of broader pulse width and yielding 40% more power per pulse. The two trials conducted with the commercial MAGSTIM device failed to trigger seizures in the primates. The custom device, however, was capable of inducing seizures in both subjects in all trials, therefore illustrating the need for more powerful stimulation than a standard TMS device could deliver in order to elicit seizures.

Further studies comparing electroconvulsive shock (ECS), the animal equivalent of ECT, with MST and Sham on non-human primates were conducted in order to assess the safety of the procedure. Results pointed that MST has significantly less impact than ECS on functions such as spatial memory, time to task completion, and anterograde memory. In fact, there was no difference between MST and Sham, even when with the use of higher-intensity stimulus, with pulse frequency of up to 100 Hz [35–37]. Postmortem studies conducted on the experimentation animals did not reveal morphologic changes or histological lesions [38, 39].

Soon after the first description of MST in animals, initial human reports were published. In 2001, Lisanby et al. [40] described the first use of MST in a 20-year-old patient with treatment-resistant episode of MDD, who had undergone for the previous three years several pharmacological trials, with different classes of antidepressant drugs, without success, being referred to ECT. The patient received four sessions of MST before following up with conventional ECT. Generalized tonic-clonic seizures were successfully elicited in the four MST treatments, with Hamilton Depression Rating Scale scores decreasing from 20 at baseline to 13 after the fourth session. No severe adverse effects were reported and Mini Exam of Mental State scores remained unaltered throughout the trial.

In 2003, Kosel et al. [41] reported a second patient treated with MST for treatment-resistant MDD. This time, a 66-year-old patient was submitted to a full trial of MST, with 12 sessions, until remission was achieved. Hamilton Depression Rating Scale (HDRS) scores dropped from 33 to 6 and Beck Depression Inventory (BDI) dropped from 40 to 11. The sessions were well-tolerated, with no somatic or cognitive complaints. A more comprehensive cognitive assessment battery was employed, and the patient did not show any significant cognitive deficits following MST. Actually, there was an improvement on some cognitive tests following treatment.

Since the mechanism of action of MST involves the induction of seizures, the use of general anesthesia with muscle relaxation and clinical support is necessary, much like in ECT. The use of a bite block, which is mandatory for patients receiving ECT, on the other hand, is not necessary since there is no direct stimulation of the masseter muscle by shunted electric current. However, the loud “clicking” noise causing the coil might require the use of earplugs by both patients and staff. Different coils have been tested, with the nonfocal round coil and the double cone coil reported to be reliable in seizure induction, while the more focal “figure of 8” coil was considered inefficient in inducing seizure. Another concern related to the coils is the heating of the equipment, which is more pronounced than in TMS coils and required previous cooling and the use of heat-resistant materials [34].

The seizure induced by MST is quite different from that produced by ECT. Magnetic pulses, such as those employed by rTMS and MST, are capable of focusing the stimulation to a specific area, since they pass unhindered into the brain without resistance and are not shunted through scalp, skull, and CRL like ECT’s electric stimulus. On the other hand, magnetic pulses generated by most commercially available coils only penetrate a few centimeters deep, while electric current can reach deeper structures more easily. Therefore, a major difference between MST and ECT is the former’s capacity to focus stimulation, with MST-induced seizures originating on superficial regions of the cortex, unlike ECT, where electrical current passes deep through the brain [22]. Consequently, it is possible that MST may produce similar therapeutic benefits to ECT without inducing memory-related side effects, as there is no direct electrical stimulation of medial temporal lobe structures, such as the hippocampus, which are implicated in ECT-related memory impairment [42].

2. Aims

Our objective was to review current clinical evidence on magnetic seizure therapy, its effectiveness on unipolar and bipolar depression, and its side effects profile, with special emphasis on the cognitive aspects. Whenever possible, comparisons with ECT were drawn in order to establish similarities and differences between the methods.

3. Methods

3.1. Literature Review. In order to systematically review the current literature on the use of magnetic seizure therapy in the treatment of mood disorders in accordance with the PRISMA guidelines, the authors performed searches in the Ovid and MedLine databases using the following terms: (1) “Magnetic Seizure Therapy” OR (2) “Magnetic Seizure Therapy” AND Depression OR (3) “Magnetic Seizure Therapy” and Bipolar. Articles dating from 1985, the year the first papers on Transcranial Magnetic Stimulation were published, to August 2014 were selected. The results were managed with Mendeley Desktop (version 1.10.1.0, © Mendeley, Ltd.) software.

3.2. Eligibility Criteria. The inclusion and exclusion criteria for studies were as follows: inclusion: (a) manuscript in English; (b) clinical trials, either open-labeled or blinded studies; (c) studies on human subjects and exclusion: (a) virtual models; (b) animal studies; (c) case reports.

3.3. Studies Overview. Our initial search yielded 75 references, 64 of which were initially excluded, based on eligibility criteria, leaving 11 articles. In a subsequent analysis, 3 references were excluded after the abstracts were reviewed and did not meet eligibility criteria. Ultimately, 8 studies were included.

3.4. Data Extraction. The following variables were extracted per a structured checklist that we developed: (a) overview: study design, authors, year of publication, technique, summary, and other relevant data; (b) demographics: total sample (number) and intervention groups; (c) assessment of mood disorder: method of diagnosis (clinical or structured interviews); and (d) outcomes: description of each study’s results.

3.5. Quality Assessment. To assess the methodological heterogeneity between studies, each report was evaluated with regard to quality, focusing on 2 critical methodological issues: (a) internal validity: for clinical studies, whenever possible the authors followed the Cochrane guidelines to determine the risk of bias in randomization (selection bias), blinding and control comparison (performance bias), and outcome assessment and reporting (attrition, measurement, and reporting biases); however, since a small number of double-blind randomized clinical trials were found, open-label studies were included as well; (b) construct validity: we determined whether the operational criteria for mood disorders were appropriate, that is, whether each study fulfilled the following criteria: (i) clinical studies that focused on MST and (ii) articles on mood disorders.

4. Results

A total of eight studies were identified by our search. A summary of results is available on Table 1. Outcomes on clinical improvement and cognitive side effects are described separately below.

4.1. Clinical Improvement. The first clinical trial assessing MST’s effectiveness compared to ECT in reducing depressive symptoms was reported by White et al. [43]. Depressive symptoms were evaluated with the HDRS before and after the series of 10 to 12 treatments. Researchers reported that both methods resulted in a decrease in depressive scores, and ECT reduced mean HDRS from 30 at baseline to 6 after treatment, while on the MST group mean HDRS at baseline was 32, dropping to 14 at the end of the trial. Results suggested that while MST demonstrated a safer cognitive profile, its effectiveness was still below ECT. Only 53% of the patients treated with MST achieved a 50% or greater improvement on the depression rating scores. Considering that ECT responses have been previously associated with electric dose in relation to the seizure threshold, a possible explanation for MST’s

TABLE 1: Clinical studies.

Author	Objective	Subjects	MST device and parameters	ECT device and parameters	Study design	Anesthetics	Cognitive and physiologic outcomes	Clinical outcomes
Lisanby et al., 2003 [22]	Assert the safety and feasibility of MST for MDD ¹	N = 10 (MDD)	50 Hz modified Magstim; first session titration for ST ² , followed by sessions 0.5 ms PW ³ and 60 Hz at 100% output	Mecta 5000 Q; 0.5 ms pulse width; 9 patients RUL ⁴ -ECT x6 ST + 1 patient BL ⁵ -ECT 2.5x ST	Double-blind within subject crossover MST x ECT	Atropine 0.4 mg/Kg IV; methohexital 0.75 mg/Kg IV; succinylcholine 0.75 mg/Kg IV	MST superior to ECT on multiple cognitive domains; MST elicited shorter seizures	N/A
White et al., 2006 [43]	Evaluation of anesthetic aspects of MST	N = 20 (MDD)	50 Hz modified Magstim; first session titration for ST, followed by sessions at 1.5x ST	Mecta 5000 Q; BF ⁶ -ECT; 0.5 ms PW; 2.5x ST	Double-blind randomized trial MST x ECT	Etomidate 0.15–0.20 mg/Kg IV; succinylcholine 0.5–1.0 mg/Kg IV; glycopyrrolate 2.5 mcg/Kg IV; ketorolac 0.4 mg/Kg	MST resulted in lower variation on BIS ⁷ and faster reorientation	ECT reduced HAM-D ⁸ from 30 to 6; MST reduced HAM-D from 32 to 14 after 10–12 sessions
Kirov et al., 2008 [47]	Assessment of reorientation time after HD-MST ⁹	N = 11	100 Hz modified Magstim; PW 0.34–0.4 ms, 10 s stimulus for all patients	Not specified	Double-blind crossover MST x ECT	Etomidate 0.15–0.3 mg/Kg IV; succinylcholine 0.5–1.0 mg/Kg IV	MST faster reorientation (7:12 min) compared to ECT (26:35 min)	N/A
Kayser et al., 2011 [44]	Effectiveness and safety of MST compared to ECT	N = 20 (16 MDD, 3 BP-II ¹⁰ and 1 BP-I ¹¹)	100 Hz MagVenture MST MagPro; 0.37 ms PW;	Thymatron IV; 0.5 PW, RUL-ECT; 3x ST	Double-blind randomized trial MST x ECT	Propofol 1.5–2.5 mg/Kg IV; succinylcholine 1–1.5 mg/Kg IV	No cognitive loss on either group	MST: 60% response and 30% remission; ECT 40% response
Kayser et al., 2013 [48]	Assessment of cognitive and seizure characteristics of HD-MST and ECT	N = 7 (6 MDD, 1 BP-II)	100 Hz MagVenture MST MagPro;	Thymatron IV; 0.5 ms PW, 0.9 A, 30–120 Hz; 5 patients RUL-ECT 6x ST, 2 patients BL-ECTG 3x ST	Open-label, follow-up of MST after failure to respond to ECT	Propofol 1.0–1.5 mg/Kg IV; 1.0–1.5 mg/Kg succinylcholine IV	Shorter reorientation after MST; seizures similar, but shorter after MST	N/A
Hoy et al., 2013 [46]	Effects of MST on brain glucose metabolism	N = 10 (MDD)	100 Hz MagVenture MST; 400 pulses above ST	N/A	Open label	Propofol (mean dose 122.13 mg IV); succinylcholine (mean dose 53.61 mg IV)	Glucose metabolism increased in several areas	57% of response after treatment
Fitzgerald et al., 2013 [45]	Effectiveness and safety of MST	N = 13 (MDD)	100 Hz MagVenture MST MagPro; 10 s stimulus for first patient; 400 pulses above ST for all others	N/A	Open-label study	Propofol (mean dose 124.0 ± 24.1 mg IV); succinylcholine (mean dose 52.7 ± 12.2 mg IV)	Fast reorientation with patients reporting awakening under muscle relaxation	Five patients responded, two of which achieved remission
Polster et al., 2014 [49]	Compare acute memory retrieval of MST and ECT	N = 30 (20 MDD + 10 controls)	100 Hz MagVenture MST MagPro; suprathreshold stimulation; 2x week	Thymatron IV; 0.5 ms PW, RUL-ECT; 5x ST; 2x week	Open-label study	Propofol 1.5 mg/Kg IV; succinylcholine 1.0 mg/Kg V	Delayed recall disturbed after ECT but not after MST	N/A

Notes. ¹Major depressive disorder; ²seizure threshold; Hamilton Depression Rating Scale; ³pulse width; ⁴right unilateral electrodes; ⁵bifrontal electrodes; ⁶bifrontal electrodes; ⁷electroencephalographic bispectral index; ⁸Hamilton Depression Rating Scale; ⁹high-dose magnetic seizure therapy; ¹⁰bipolar disorder, Type I; ¹¹bipolar disorder, Type II.

lower efficacy could be the fact that treatments were delivered only on average 1.3 times above magnetic seizure threshold. The authors suggested that better response rates could have been achieved with higher stimulation intensity; however, MST devices available at the time could only produce short trains of 8 seconds and 50 Hz at maximum output.

In an attempt to improve effectiveness of MST, it has been proposed that more intense stimuli could be necessary, which could be achieved by employing higher frequency, up to 100 Hz, and longer trains. Such techniques became known as high-dose MST (HD-MST). The first comparison between effectiveness of HD-MST and ECT was published in 2011 by Kayser et al. [44] in patients on a treatment-resistant depressive episode of BD and MDD. Primary outcome measure was response, defined by 50% reduction of Montgomery and Asberg Depression Rating Scale (MADRS) or remission, defined by MADRS score of less than 10. Other clinical measures included HDRS scores, Hamilton Anxiety Scale (HAM-A), BDI, and the 90-Item Symptom Checklist (SCL-90). Out of ten patients on the MST group, six were responders, three of which achieved remission. In the ECT group, four patients were responders, all of which achieved remission. No significant difference in effectiveness of MST and ECT was found. It is noteworthy that ECT procedures employed by the researchers differed from the usual practice inasmuch as low down RUL-ECT was used, which reportedly could be less effective than high-dose RUL-ECT.

Further investigation of HD-MST and its impact on depressive symptoms was reported by Fitzgerald et al. in 2013 [45]. Clinical assessment was done with the MADRS, the primary outcome variable, as well as the 17-item HAM-D, BDI, BPRS, and the CORE rating of melancholia. Response was defined as a reduction of 50% in the MADRS. Scores were rated at baseline, after six sessions of MST and after the end of treatment. Out of 13 patients, five patients met response criteria, of which two achieved remission. One patient dropped out of the study due to a desire to receive ECT. Average MADRS scores dropped from 39.0 to 26.5, HAMD decreased from 26.7 to 19.2, BDI went from 35.8 to 26.7, and BPRS dropped from 20.7 to 13.6. No differences were detected between responders and nonresponders. The authors note that the response to MST in this sample was significantly inferior to the reported effectiveness of ECT, which generally yields remission rates of up to 80%.

The first investigation of regional glucose brain metabolism in MST-treated patients was reported by Hoy et al. in 2013 [46]. Ten patients suffering from MDD were selected for an open-label study. Patients received high-dose MST three times a week, and clinical measures were performed at baseline, after every 6 treatments and at the end of the MST treatment. The primary clinical outcome variable for response was MADRS score, and response was defined as 50% reduction on the score, while reduction of 20–50% was considered partial response and less than 20% nonresponse. Cognitive functions were assessed. Fluorine-18-labeled deoxyglucose (FDG) PET/CT was performed in the week preceding the first MST treatment and a few days (average 3.8) after completion of the MST treatment course. Out of ten patients, four were considered

responders. Average MADRS scores dropped from 40 at the baseline to 27.2 at endpoint. Neuropsychological assessment showed no cognitive adverse effects to the treatment. Significant relative glucose metabolism increases were seen in the globus pallidus, substantia nigra, putamen, and the orbital frontal cortex. Other findings, though not at the same level of significance, include increases in the glucose metabolism of the medial frontal cortex and the dorsolateral prefrontal cortex. There were no significant overall decreases in activity after MST. Differences between responders and nonresponders were only significant at a trend level and no relation was found between changes in relative glucose metabolism and changes in depression scores. Authors ponder that such findings could be due to the small sample.

4.2. Cognitive Side Effects. In 2003, Lisanby et al. reported the first series of patients submitted to MST [22]. A total of 10 patients suffering from MDD and referred to an ECT course were selected for a randomized, within-subject trial. Patients were blind to which treatment, MST or ECT, they would receive. The first two sessions of each patient were used to determine seizure threshold for both MST and ECT, followed by two sessions of suprathreshold stimulation with MST and ECT, and finally ECT sessions for the remaining treatments. Due to the high-frequency clicking noise intrinsic to magnetic stimulation, earplugs were worn by patients during both MST and ECT sessions. A bite-block was inserted immediately prior to seizure elicitation to protect the teeth, even if MST did not produce the marked jaw contraction as typically seen with ECT, since no current passes through the masseter. MST was administered with a modified Magstim stimulator, with a pulse width of 0.5 ms and 60 Hz at 100% output. All suprathreshold MST sessions were all given at maximal stimulator output (400 pulses). Tonic-clonic seizures similar to those seen with conventional ECT were elicited on all MST sessions, albeit seizure duration was shorter on average for MST (40.9 s on threshold and 49.5 s on suprathreshold stimulation) compared to ECT (101.0 s on threshold and 74.4 s on suprathreshold stimulation). A neuropsychological battery sampling multiple cognitive domain was administered by a blind rater at baseline and immediately before and after each of the four test sessions, evaluating cognitive functions such as memory, orientation, and attention, as well as the Squire Memory Test (Sentences), the Buschke Selective Reminding Test, and a Complex Figure Test. Patients had fewer subjective side effects and recovered orientation more quickly with MST than ECT. MST was also superior to ECT on measures of attention, retrograde amnesia, and category fluency.

To further investigate the cognitive side effects of MST compared to ECT, White et al. [43] conducted a study evaluating the amount of time patients took to recover orientation after each procedure, as well as reduction of depressive symptoms scores. Ten patients with MDD were submitted to MST and results compared to other 10 case-matched patients undergoing ECT treatment in an open-parallel study design. All patients underwent a series of 10–12 treatments of either MST or ECT. Time to recover orientation was assessed by a blinded observer in the postanesthesia recovery area who asked simple questions such as name,

place, and day of the week after each session. Orientation recovery time was considerably shorter on the MST group, averaging 4 minutes, while patients submitted to ECT took an average of 18 minutes to correctly answer the questions. Such results suggested that MST could present a safer cognitive profile compared to ECT.

The first report of HD-MST on humans was published in 2008 by Kirov et al. [47]. Eleven patients suffering from TR MDD were enrolled for a within-subject open study comparing orientation recovery time of a single ECT and a single HD-MST session. Recovery of orientation after treatments was assessed by asking the patient for their name, date of birth, age, place, and day of the week. The point of orientation recovery was defined as the time when a patient was able to recall four of these five items. Recovery of orientation was much faster after MST than after ECT. The mean time to recovery after successful seizures elicited by MST was 7 min 12 s, against 26 min 35 s for ECT. The authors did not report effectiveness data on this study, since this was not the study focus and only one MST session was performed on each patient.

Another study, this time comparing the effectiveness, electrophysiological characteristics, and cognitive side effects of HD-MST and ECT, was published in 2011 by Kayser et al. [44]. Twenty patients in a treatment-resistant depressive episode were enrolled and blindly randomized in two groups of ten, treated with either ECT or MST. It is noteworthy that for the first time patients suffering from bipolar disorder were included in a MST trial, two patients with Type II BD receiving ECT and one Type I and one Type II patients on the MST group. A neuropsychological battery was employed before and after treatment to evaluate such cognitive domains as memory, learning, executive function, language, and processing speed. Also, after each session patients were assessed immediately in order to evaluate orientation. Recovery was defined as the time when patients opened their eyes and breathed independently, while reorientation time was assessed by asking the patient for her/his names, date of birth, age, place, and day of the week. Recovery and reorientation were faster in the MST group, at 1 min 42 s and 2 m 16 s respectively, compared to the ECT group, with recovery at 4 min 3 s and reorientation at 8 min 21 s. Except for that, no significant differences in cognitive side effects were found between groups. EEG activity was similar in both groups, consisting of high-amplitude synchronized theta activity and equal postictal suppression. However, on the MST group seizure duration was briefer and some patients showed delayed ictal EEG activity and duration of motor and ictal activity in MST-treated patients had about the same length. It is important to notice that the ECT procedures in this paper are quite different from other studies [5]. The authors used right unilateral electrodes (RUL-ECT) but chose to apply stimulus three times above seizure threshold, which is reported to be less effective than high-dose RUL-ECT. On the other hand, lower electrical doses are associated with less cognitive impairment than high-dose ECT, which could explain the absence of significant cognitive side effects on the ECT group.

Kayser et al. further explored seizure characteristics and cognitive aspects of HD-MST compared with ECT [48]. Seven patients in a treatment-resistant depressive episode were enrolled, six suffering from MDD and one from bipolar disorder Type II. Such patients had failed to respond to a 12-session MST course and were then referred to ECT 12 ECT sessions in an open-label, within-subject trial. None of the patients responded to the 12 sessions of ECT. Reorientation time was, once again, shorter, shorter after MST than after ECT. Except for the fact that seizures lasted longer on patients receiving ECT, the authors found no significant differences in visible motor seizures and EEG activities between the two procedures, including postictal suppression, a measure reported to predict response to ECT.

In 2013, Fitzgerald et al. [45] reported an open-label trial in which 13 patients with TR-MDD received MST, assessing its effectiveness and side effects. MST was applied three times per week, each patient receiving up to 18 treatments. A comprehensive neuropsychological battery was employed [47], and patients were evaluated at baseline, after six sessions and lastly after the end of treatments. Out of 13 patients, 12 completed the trial; the remaining subject chose to abandon the study due to poor response and desire to receive ECT. Average time to reorientation was 82.8 seconds. In fact, the fast recovery from the procedure might have created an unexpected side effect: four patients awoke from the procedure while still under effect of muscle relaxation. Neuropsychological tests showed no cognitive impairment after MST; in fact nonsignificant improvements were detected in several domains. One patient reported severe headaches. Unlike previous reports [44, 48], the authors reported EEG patterns unlike those expected on ECT patients. Not only were the motor seizures shorter, but also ictal activity was of less amplitude and there was typically much less postictal suppression. In some patients, ictal activity was not always apparent on the EEG despite a clear motor seizure. The authors also note that the response to MST in this sample was significantly inferior to the reported effectiveness of ECT, which generally yields remission rates of up to 80%.

The cognitive side effects of MST were recently explored by Polster et al. [49], who compared its impact on acute memory retrieval to ECT. Twenty patients suffering from treatment-resistant MDD were randomly assigned to two groups, one treated with MST and the other with ECT. A further ten healthy controls were included for comparison with the treatment groups. Memory assessment consisted of a customized test. On each of the 2 treatment control days and 2 treatment-free control days, the patients were given 3 consecutive learning trials in the morning to learn 40 words. Words were clustered into pairs and assigned to a hypernymic category for additional differentiation between storage or retrieval disruption of memory. This enabled the recording of cued recall providing information about the category. After treatment, patients were initially asked to remember all 40 of the words by themselves (delayed recall). Subsequently, they were provided with the name of each hypernymic category to enable them to recall all 40 words independently from delayed recall, again. The authors point that if patients extraordinarily benefit from these cues, this is

indicative of a retrieval-based rather than a storage-focused memory disruption. By comparing memory performance on treatment days to control days, treatment-induced memory disruption was evaluated. To enable evaluation of treatment caused effects on a particular subject, the patients were treated with MST or ECT on 2 of the 4 testing days, whereas the other 2 days served as control. After ECT, delayed recall was disturbed, whereas after MST, it was not. However, this difference in performance was no longer apparent upon cue application.

5. Discussion

The results of this review show that MST is as effective as ECT in inducing generalized tonic-clonic seizures, both in animals and in humans. However, there are considerable differences in the mechanism of induction, since magnetic pulses are not shunted by the scalp and skull, unlike ECT's electric current, allowing for a more focused and superficial stimulation, which on its turn might have implications for its electrophysiological aspects, effectiveness, and side effects. MST-induced seizures are shorter lasting. In most animal studies and some clinical studies MST also led to less postictal suppression and less EEG amplitude compared to ECT-induced seizures, although two studies reported similar EEG characteristics for both groups [44, 48]. Such results could possibly be related to the use of RUL-ECT and low electric dose, which are associated with less cognitive loss but less effectiveness as well.

The clinical studies so far reported all support that MST is an effective treatment for depressive episodes, with response rates ranging from 40% to 60% and remission rates ranging from 15% to 30%. It is noteworthy that most trials were conducted on patients suffering from TRD, who had failed previous therapeutic strategies and therefore had a worse prognosis. On the other hand, such results are still far from those expected from ECT, which is reported to lead to remission rates of 50% to 70% on the same conditions. The results could be related to the parameters of MST, such as frequency of pulses and total pulse count. High-dose MST (HD-MST) is an attempt to bridge this gap, and further studies are being conducted in order to improve its effectiveness.

Both the neurocognitive effects and the effectiveness of ECT are related in part to the stimulus parameters with higher doses conferring increased adverse effects but perhaps better response rates on the other hand. For example, 6 × seizure thresholds RUL-ECT can achieve results similar to BL-ECT [50–53]. Following that rationale, the effectiveness of MST could, theoretically, be improved by increasing the stimulus' dose, which can be achieved by administering a larger number of pulses in the MST train. Early MST equipment employed pulse frequencies of 40 Hz to 50 Hz and train lengths of up to 8 seconds, for a maximum of 400 pulses. Specific equipment, capable of delivering stimulus at 100 Hz and for up to 10 seconds (1000 pulses), was developed in order to test such hypothesis [34]. Preclinical studies suggested that HD-MST delivered under such parameters could be safe from

a cognitive point of view, with no histological damage, either compared to low-dose MST, Sham, or ECS [36, 37, 39].

MST shows a considerable advantage over ECT on its side effects profile and induced cognitive loss. Such difference was evident from the early preclinical studies comparing it to ECS, subjects showing significantly better cognitive scores after MST than ECS. On human subjects, reorientation time after MST ranges from 2 minutes to 8 minutes, while it takes from 18 minutes to 26 minutes after ECT, a considerable improvement. Other cognitive functions, such as retrograde and anterograde memory, language, and praxis, seem to be unaffected by MST. ECT, on the other hand, is notorious for inducing cognitive loss, which might be its most significant drawback.

Mood disorders are complex and varied in its symptoms and characteristics. All clinical studies revised here focused on depressive episodes and the majority of enlisted patients suffered from MDD, with very few cases of BD. Considering the challenges of treating depressive episodes in bipolar disorder and the low number of approved effective treatments, MST warrants further investigation as an alternative treatment for such cases. Furthermore, all studies which included BD patients included them on a sample majorly composed of MDD patients; no study so far has focused on bipolar depression specifically. Also, there are no reports of MST for manic episodes of BD. Considering ECT's record of effectiveness on manic states, the use of MST on such cases could be a focus for future research.

Being such a novel and still experimental technique, MST studies suffer from small samples and low statistical power. Also there is considerable variance in the MST technique employed by the researchers, which is unsurprising, considering that commercially available MST devices have only recently become available. However, there is little standardization of anesthetic methods, rating scales, cognitive assessments, and the protocol for ECT control. There is currently only one double-blind randomized clinical trial comparing the effectiveness and side effects of MST compared to ECT, and all the others are either case reports, open-label studies, or crossover studies. Future research, with larger samples, of double-blind design, and more consistent methods will allow for more statistic power and better understanding of the technique.

The findings reported on this review suggest that MST might be an effective and safe alternative for the treatment of mood disorders, specifically on treatment-resistant depression, with a safer and more tolerable side effects profile than the current first choice, ECT. However, further studies are necessary to improve the assessment of its potential effectiveness and expand current understanding of its mechanisms.

Conflict of Interests

Eric Cretaz declared no conflict of interests.

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References

- [1] K. N. Fountoulakis, "The emerging modern face of mood disorders: a didactic editorial with a detailed presentation of data and definitions," *Annals of General Psychiatry*, vol. 9, article 14, 2010.
- [2] P. Sienaert, L. Lambrichts, A. Dols, and J. de Fruyt, "Evidence-based treatment strategies for treatment-resistant bipolar depression: a systematic review," *Bipolar Disorders*, vol. 15, no. 1, pp. 61–69, 2013.
- [3] The UK ECT Review Group, "Efficacy and safety of electroconvulsive therapy in depressive disorders: a systematic review and meta-analysis," *The Lancet*, vol. 361, no. 9360, pp. 799–808, 2003.
- [4] M. M. Husain, A. J. Rush, M. Fink et al., "Speed of response and remission in major depressive disorder with acute electroconvulsive therapy (ECT): a consortium for research in ECT (CORE) report," *Journal of Clinical Psychiatry*, vol. 65, no. 4, pp. 485–491, 2004.
- [5] C. H. Kellner, R. G. Knapp, G. Petrides et al., "Continuation electroconvulsive therapy vs pharmacotherapy for relapse prevention in major depression: a multisite study from the consortium for research in electroconvulsive therapy (CORE)," *Archives of General Psychiatry*, vol. 63, no. 12, pp. 1337–1344, 2006.
- [6] B. Dierckx, W. T. Heijnen, W. W. van den Broek, and T. K. Birkenhäger, "Efficacy of electroconvulsive therapy in bipolar versus unipolar major depression: a meta-analysis," *Bipolar Disorders*, vol. 14, no. 2, pp. 146–150, 2012.
- [7] R. M. Hiremani, J. Thirthalli, B. S. Tharayil, and B. N. Gangadhar, "Double-blind randomized controlled study comparing short-term efficacy of bifrontal and bitemporal electroconvulsive therapy in acute mania," *Bipolar Disorders*, vol. 10, no. 6, pp. 701–707, 2008.
- [8] M. Barekatin, L. Jahangard, M. Haghghi, and F. Ranjesh, "Bifrontal versus bitemporal electroconvulsive therapy in severe manic patients," *Journal of ECT*, vol. 24, no. 3, pp. 199–202, 2008.
- [9] T. S. P. Mohan, P. Tharyan, J. Alexander, and N. S. Raveendran, "Effects of stimulus intensity on the efficacy and safety of twice-weekly, bilateral electroconvulsive therapy (ECT) combined with antipsychotics in acute mania: a randomised controlled trial," *Bipolar Disorders*, vol. 11, no. 2, pp. 126–134, 2009.
- [10] F. Rezaei, K. Nasserli, G. R. Esfandiari, S. M. H. Sadeghi, M. Fathie, and F. Gharibi, "Remifentanyl added to propofol for induction of anesthesia can reduce reorientation time after electroconvulsive therapy in patients with severe mania," *Journal of ECT*, vol. 28, no. 2, pp. 124–127, 2012.
- [11] M. Haghghi, H. Bajoghli, G. Bigdelou, L. Jahangard, E. Holsboer-Trachsler, and S. Brand, "Assessment of cognitive impairments and seizure characteristics in electroconvulsive therapy with and without sodium valproate in manic patients," *Neuropsychobiology*, vol. 67, no. 1, pp. 14–24, 2013.
- [12] B. K. Gardner and D. W. O'Connor, "A review of the cognitive effects of electroconvulsive therapy in older adults," *Journal of ECT*, vol. 24, no. 1, pp. 68–80, 2008.
- [13] J. Prudic, S. Peyser, and H. A. Sackeim, "Subjective memory complaints: a review of patient self-assessment of memory after electroconvulsive therapy," *Journal of ECT*, vol. 16, no. 2, pp. 121–132, 2000.
- [14] A. Ingram, M. M. Saling, and I. Schweitzer, "Cognitive side effects of brief pulse electroconvulsive therapy: a review," *Journal of ECT*, vol. 24, no. 1, pp. 3–9, 2008.
- [15] L. M. Fraser, R. E. O'Carroll, and K. P. Ebmeier, "The effect of electroconvulsive therapy on autobiographical memory: a systematic review," *Journal of ECT*, vol. 24, no. 1, pp. 10–17, 2008.
- [16] A. Stoppe, M. Louzã, M. Rosa, G. Gil, and S. Rigonatti, "Fixed high-dose electroconvulsive therapy in the elderly with depression: a double-blind, randomized comparison of efficacy and tolerability between unilateral and bilateral electrode placement," *Journal of ECT*, vol. 22, no. 2, pp. 92–99, 2006.
- [17] S. H. Lisanby, J. H. Maddox, J. Prudic, D. P. Devanand, and H. A. Sackeim, "The effects of electroconvulsive therapy on memory of autobiographical and public events," *Archives of General Psychiatry*, vol. 57, no. 6, pp. 581–590, 2000.
- [18] D. Rose, T. Wykes, M. Leese, J. Bindman, and P. Fleischmann, "Patients' perspectives on electroconvulsive therapy: systematic review," *British Medical Journal*, vol. 326, no. 7403, pp. 1363–1365, 2003.
- [19] S. C. Schulze-Rauschenbach, U. Harms, T. E. Schlaepfer, W. Maier, P. Falkai, and M. Wagner, "Distinctive neurocognitive effects of repetitive transcranial magnetic stimulation and electroconvulsive therapy in major depression," *British Journal of Psychiatry*, vol. 186, pp. 410–416, 2005.
- [20] H. A. Sackeim, J. Prudic, M. S. Nobler et al., "Effects of pulse width and electrode placement on the efficacy and cognitive effects of electroconvulsive therapy," *Brain Stimulation*, vol. 1, no. 2, pp. 71–83, 2008.
- [21] A. Merkl, I. Heuser, and M. Bajbouj, "Antidepressant electroconvulsive therapy: mechanism of action, recent advances and limitations," *Experimental Neurology*, vol. 219, no. 1, pp. 20–26, 2009.
- [22] S. H. Lisanby, B. Lubner, T. E. Schlaepfer, and H. A. Sackeim, "Safety and feasibility of magnetic seizure therapy (MST) in major depression: randomized within-subject comparison with electroconvulsive therapy," *Neuropsychopharmacology*, vol. 28, no. 10, pp. 1852–1865, 2003.
- [23] S. H. Lisanby, B. Lubner, H. A. Sackeim, A. D. Finck, and C. Schroeder, "Deliberate seizure induction with repetitive transcranial magnetic stimulation in nonhuman primates," *Archives of General Psychiatry*, vol. 58, no. 2, pp. 199–200, 2001.
- [24] Z.-D. Deng, S. H. Lisanby, and A. V. Peterchev, "Electric field strength and focality in electroconvulsive therapy and magnetic seizure therapy: a finite element simulation study," *Journal of Neural Engineering*, vol. 8, no. 1, Article ID 016007, 2011.
- [25] A. T. Barker, R. Jalinous, and I. L. Freeston, "Non-invasive magnetic stimulation of human motor cortex," *The Lancet*, vol. 1, no. 8437, pp. 1106–1107, 1985.
- [26] B. Micallef-Trigona, "Comparing the effects of repetitive transcranial magnetic stimulation and electroconvulsive therapy in

- the treatment of depression: a systematic review and meta-analysis," *Depression Research and Treatment*, vol. 2014, Article ID 135049, 8 pages, 2014.
- [27] J. L. Moreines, S. M. McClintock, and P. E. Holtzheimer, "Neuropsychologic effects of neuromodulation techniques for treatment-resistant depression: a review," *Brain Stimulation*, vol. 4, no. 1, pp. 17–27, 2011.
- [28] S. H. Lisanby, T. Moscrip, O. Morales, B. Luber, C. Schroeder, and H. A. Sackeim, "Neurophysiological characterization of magnetic seizure therapy (MST) in non-human primates," *Supplements to Clinical Neurophysiology*, vol. 56, pp. 81–99, 2003.
- [29] A. Conca, P. König, and A. Hausmann, "Transcranial magnetic stimulation induces 'pseudoabsence seizure,'" *Acta Psychiatrica Scandinavica*, vol. 101, no. 3, pp. 246–249, 2000.
- [30] A. Pascual-Leone, C. M. Houser, K. Reese et al., "Safety of rapid-rate transcranial magnetic stimulation in normal volunteers," *Electroencephalography and Clinical Neurophysiology*, vol. 89, no. 2, pp. 120–130, 1993.
- [31] E. M. Wassermann, L. G. Cohen, S. S. Flitman, R. Chen, and M. Hallett, "Seizures in healthy people with repeated "safe" trains of transcranial magnetic stimuli," *The Lancet*, vol. 347, no. 9004, pp. 825–826, 1996.
- [32] H. A. Sackeim, "Magnetic stimulation and ECT," *Convulsive Therapy*, vol. 10, pp. 255–258, 1994.
- [33] B. Luber, S. M. McClintock, and S. H. Lisanby, "Applications of transcranial magnetic stimulation and magnetic seizure therapy in the study and treatment of disorders related to cerebral aging," *Dialogues in Clinical Neuroscience*, vol. 15, no. 1, pp. 87–98, 2013.
- [34] S. B. Rowny, K. Benzl, and S. H. Lisanby, "Translational development strategy for magnetic seizure therapy," *Experimental Neurology*, vol. 219, no. 1, pp. 27–35, 2009.
- [35] T. D. Moscrip, H. S. Terrace, H. A. Sackeim, and S. H. Lisanby, "A primate model of anterograde and retrograde amnesia produced by convulsive treatment," *Journal of ECT*, vol. 20, no. 1, pp. 26–36, 2004.
- [36] T. Spellman, S. M. McClintock, H. Terrace, B. Luber, M. M. Husain, and S. H. Lisanby, "Differential effects of high-dose magnetic seizure therapy and electroconvulsive shock on cognitive function," *Biological Psychiatry*, vol. 63, no. 12, pp. 1163–1170, 2008.
- [37] S. M. McClintock, N. K. DeWind, M. M. Husain et al., "Disruption of component processes of spatial working memory by electroconvulsive shock but not magnetic seizure therapy," *International Journal of Neuropsychopharmacology*, vol. 16, no. 1, pp. 177–187, 2013.
- [38] A. J. Dwork, V. Arango, M. Underwood et al., "Absence of histological lesions in primate models of ECT and magnetic seizure therapy," *The American Journal of Psychiatry*, vol. 161, no. 3, pp. 576–578, 2004.
- [39] A. J. Dwork, J. R. Christensen, K. B. Larsen et al., "Unaltered neuronal and glial counts in animal models of magnetic seizure therapy and electroconvulsive therapy," *Neuroscience*, vol. 164, no. 4, pp. 1557–1564, 2009.
- [40] S. H. Lisanby, T. E. Schlaepfer, H. U. Fisch, and H. A. Sackeim, "Magnetic seizure therapy of major depression," *Archives of General Psychiatry*, vol. 58, no. 3, pp. 303–305, 2001.
- [41] M. Kosel, C. Frick, S. H. Lisanby, H.-U. Fisch, and T. E. Schlaepfer, "Magnetic seizure therapy improves mood in refractory major depression," *Neuropsychopharmacology*, vol. 28, no. 11, pp. 2045–2048, 2003.
- [42] S. H. Lisanby, "Update on magnetic seizure therapy: a novel form of convulsive therapy," *The Journal of ECT*, vol. 18, no. 4, pp. 182–188, 2002.
- [43] P. F. White, Q. Amos, Y. Zhang et al., "Anesthetic considerations for magnetic seizure therapy: a novel therapy for severe depression," *Anesthesia and Analgesia*, vol. 103, no. 1, pp. 76–80, 2006.
- [44] S. Kayser, B. H. Bewernick, C. Grubert, B. L. Hadrysiewicz, N. Axmacher, and T. E. Schlaepfer, "Antidepressant effects of magnetic seizure therapy and electroconvulsive therapy, in treatment-resistant depression," *Journal of Psychiatric Research*, vol. 45, no. 5, pp. 569–576, 2011.
- [45] P. B. Fitzgerald, K. E. Hoy, S. E. Herring, A. M. Clinton, G. Downey, and Z. J. Daskalakis, "Pilot study of the clinical and cognitive effects of high-frequency magnetic seizure therapy in major depressive disorder," *Depression and Anxiety*, vol. 30, no. 2, pp. 129–136, 2013.
- [46] K. E. Hoy, R. H. Thomson, M. Cherk, K. S. K. Yap, Z. J. Daskalakis, and P. B. Fitzgerald, "Effect of magnetic seizure therapy on regional brain glucose metabolism in major depression," *Psychiatry Research—Neuroimaging*, vol. 211, no. 2, pp. 169–175, 2013.
- [47] G. Kirov, K. P. Ebmeier, A. I. F. Scott et al., "Quick recovery of orientation after magnetic seizure therapy for major depressive disorder," *British Journal of Psychiatry*, vol. 193, no. 2, pp. 152–155, 2008.
- [48] S. Kayser, B. H. Bewernick, R. Hurlmann, M. Soehle, and T. E. Schlaepfer, "Comparable seizure characteristics in magnetic seizure therapy and electroconvulsive therapy for major depression," *European Neuropsychopharmacology*, vol. 23, no. 11, pp. 1541–1550, 2013.
- [49] J. D. Polster, S. Kayser, B. H. Bewernick, R. Hurlmann, and T. E. Schlaepfer, "Effects of electroconvulsive therapy and magnetic seizure therapy on acute memory retrieval," *The Journal of ECT*, 2014.
- [50] H. A. Sackeim, J. Prudic, D. P. Devanand et al., "A prospective, randomized, double-blind comparison of bilateral and right unilateral electroconvulsive therapy at different stimulus intensities," *Archives of General Psychiatry*, vol. 57, no. 5, pp. 425–434, 2000.
- [51] H. A. Sackeim, B. Luber, J. R. Moeller, J. Prudic, D. P. Devanand, and M. S. Nobler, "Electrophysiological correlates of the adverse cognitive effects of electroconvulsive therapy," *Journal of ECT*, vol. 16, no. 2, pp. 110–120, 2000.
- [52] H. A. Sackeim, J. Prudic, D. P. Devanand et al., "Effects of stimulus intensity and electrode placement on the efficacy and cognitive effects of electroconvulsive therapy," *The New England Journal of Medicine*, vol. 328, no. 12, pp. 839–846, 1993.
- [53] A. Quante, A. Luborzewski, E.-L. Brakemeier, A. Merkl, H. Danker-Hopfe, and M. Bajbouj, "Effects of 3 different stimulus intensities of ultrabrief stimuli in right unilateral electroconvulsive therapy in major depression: a randomized, double-blind pilot study," *Journal of Psychiatric Research*, vol. 45, no. 2, pp. 174–178, 2011.

Review Article

The Potential Role of the NLRP3 Inflammasome as a Link between Mitochondrial Complex I Dysfunction and Inflammation in Bipolar Disorder

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Mitochondrial dysfunction and activation of the inflammatory system are two of the most consistently reported findings in bipolar disorder (BD). More specifically, altered levels of inflammatory cytokines and decreased levels of mitochondrial complex I subunits have been found in the brain and periphery of patients with BD, which could lead to increased production of mitochondrial reactive oxygen species (ROS). Recent studies have shown that mitochondrial production of ROS and inflammation may be closely linked through a redox sensor known as nod-like receptor pyrin domain-containing 3 (NLRP3). Upon sensing mitochondrial release of ROS, NLRP3 assembles the NLRP3 inflammasome, which releases caspase 1 to begin the inflammatory cascade. In this review, we discuss the potential role of the NLRP3 inflammasome as a link between complex I dysfunction and inflammation in BD and its therapeutic implications.

1. Introduction

Bipolar disorder (BD) is the sixth leading cause of disability worldwide (WHO) with a chronic course, where 25–50% of patients with BD attempt suicide and 50–67% of the patients experience at least one relapse [1]. Despite the urgent need to develop more effective treatments for this disorder, progress has been limited due to a lack of understanding of its pathology.

A growing number of studies are demonstrating mitochondrial dysfunction, especially that of complex I and inflammation in patients with BD [2–7]. For example, a recent review examining microarray findings in BD reported a decrease in complex I subunits that are responsible for transportation of electrons in patients with BD, which could result in increased leakage of electrons and production of reactive oxygen species (ROS) [6]. Altered levels of inflammatory cytokines were also shown in the brain and periphery of patients with BD, including IL-6, TNF α , IFN- γ , and

IL-1 β , suggesting that activation of the inflammatory system may also play a role in the pathophysiology of BD. Recent studies suggest that mitochondrial production of ROS may be linked to inflammatory activation [8, 9]. In fact, inhibition of complex I and subsequent increase in ROS production lead to increased levels of inflammatory factors such as IL-1 β , caspase 1, and NF- κ B [9, 10].

A potential link between mitochondrial dysfunction and inflammation may be the nod-like receptor pyrin domain-containing 3 (NLRP3) inflammasome, which is a redox sensor that can potentiate the activation of the inflammatory cascade by releasing caspase 1. Indeed, complex I inhibition resulted in the activation of the NLRP3 inflammasome, and decreasing mitochondrial ROS production was able to eliminate inflammasome activation [9, 11]. Therefore, the aim of this review is to explore the link between mitochondrial generation of oxidative stress and inflammation in BD, with a focus on the NLRP3 inflammasome.

2. BD and Mitochondrial Dysfunction: A Brief Overview

Mitochondria are energy producing organelles in the cell that generate ATP by transporting electrons through electron transport chain (ETC) complexes I-V. Moreover, they regulate calcium levels and apoptotic processes. Mitochondria are also the main producers of ROS [12]. Complex I, which is responsible for oxidizing NADH and transferring electrons to ubiquinone [13], contains four main subcomplexes: γ , β , α - λ , and λ that regulate its activity and ROS generation [14]. The α - λ and λ subcomplexes are located on the hydrophilic arm of complex I, which is responsible for electron transfer, and γ and β subcomplexes are located at the hydrophobic arm, which is responsible for proton pumping [13]. A recent review of microarray studies revealed that patients with BD have decreased mRNA levels of iron-sulfur cluster containing subunits in the hydrophilic portions that are specifically involved in electron transfer, including *NDUFVI*, *NDUFSI*, *NDUFS8*, and *NDUFS7* [6, 15]. On the other hand, the same review revealed that while patients with SCZ have some alterations in mRNA levels of complex I subunits, they do not have alterations in the subunits that are directly involved in the electron transfer process [6, 16, 17]. In support of the microarray findings, decreased protein levels of *NDUFS7* and complex I activity were also reported in patients with BD [7, 18]. These findings suggest that patients with BD may be more vulnerable to having increased levels of electron leakage compared to the normal population or patients with SCZ [6]. Leaked electrons from complex I can react with molecular oxygen to produce the superoxide anion, which can escape the mitochondria to undergo a series of reactions to form powerful ROS such as the hydroxyl radical [12]. Oxidative damage to lipids, DNA, and proteins in patients with BD is some of the most consistently reported alterations in BD, which is in agreement with these findings [2, 3, 19].

Superoxide anion and other ROS also play important roles as signaling molecules in the cell through redox sensors that undergo conformational changes, oligomerization, and/or translocation upon detecting ROS or downstream products of ROS release [20]. Nrf2, for example, migrates to the nucleus upon sensing ROS production, and thioredoxin undergoes a structural change upon being modified by ROS [20]. Recent studies have demonstrated that mitochondria may be a potent activator of the immune system through its ability to generate ROS and its interaction with redox sensors in the inflammatory system, such as NLRP3 [8, 9]. These findings suggest that mitochondrial dysfunction in BD may at least be partly responsible for cytokine activation in the central nervous system (CNS) and periphery of patients with BD.

3. BD and Inflammation

Alterations in the inflammatory pathway in patients with BD have been reported since 1995, when Maes et al. [21] found increased sIL-6R and sIL-2R levels in patients with mania. Indeed, medical complications related to activation

of the inflammatory system such as cardiovascular diseases, diabetes, and obesity are frequently diagnosed in patients with BD [22–24]. Furthermore, patients with BD generally have an earlier onset of cardiovascular diseases [22]. Such findings have inspired the microglial theory, which states that proinflammatory cytokines produced as a result of microglial activation result in disruption of neuroprotective functions, leading to increased vulnerability in BD [23].

Majority of the studies examining inflammation in BD have focused on peripheral samples such as plasma and serum [25–47]. A summary of the findings discussed here can be found in Tables 1, 2, 3, and 4. Multiple studies have reported increased levels of sIL-2R, sIL-6R, TNF- α , sTNFR1, IL-1, IL-12, and TGF- β in BD, while mixed results have been reported for other inflammatory factors, including IL-4, IL-2, IL-8, and IFN- γ [25, 27, 30, 32, 38, 40, 42, 44–47]. In this review, we will focus on TNF- α and IL-6 for the periphery and the IL-1 pathway for the CNS, as these factors have been consistently reported to be altered in patients with BD.

Despite the large number of studies examining inflammation in BD, there is a lack of agreement regarding the direction of alteration and the cytokines which are altered [23]. However, TNF- α and related factors such as sTNFR1 (soluble tumor necrosis factor receptor-1) have been consistently found to be elevated in the periphery of patients with BD [26, 29, 32, 35, 37, 40–42, 44–47]. TNF- α is proinflammatory cytokine, which is produced mainly by activated macrophages, CD4+ lymphocytes, and natural killer cells [48, 49]. Upon binding to its receptors, TNFR1 and TNFR2, TNF- α can trigger the activation of NF- κ B and MAPK pathways [45, 50].

IL-6, which is a proinflammatory cytokine secreted by T cells and macrophages, was also found to be increased in peripheral samples from patients with BD in the majority of studies that were examined in this review. Indeed, IL-6 is one of the cytokines most commonly reported to be altered in BD [29, 31, 32, 40, 42]. IL-6 can mediate fever and acute phase responses. It can also cross the blood brain barrier and trigger the activation of prostaglandin synthesis, which has been implicated in BD [51].

To our knowledge, only three studies have examined inflammation in the CNS in BD [50, 52, 53]. Dean et al. [50] focused on TNF- α related factors and pathways in different brain areas (BA24 and BA46) using postmortem brain. Increased concentration of tmTNF- α was observed in BA24 for BD, but not in BA46. TNFR2 was found to be decreased in BD subjects [50]. Rao and colleagues [52] focused on the IL-1 pathway and markers of microglial activation using postmortem prefrontal cortex from patients with BD. Higher protein and mRNA levels of IL-1 β , IL-1R, and MyD88 and microglial/astrocyte markers GFAP and iNOS were found in patients with BD [52]. This was in contrast to Dean et al. [50] who could not detect IL-1 β in their samples [50]. Findings from Söderlund and others [53] were consistent with Rao et al.'s study [52], showing elevated IL-1 β levels in the cerebral spinal fluid (CSF) of patients with BD compared to healthy controls. Also, patients who recently had manic or hypomanic episodes showed elevated IL-1 β levels compared

TABLE 1: Characteristics of studies examining cytokine alterations in peripheral samples of patients with bipolar disorder.

Author name	Year	Sample size	Sample	Technique used	Cytokines examined
Tsai et al. [25]	2001	31 manic, 31 remission, 31 control	Plasma	ELISA	sIL-2R, sIL-6R
Su et al. [27]	2002	20 BD-I manic, 15 control	Plasma		Stimulated sIL-2R, IL-10, IFN-g
Wadee et al. [28]	2002	45 BD-I manic, 45 control	Serum		CRP
Kim et al. [92]	2002	25 mania, 85 control	Plasma	ELISA	IL-12
Breunis et al. [30]	2003	172 BD I and II, 66 matched control	Serum		sIL-2R
Boufidou et al. [31]	2004	BD I and II, 40 Li treated euthymic, 10 medication naive, and 20 controls	Plasma	ELISA	Stimulated IL-2, IL-6, IL-10, IFN-g
Kim et al. [93]	2004	70 mania, 96 control	Plasma	ELISA	IFN-g, IL-4, TGF- β 1
Liu et al. [94]	2004	29 BD-I manic, 20 controls	Plasma		Stimulated IL-1RA, IL-2, IL-4, IL-10, IFN-g
Knijff et al. [95]	2006	54 BD-I and II, 10 controls	Peripheral blood	FACS	Stimulated IL-2R w/dexamethasone suppression
O'brien et al. [32]	2006	21 control, 12 manic, 9 depressed	Plasma	ELISA	IL-6, IL-8, IL-10, TNF-alpha, sIL-6R
Dickerson et al. [96]	2007	122 BD-I and II, 165 controls	Serum		CRP
Huang and Lin [34]	2007	13 BD-I manic, 23 MDD, 31 controls	Serum		hsCRP
Kim et al. [29]	2007	37 BD-I manic, 74 controls	Plasma	ELISA	Stimulated IL-2, IL-4, IL-10, TNF-a, and IFN-g
Knijff et al. [97]	2007	80 BD-I and II, 59 controls	Serum	ELISA	IL-1b, I-6
Ortiz-Domínguez et al. [42]	2007	33 controls, 20 patients, 10 in manic phase, 10 in depressed phase,	Serum	ELISA	TNF-a, IL-6, IL-1b, IL-2, and IL-4.
Cunha et al. [98]	2008	30 mania, 30 depressed, 20 euthymic, 32 controls	Serum		hsCRP
Kauer-Sant'Anna et al. [35]	2009	60 matched controls, 30 early stage, 30 late stage	Serum	ELISA	BDNF, TNF-a, IL-6 and IL-1
Guloksuz [99]	2010	31 euthymic, 16 control	Serum	Flow cytometry	IL-2, IL-4, IL-5, IL-10, IFN-g, TNF-a
Britzke and Teixeira [100]	2010	30 euthymic, 30 control	Serum	ELISA	sTNFR1, sTNFR2
Kapczinski et al. [36]	2011	20 manic, 20 depressed, 250 euthymic, 80 control	Serum	ELISA	TNF-a, IL-6, IL-10
Drexhage et al. [47]	2010	38 Euthymic, 22 control	Serum	Flow cytometry, ELISA for sIL-2R	IFN-g, IL-17A, IL-10, IL-6, IL-4, IL-5, IL-8, TNF-a, IL-1b, sIL-2R
Hope et al. [44]	2011	17 "Elevated", 58 Depressed, 26 Euthymic, 239 control	Plasma	EIA	sTNF-RI, IL1-Ra, IL-6
Barbosa et al. [101]	2011	34 manic, 19 Euthymic, 38 control	Plasma	ELISA	sTNF-RI, IL1-Ra, IL-6
Guloksuz et al. [45]	2012	45 euthymic with subsyndromal symptoms (BD+), 23 without subsyndromal symptoms (BD-), 23 control	Plasma	ELISA	Soluble tumor necrosis factor receptor-1 (sTNF-RI), soluble interleukin-6 receptor (sIL-6R), soluble interleukin-2 receptor (sIL-2R)
Barbosa et al. [37]	2012	25 euthymic, 25 control	Plasma	ELISA	TNF-a, sTNFR1, sTNFR2
Barbosa et al. [37]	2012	30 euthymic, 30 control	Plasma	ELISA	TNF-a, sTNFR1, sTNFR2
Remlinger-Molenda et al. [40]	2012	121 euthymic, 78 control	Serum	cytometry	IL-6, TNF-a, IL-10, IFN-g, IL-2, IL-1b
Cetin et al. [41]	2012	45 euthymic, 23 control	Plasma	ELISA	sTNF-RI, sIL-6R
Tsai et al. [26]	2012	33 manic, 33 remission, 33 control	Plasma	ELISA	IL1-Ra, sTNF-RI

TABLE 2: Characteristics of studies examining inflammatory cytokines in the central nervous system of patients with BD.

Author name	Article year	Sample size	Sample	Technique used	Cytokines examined
Rao et al. [52]	2010	10 BD, 10 control	Postmortem frontal cortex BA 24 and BA 46	Western plot, RT PCR, immunohistochemistry	NMDA receptors, NR-1 and NR-3A, IL-1 β , IL-1R, MyD88, NF-kB (p50, p65), GFAP, iNOS, c-fos and CD11b, TNF α , neuronal nNOS,
Söderlund et al. [53]	2011	BD euthymic patients, r type I ($n = 15$) or type II ($n = 15$)	CSF	An immunoassay-based protein array multiplex system	IL-1b, IL-6,
Dean et al. [50]	2013	10 MDD, 10 BD, 19 SZ, 30 control	Postmortem CNS tissue, BA24 and BA46	Western plot, RT PCR	tmTNF-a, sTNF-a, TNF mRNA, TNFR1, TNFR2, IL-1beta, synaptophysin, PSD95, GFAP43, GFAP41, CD11b and pro-IL1B

to those who did not [53]. Alterations in cytokine balance in the brain can lead to changes in neurotransmitter levels including dopamine [54, 55], cause microglial activation [56], and activate apoptotic processes [3, 57], all of which have been reported in patients with BD [3, 52, 58].

Interestingly, there has been a lack of agreement between the results found in peripheral samples and the CNS. For example, while TNF- α levels are not reported to differ between patients with BD and nonpsychiatric controls in the CNS, its levels are consistently reported to be altered in patients with BD using peripheral samples [29, 32, 35, 37, 40, 42, 47, 50, 52]. Moreover, while increased levels of cytokines in the IL-1 pathway have been reported in the central nervous system, studies examining peripheral samples have not reported alterations in this pathway [23, 40, 42, 47, 52, 53]. The difference between cytokine pathways activated in the periphery and the CNS in BD may be due to the presence of diseases that affect the periphery to a greater extent than the CNS, such as atherosclerosis [24]. The different immune cells that reside in the brain and outside of the blood brain barrier may also be underlying the differences in cytokine profile. On the other hand, the fact that inflammatory activation is found both in the CNS and periphery of patients with BD suggests that the same underlying factor may be causing their activation. Decreased expression of complex I subunits and subsequent generation of mitochondrial ROS may underlie activation of central and peripheral immune cells through the NLRP3 inflammasome [9] (Figure 1).

4. The NLRP3 Inflammasome

Recently, studies have shown that oxidative stress and mitochondrial dysfunction have important roles in regulating immune cells of the CNS and the periphery [59]. NLRP3 is a pattern recognition receptor in the inflammatory system that was shown to act as a redox sensor [9]. Cytosolic and membrane-associated pattern recognition receptors can detect danger signals induced by physical and psychological

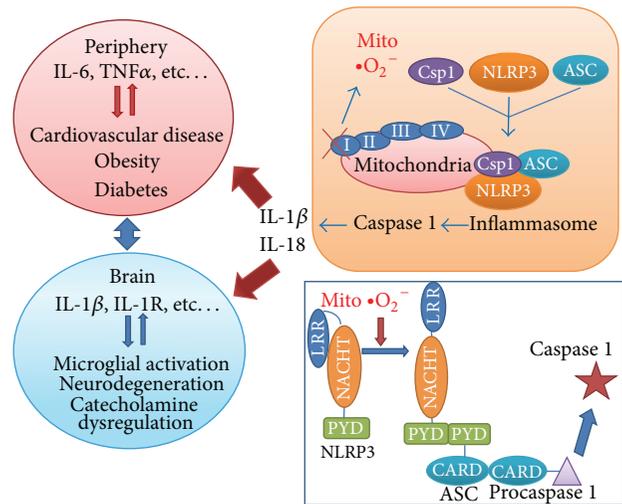


FIGURE 1: Mitochondrial complex I dysfunction in patients with BD could lead to increased release of superoxide anions, resulting in greater reactive oxygen species (ROS) production. This release of ROS causes a conformational change in NLRP3 such that the pyrin domain (PYD) becomes available to recruit ASC. The combining of NLRP3 and ASC allows for the recruitment of caspase 1 (csp1) through ASC's CARD domain, causing the formation of the NLRP3 inflammasome. The inflammasome then migrates to the mitochondria, allowing it to be close to the site of damage. Activated NLRP3 inflammasome releases caspase 1 into the cytosol, which then cleaves and activates two downstream cytokines, IL-1 β and IL-18, causing them to be released into the extracellular space. These two cytokines cause the activation of downstream pathways, which may differ depending on the type of immune cell. Indeed, NLRP3 inflammasome activation may underlie the different patterns of cytokine activation observed in the brain and peripheral samples of patients with BD, where alterations in cytokines pertaining to the IL-1 pathway have been reported for the brain, while a more general pattern of cytokine activation involving IL-6 and TNF- α has been reported in the periphery. Cytokine activation in the periphery can lead to various immune disorders, including cardiovascular disease and diabetes, while, in the brain, it could lead to alterations in neurotransmitters and neurodegeneration.

TABLE 4: Summary of findings from studies examining cytokine alterations in the central nerve system of patients with bipolar disorder.

Outcome	N studies	BD vs. controls	References
NR-1 (mRNA and Protein)	1	+	[52]
NR-2A (mRNA and Protein)	1	+	[52]
IL-1 β (mRNA and Protein)	3	+	[50, 52, 53]
IL-1R (mRNA and Protein)	1	+	[52]
MyD88 (mRNA and Protein)	1	+	[52]
GFAP (mRNA and Protein)	2	+	[50, 52]
iNOS (mRNA and Protein)	1	+	[52]
TNF α	2	+	[50, 52]
IL-6	1	-	[53]
tmTNFa	1	+ (at BA 24)	[50]
Astrocyte	1	+	[52]
Microglia markers	1	+	[52]
TNFR2	1	-	[50]

stress [60]. Membrane associated pattern recognition receptors include toll-like receptors that recognize pathogen associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), which then leads to the release of proinflammatory cytokines such as IL-1 β , TNF- α , and IL-6 [61]. Cytosolic pattern recognition receptors include NOD-like receptors, RIG-like receptors, and DNA sensors [62]. NLRP3 is the most widely studied receptor in the NOD-like receptor family [61]. NLRP3 contains a pyrin domain, a C-terminal leucine-rich domain, and a central nucleotide binding domain [61, 63]. NLRP3 is implicated in a wide variety of inflammatory conditions as it is activated by many different triggers including microbial infection, lipopolysaccharide, tissue damage, ATP, nigericin, and monosodium urate [64, 65]. When NLRP3 is inactive, it resides in the cytoplasm with its leucine-rich domain bound to the central nucleotide binding domain, preventing oligomerization [64]. Upon activation, NLRP3 migrates to the mitochondria associated endoplasmic reticulum membranes and the mitochondria [9]. This was demonstrated by increased colocalization between NLRP3 and mitotracker, which is a fluorescent marker for the mitochondria, and increased levels of NLRP3 in the mitochondrial fraction as well as mitochondria-associated membranes following NLRP3-inflammasome activation [9]. Since ROS are highly reactive and can only travel short distances, it would be ideal for NLRP3 to be localized the mitochondria where ROS is released [9, 66]. However, more studies are needed to test the generalizability of these findings in different systems and in humans. Activation of NLRP3 also causes NLRP3 oligomerization and recruitment of apoptosis-associated speck-like protein containing

a CARD (ASC) through pyrin-pyrin domain interaction [9]. Procaspase 1 is also recruited through a CARD-CARD interaction between ASC and procaspase 1, completing the process of NLRP3 inflammasome assembly and activation [63]. NLRP3 inflammasome then releases caspase 1, also known as IL-1 β converting enzyme. Caspase 1 cleaves pro-IL-1 β and pro-IL-18 to their mature biologically active forms [67]. IL-1 β is then released from the cells and binds to the IL-1 type-I receptor, a plasma membrane receptor, and IL-1 receptor-accessory protein to trigger the inflammatory cascade involving downstream signaling molecules such as MYD88 and NF- κ B [68]. This leads to increased expression and activation of other inflammatory mediators such as IL-6, TNF- α , and prostaglandin E2 [69, 70].

5. Mitochondrial Dysfunction and the NLRP3 Inflammasome

Since many different PAMPs and DAMPs can activate NLRP3, it is unlikely that its ligand binding site recognizes all the molecules known to trigger the assembly of the NLRP3 inflammasome [71]. Mitochondrial dysfunction and subsequent production of ROS have received much attention as the common pathway by which different PAMPs and DAMPs trigger inflammasome activation [8, 9, 11, 72]. For example, addition of rotenone, a complex I inhibitor, induces a dose-dependent increase in IL-1 β secretion [73], while in *Nlrp3* KO mice, the addition of a mitochondrial ETC inhibitor fails to increase IL-1 β and caspase 1 release [9]. Furthermore, inhibiting liposome-induced mitochondrial ROS release was followed by a decrease in the level of NLRP3-inflammasome activation [11]. While the exact pathway by which mitochondrial ROS leads to NLRP3 inflammasome activation and assembly remains elusive, two possible mechanisms have been proposed: thioredoxin-interacting protein- (TXNIP-) NLRP3 interaction and mitochondrial DNA (mtDNA) release [9, 65].

TXNIP is a tumor suppressor gene and its primary role is to inhibit the redox protein thioredoxin to suppress cell proliferation [9, 74]. Mitochondrial ROS production causes the dissociation between TXNIP and thioredoxin in the mitochondria, causing migration of TXNIP to the cytoplasm, which allows it to directly bind and activate cytoplasmic NLRP3 [74]. Zhou et al. (2011) showed that inflammation stimulating substances such as monosodium urate (MSU), silica, and ATP produce significantly less caspase 1 and IL-1 β in TXNIP deficient mice, indicating decreased level of NLRP3-inflammasome activation [9]. In addition, in a high glucose concentration environment, islet cells from *Txnip*^{-/-} and *Nlrp3*^{-/-} mice showed reduced level of IL-1 β secretion compared to wild-type mice [9]. TXNIP was also observed to be increased in patients with type II diabetes by a number of different studies [75–77]. Patients with BD are three times more likely to be diagnosed with type II diabetes compared to the general population [78], suggesting that NLRP3 inflammasome activation mediated by TXNIP could be underlying increased peripheral and CNS inflammation in patients with BD.

Another potential mediator between mitochondrial ROS and NLRP3 inflammasome assembly is mtDNA. The role of mtDNA release from the mitochondria to the cytoplasm in NLRP3-inflammasome activation has been suggested following the observation that mtDNA directly binds and activates the NLRP3-inflammasome [10, 79]. Opening of mitochondrial membrane permeability transition pores (MPTs), which allows for mtDNA to escape the mitochondria, is often preceded by mitochondrial ROS production [80]. Also, adding ATP and lipopolysaccharide, which are two well-known stimulators of the NLRP3-inflammasome, increases mitochondrial ROS production and oxidized mtDNA levels in NLRP3 immunoprecipitates [79]. Importantly, it was also found that adding NLRP3 stimuli into cells lacking mtDNA (p0 cells) does not result in IL-1 β secretion [79], and that the addition of mito-TEMPO, a mitochondrial-ROS scavenger, to bone marrow derived macrophages inhibits IL-1 β and IL-18 secretion in a dose-dependent manner [81, 82]. Furthermore, preventing the opening of MPTs through the addition of cyclosporine A and thereby preventing mtDNA release inhibit LPS- and ATP-induced IL-1 β secretion [10].

While the exact mechanism for how mitochondrial dysfunction triggers the assembly of the NLRP3 inflammasome remains to be elucidated, recent studies suggest that release of mitochondrial ROS plays a significant role in this pathway, either through activation of an intermediate redox sensor, such as TXNIP, or by activating apoptotic pathways causing the opening of MPTs [9, 10]. These findings suggest that amelioration of mitochondrial ROS production may aid in decreasing NLRP3-inflammasome activation, which could contribute to decreasing cytokine release in patients with BD.

6. Perspectives

With the discovery of immunological alterations in BD, much attention has been given to the possibility of implementing anti-inflammatory agents to treat symptom severity and cognitive decline [24]. An anti-inflammatory drug that was examined in patients with BD is celecoxib, which is a cyclooxygenase-2 inhibitor. Studies performed on rats showed that celecoxib can decrease IL-1 β concentration in the hypothalamus, prefrontal cortex, and the hippocampus [83, 84]. Celecoxib was also shown to have a significant antidepressant effect in patients with BD, suggesting that anti-inflammatory medications targeting IL-1 β may be helpful for patients with BD [85]. Aspirin (acetylsalicylic acid), which also inhibits the activity of cyclooxygenase 2 as well as cyclooxygenase 1, is also receiving much attention as a potential treatment option for bipolar depression [86, 87]. Cyclooxygenase enzymes are involved in the arachidonic acid cascade, which can lead to the activation of neuroinflammation pathways [88, 89]. Indeed, low-dose aspirin was found to decrease medication events (change in type of drug, increase in dose, or the number of prescribed drugs) in patients with BD, suggesting that aspirin may aid in stabilizing the symptoms [87].

Since NLRP3 inflammasome is strongly linked to mitochondrial dysfunction and subsequent production of

ROS, improving mitochondrial function may contribute to decreasing inflammation in BD. A potential treatment is melatonin, which is a well-established antioxidant and an anti-inflammatory agent that was also demonstrated to target and accumulate in the mitochondria, improve mitochondrial respiration, and inhibit lipopolysaccharide-induced cytokine release [90, 91]. Interestingly, melatonin was found to be decreased in patients with BD, which may underlie disruptions in sleep patterns frequently observed in these patients. These findings suggest that melatonin may aid in decreasing mitochondrial ROS production and subsequent NLRP3 inflammasome activation in BD.

7. Conclusion

Mitochondrial complex I dysfunction and chronic inflammation are two of the most consistent findings in BD [2, 3]. Mitochondria are potent activators of the immune system, and this may occur in part through the NLRP3 inflammasome, which is assembled and activated following mitochondrial release of ROS [9]. Since complex I dysfunction in BD could lead to increased production of mitochondrial ROS, NLRP3 inflammasome mediated activation of the inflammatory system may underlie increased cytokine release in the CNS and periphery of patients with BD. Future studies examining the role of the NLRP3 inflammasome in BD will contribute to elucidating the link between two prominent pathophysiological alterations in this disorder, which may reveal pathways that can be used for the development of novel therapeutic interventions that can target both systems to improve symptomatology and cognitive functioning.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

- [1] K. N. Fountoulakis, X. Gonda, M. Siamouli, and Z. Rihmer, "Psychotherapeutic intervention and suicide risk reduction in bipolar disorder: a review of the evidence," *Journal of Affective Disorders*, vol. 113, no. 1-2, pp. 21-29, 2009.
- [2] H. B. Clay, S. Sullivan, and C. Konradi, "Mitochondrial dysfunction and pathology in bipolar disorder and schizophrenia," *International Journal of Developmental Neuroscience*, vol. 29, no. 3, pp. 311-324, 2011.
- [3] M. Berk, F. Kapczinski, A. C. Andreazza et al., "Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors," *Neuroscience & Biobehavioral Reviews*, vol. 35, no. 3, pp. 804-817, 2011.
- [4] T. Kato and N. Kato, "Mitochondrial dysfunction in bipolar disorder," *Bipolar Disorders*, vol. 2, no. 3, pp. 180-190, 2000.
- [5] C. Konradi, S. E. Sullivan, and H. B. Clay, "Mitochondria, oligodendrocytes and inflammation in bipolar disorder: evidence from transcriptome studies points to intriguing parallels with multiple sclerosis," *Neurobiology of Disease*, vol. 45, no. 1, pp. 37-47, 2012.

- [6] G. Scola, H. K. Kim, L. T. Young, and A. C. Andreazza, "A fresh look at complex i in microarray data: clues to understanding disease-specific mitochondrial alterations in bipolar disorder," *Biological Psychiatry*, vol. 73, no. 2, pp. e4–e5, 2013.
- [7] A. C. Andreazza, L. Shoo, J. F. Wang, and L. Trevor Young, "Mitochondrial complex I activity and oxidative damage to mitochondrial proteins in the prefrontal cortex of patients with bipolar disorder," *Archives of General Psychiatry*, vol. 67, no. 4, pp. 360–368, 2010.
- [8] M. J. López-Armada, R. R. Riveiro-Naveira, C. Vaamonde-García, and M. N. Valcárcel-Ares, "Mitochondrial dysfunction and the inflammatory response," *Mitochondrion*, vol. 13, no. 2, pp. 106–118, 2013.
- [9] R. Zhou, A. S. Yazdi, P. Menu, and J. Tschopp, "A role for mitochondria in NLRP3 inflammasome activation," *Nature*, vol. 469, pp. 221–225, 2011.
- [10] K. Nakahira, J. A. Haspel, V. A. K. Rathinam et al., "Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome," *Nature Immunology*, vol. 12, no. 3, pp. 222–230, 2011.
- [11] Z. Zhong, Y. Zhai, S. Liang et al., "TRPM2 links oxidative stress to NLRP3 inflammasome activation," *Nature Communications*, vol. 4, article 1611, 2013.
- [12] B. Halliwell, "Reactive oxygen species and the central nervous system," *Journal of Neurochemistry*, vol. 59, no. 5, pp. 1609–1623, 1992.
- [13] D. R. Green and G. Kroemer, "The pathophysiology of mitochondrial cell death," *Science*, vol. 305, no. 5684, pp. 626–629, 2004.
- [14] G. Lenaz, "The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology," *IUBMB Life*, vol. 52, no. 3–5, pp. 159–164, 2002.
- [15] R. J. R. Janssen, L. G. Nijtmans, L. P. van den Heuvel, and J. A. M. Smeitink, "Mitochondrial complex I: structure, function and pathology," *Journal of Inherited Metabolic Disease*, vol. 29, no. 4, pp. 499–515, 2006.
- [16] M. P. Vawter, H. Tomita, F. Meng et al., "Mitochondrial-related gene expression changes are sensitive to agonal-pH state: implications for brain disorders," *Molecular Psychiatry*, vol. 11, no. 7, pp. 663–679, 2006.
- [17] S. Prabakaran, J. E. Swatton, M. M. Ryan et al., "Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress," *Molecular Psychiatry*, vol. 9, no. 7, pp. 684–697, 2004.
- [18] A. C. Andreazza, J. F. Wang, F. Salmasi, L. Shao, and L. T. Young, "Specific subcellular changes in oxidative stress in prefrontal cortex from patients with bipolar disorder," *Journal of Neurochemistry*, vol. 127, no. 4, pp. 552–561, 2013.
- [19] A. C. Andreazza, M. Kauer-Sant'Anna, B. N. Frey et al., "Oxidative stress markers in bipolar disorder: a meta-analysis," *Journal of Affective Disorders*, vol. 111, no. 2–3, pp. 135–144, 2008.
- [20] D. P. Jones, "Redox sensing: orthogonal control in cell cycle and apoptosis signalling," *Journal of Internal Medicine*, vol. 268, no. 5, pp. 432–448, 2010.
- [21] M. Maes, E. Bosmans, J. Calabrese, R. Smith, and H. Y. Meltzer, "Interleukin-2 and interleukin-6 in schizophrenia and mania: effects of neuroleptics and mood stabilizers," *Journal of Psychiatric Research*, vol. 29, no. 2, pp. 141–152, 1995.
- [22] A. M. Kilbourne, J. R. Cornelius, X. Han et al., "Burden of general medical conditions among individuals with bipolar disorder," *Bipolar Disorders*, vol. 6, no. 5, pp. 368–373, 2004.
- [23] K. Munkholm, J. V. Braüner, L. V. Kessing, and M. Vinberg, "Cytokines in bipolar disorder vs. healthy control subjects: a systematic review and meta-analysis," *Journal of Psychiatric Research*, vol. 47, no. 9, pp. 1119–1133, 2013.
- [24] B. I. Goldstein, A. Fagiolini, P. Houck, and D. J. Kupfer, "Cardiovascular disease and hypertension among adults with bipolar I disorder in the United States," *Bipolar Disorders*, vol. 11, no. 6, pp. 657–662, 2009.
- [25] S.-Y. M. Tsai, Y.-Y. Yang, C.-J. Kuo, C.-C. Chen, and S.-J. C. Leu, "Effects of symptomatic severity on elevation of plasma soluble interleukin-2 receptor in bipolar mania," *Journal of Affective Disorders*, vol. 64, no. 2–3, pp. 185–193, 2001.
- [26] H.-C. Tsai, M.-K. Lu, Y.-K. Yang et al., "Empirically derived subgroups of bipolar I patients with different comorbidity patterns of anxiety and substance use disorders in Han Chinese population," *Journal of Affective Disorders*, vol. 136, no. 1–2, pp. 81–89, 2012.
- [27] K.-P. Su, S.-J. C. Leu, Y.-Y. Yang, W. W. Shen, Y.-M. Chou, and S.-Y. M. Tsai, "Reduced production of interferon-gamma but not interleukin-10 in bipolar mania and subsequent remission," *Journal of Affective Disorders*, vol. 71, no. 1–3, pp. 205–209, 2002.
- [28] A. A. Wadee, R. H. Kuschke, L. A. Wood, M. Berk, L. Ichim, and M. Maes, "Serological observations in patients suffering from acute manic episodes," *Human Psychopharmacology*, vol. 17, no. 4, pp. 175–179, 2002.
- [29] Y.-K. Kim, H.-G. Jung, A.-M. Myint, H. Kim, and S.-H. Park, "Imbalance between pro-inflammatory and anti-inflammatory cytokines in bipolar disorder," *Journal of Affective Disorders*, vol. 104, no. 1–3, pp. 91–95, 2007.
- [30] M. N. Breunis, R. W. Kupka, W. A. Nolen et al., "High numbers of circulating activated T cells and raised levels of serum IL-2 receptor in bipolar disorder," *Biological Psychiatry*, vol. 53, no. 2, pp. 157–165, 2003.
- [31] F. Boufidou, C. Nikolaou, B. Alevizos, I. A. Liappas, and G. N. Christodoulou, "Cytokine production in bipolar affective disorder patients under lithium treatment," *Journal of Affective Disorders*, vol. 82, no. 2, pp. 309–313, 2004.
- [32] S. M. O'Brien, P. Scully, L. V. Scott, and T. G. Dinan, "Cytokine profiles in bipolar affective disorder: focus on acutely ill patients," *Journal of Affective Disorders*, vol. 90, no. 2–3, pp. 263–267, 2006.
- [33] F. Dickerson, C. Stallings, A. Origoni et al., "A combined marker of inflammation in individuals with mania," *PLoS ONE*, vol. 8, no. 9, Article ID e73520, 2013.
- [34] T. L. Huang and F. C. Lin, "High-sensitivity C-reactive protein levels in patients with major depressive disorder and bipolar mania," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 31, no. 2, pp. 370–372, 2007.
- [35] M. Kauer-Sant'Anna, F. Kapczinski, A. C. Andreazza et al., "Brain-derived neurotrophic factor and inflammatory markers in patients with early- vs. late-stage bipolar disorder," *The International Journal of Neuropsychopharmacology*, vol. 12, no. 4, pp. 447–458, 2009.
- [36] F. Kapczinski, F. Dal-Pizzol, A. L. Teixeira et al., "Peripheral biomarkers and illness activity in bipolar disorder," *Journal of Psychiatric Research*, vol. 45, no. 2, pp. 156–161, 2011.
- [37] I. G. Barbosa, N. P. Rocha, A. S. D. Miranda et al., "Increased levels of adipokines in bipolar disorder," *Journal of Psychiatric Research*, vol. 46, no. 3, pp. 389–393, 2012.
- [38] I. G. Barbosa, N. P. Rocha, M. E. Bauer et al., "Chemokines in bipolar disorder: trait or state?" *European Archives of Psychiatry and Clinical Neuroscience*, vol. 263, no. 2, pp. 159–165, 2013.

- [39] I. G. Barbosa, I. B. Morato, A. S. de Miranda, M. E. Bauer, J. C. Soares, and A. L. Teixeira, "A preliminary report of increased plasma levels of IL-33 in bipolar disorder: further evidence of pro-inflammatory status," *Journal of Affective Disorders*, vol. 157, pp. 41–44, 2014.
- [40] A. Remlinger-Molenda, P. Wojciak, M. Michalak, J. Karczewski, and J. K. Rybakowski, "Selected cytokine profiles during remission in bipolar patients," *Neuropsychobiology*, vol. 66, no. 3, pp. 193–198, 2012.
- [41] T. Cetin, S. Guloksuz, E. A. Cetin et al., "Plasma concentrations of soluble cytokine receptors in euthymic bipolar patients with and without subsyndromal symptoms," *BMC Psychiatry*, vol. 12, article 158, 2012.
- [42] A. Ortiz-Domínguez, M. E. Hernández, C. Berlanga et al., "Immune variations in bipolar disorder: phasic differences," *Bipolar Disorders*, vol. 9, no. 6, pp. 596–602, 2007.
- [43] A. B. M. Cunha, B. N. Frey, A. C. Andreazza et al., "Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes," *Neuroscience Letters*, vol. 398, no. 3, pp. 215–219, 2006.
- [44] S. Hope, I. Dieset, I. Agartz et al., "Affective symptoms are associated with markers of inflammation and immune activation in bipolar disorders but not in schizophrenia," *Journal of Psychiatric Research*, vol. 45, no. 12, pp. 1608–1616, 2011.
- [45] S. Guloksuz, K. Altinbas, E. Aktas Cetin et al., "Evidence for an association between tumor necrosis factor-alpha levels and lithium response," *Journal of Affective Disorders*, vol. 143, no. 1–3, pp. 148–152, 2012.
- [46] E. Brietzke, R. Stabellini, R. Grassi-Oliveira, and B. Lafer, "Cytokines in bipolar disorder: recent findings, deleterious effects but promise for future therapeutics," *CNS Spectrums*, vol. 16, no. 7, pp. 157–168, 2011.
- [47] R. C. Drexhage, E. M. Knijff, R. C. Padmos et al., "The mononuclear phagocyte system and its cytokine inflammatory networks in schizophrenia and bipolar disorder," *Expert Review of Neurotherapeutics*, vol. 10, no. 1, pp. 59–76, 2010.
- [48] E. A. Carswell, L. J. Old, R. L. Kassel, S. Green, N. Fiore, and B. Williamson, "An endotoxin induced serum factor that causes necrosis of tumors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 72, no. 9, pp. 3666–3670, 1975.
- [49] A. Koj, D. Magielska-Zero, J. Bereta, A. Kurdowska, H. Rokita, and J. Gaudie, "The cascade of inflammatory cytokines regulating synthesis of acute phase proteins," *The Tokai Journal of Experimental and Clinical Medicine*, vol. 13, no. 6, pp. 255–264, 1988.
- [50] B. Dean, A. S. Gibbons, N. Tawadros, L. Brooks, I. P. Everall, and E. Scarr, "Different changes in cortical tumor necrosis factor- α -related pathways in schizophrenia and mood disorders," *Molecular Psychiatry*, vol. 18, no. 7, pp. 767–773, 2013.
- [51] W. A. Banks, A. J. Kastin, and R. D. Broadwell, "Passage of cytokines across the blood-brain barrier," *Neuroimmunomodulation*, vol. 2, no. 4, pp. 241–248, 1995.
- [52] J. S. Rao, G. J. Harry, S. I. Rapoport, and H. W. Kim, "Increased excitotoxicity and neuroinflammatory markers in postmortem frontal cortex from bipolar disorder patients," *Molecular Psychiatry*, vol. 15, no. 4, pp. 384–392, 2010.
- [53] J. Söderlund, S. K. Olsson, M. Samuelsson et al., "Elevation of cerebrospinal fluid interleukin-1 β in bipolar disorder," *Journal of Psychiatry & Neuroscience*, vol. 36, no. 2, pp. 114–118, 2011.
- [54] J. C. Felger, L. Li, P. J. Marvar et al., "Tyrosine metabolism during interferon-alpha administration: association with fatigue and CSF dopamine concentrations," *Brain, Behavior, and Immunity*, vol. 31, pp. 153–160, 2013.
- [55] J. C. Felger and A. H. Miller, "Cytokine effects on the basal ganglia and dopamine function: the subcortical source of inflammatory malaise," *Frontiers in Neuroendocrinology*, vol. 33, no. 3, pp. 315–327, 2012.
- [56] S. T. Dheen, C. Kaur, and E.-A. Ling, "Microglial activation and its implications in the brain diseases," *Current Medicinal Chemistry*, vol. 14, no. 11, pp. 1189–1197, 2007.
- [57] M. Berk, L. Berk, S. Dodd et al., "Stage managing bipolar disorder," *Bipolar Disorders*, vol. 16, no. 5, pp. 471–477, 2014.
- [58] M. Berk, S. Dodd, M. Kauer-Sant'anna et al., "Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder," *Acta Psychiatrica Scandinavica*, no. 434, pp. 41–49, 2007.
- [59] M. T. Sorbara and S. E. Girardin, "Mitochondrial ROS fuel the inflammasome," *Cell Research*, vol. 21, no. 4, pp. 558–560, 2011.
- [60] T. Boller and G. Felix, "A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors," *Annual Review of Plant Biology*, vol. 60, pp. 379–407, 2009.
- [61] C. Bryant and K. A. Fitzgerald, "Molecular mechanisms involved in inflammasome activation," *Trends in Cell Biology*, vol. 19, no. 9, pp. 455–464, 2009.
- [62] B. Beutler, Z. Jiang, P. Georgel et al., "Genetic analysis of host resistance: toll-like receptor signaling and immunity at large," *Annual Review of Immunology*, vol. 24, pp. 353–389, 2006.
- [63] L. Agostini, F. Martinon, K. Burns, M. F. McDermott, P. N. Hawkins, and J. Tschopp, "NALP3 forms an IL-1 β -processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder," *Immunity*, vol. 20, no. 3, pp. 319–325, 2004.
- [64] J. Tschopp and K. Schroder, "NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production?" *Nature Reviews Immunology*, vol. 10, no. 3, pp. 210–215, 2010.
- [65] K. Schroder, R. Zhou, and J. Tschopp, "The NLRP3 inflammasome: a sensor for metabolic danger?" *Science*, vol. 327, no. 5963, pp. 296–300, 2010.
- [66] B. Harwell, "Biochemistry of oxidative stress," *Biochemical Society Transactions*, vol. 35, no. 5, pp. 1147–1150, 2007.
- [67] D. Perregaux and C. A. Gabel, "Interleukin-1 β maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity," *The Journal of Biological Chemistry*, vol. 269, no. 21, pp. 15195–15203, 1994.
- [68] C. Eder, "Mechanisms of interleukin-1 β release," *Immunobiology*, vol. 214, no. 7, pp. 543–553, 2009.
- [69] N. B. Bryan, A. Dorfleutner, S. J. Kramer, C. Yun, Y. Rojanasakul, and C. Stehlik, "Differential splicing of the apoptosis-associated speck like protein containing a caspase recruitment domain (ASC) regulates inflammasomes," *Journal of Inflammation*, vol. 7, article 23, 2010.
- [70] A. Weber, P. Wasiliew, and M. Kracht, "Interleukin-1 β (IL-1 β) processing pathway," *Science Signaling*, vol. 3, no. 105, p. cm2, 2010.
- [71] M. Lamkanfi and V. M. Dixit, "Inflammasomes: guardians of cytosolic sanctity," *Immunological Reviews*, vol. 227, no. 1, pp. 95–105, 2009.
- [72] D. Liu, A. M. Rhebergen, and S. C. Eisenbarth, "Licensing adaptive immunity by NOD-like receptors," *Frontiers in Immunology*, vol. 4, article 486, Article ID Article 486, 2013.

- [73] N. Li, K. Ragheb, G. Lawler et al., "Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production," *The Journal of Biological Chemistry*, vol. 278, no. 10, pp. 8516–8525, 2003.
- [74] T. Lane, B. Flam, R. Lockey, and N. Kolliputi, "TXNIP shuttling: missing link between oxidative stress and inflammasome activation," *Frontiers in Physiology*, vol. 4, article 50, 2013.
- [75] H. Parikh, E. Carlsson, W. A. Chutkow et al., "TXNIP regulates peripheral glucose metabolism in humans," *PLoS Medicine*, vol. 4, no. 5, article e158, 2007.
- [76] A. H. Minn, C. Hafele, and A. Shalev, "Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces β -cell apoptosis," *Endocrinology*, vol. 146, no. 5, pp. 2397–2405, 2005.
- [77] J. Chen, G. Saxena, I. N. Mungrue, A. J. Lusis, and A. Shalev, "Thioredoxin-interacting protein: a critical link between glucose toxicity and β -cell apoptosis," *Diabetes*, vol. 57, no. 4, pp. 938–944, 2008.
- [78] C. V. Calkin, D. M. Gardner, T. Ransom, and M. Alda, "The relationship between bipolar disorder and type 2 diabetes: more than just co-morbid disorders," *Annals of Medicine*, vol. 45, no. 2, pp. 171–181, 2013.
- [79] K. Shimada, T. R. Crother, J. Karlin et al., "Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis," *Immunity*, vol. 36, no. 3, pp. 401–414, 2012.
- [80] J. J. Lemasters, T. P. Theruvath, Z. Zhong, and A.-L. Nieminen, "Mitochondrial calcium and the permeability transition in cell death," *Biochimica et Biophysica Acta: Bioenergetics*, vol. 1787, no. 11, pp. 1395–1401, 2009.
- [81] J. Trnka, F. H. Blaikie, A. Logan, R. A. Smith, and M. P. Murphy, "Antioxidant properties of MitoTEMPO and its hydroxylamine," *Free Radical Research*, vol. 43, no. 1, pp. 4–12, 2009.
- [82] J. Jiang, D. A. Stoyanovsky, N. A. Belikova et al., "A mitochondria-targeted triphenylphosphonium-conjugated nitroxide functions as a radioprotector/mitigator," *Radiation Research*, vol. 172, no. 6, pp. 706–717, 2009.
- [83] A. M. Myint, H. W. M. Steinbusch, L. Goeghegan, D. Luchtman, Y. K. Kim, and B. E. Leonard, "Effect of the COX-2 inhibitor celecoxib on behavioural and immune changes in an olfactory bulbectomised rat model of depression," *NeuroImmunoModulation*, vol. 14, no. 2, pp. 65–71, 2007.
- [84] P. Casolini, A. Catalani, A. R. Zuena, and L. Angelucci, "Inhibition of COX-2 reduces the age-dependent increase of hippocampal inflammatory markers, corticosterone secretion, and behavioral impairments in the rat," *Journal of Neuroscience Research*, vol. 68, no. 3, pp. 337–343, 2002.
- [85] F. G. Nery, E. S. Monkul, J. P. Hatch et al., "Celecoxib as an adjunct in the treatment of depressive or mixed episodes of bipolar disorder: a double-blind, randomized, placebo-controlled study," *Human Psychopharmacology*, vol. 23, no. 2, pp. 87–94, 2008.
- [86] J. Savitz, S. Preskorn, T. K. Teague, D. Drevets, W. Yates, and W. Drevets, "Minocycline and aspirin in the treatment of bipolar depression: a protocol for a proof-of-concept, randomised, doubleblind, placebo-controlled, 2 \times 2 clinical trial," *BMJ Open*, vol. 2, no. 1, Article ID 000643, 2012.
- [87] P. Stolk, P. C. Souverein, I. Wilting et al., "Is aspirin useful in patients on lithium? A pharmacoepidemiological study related to bipolar disorder," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 82, no. 1, pp. 9–14, 2010.
- [88] G. R. Weerasinghe, S. I. Rapoport, and F. Bosetti, "The effect of chronic lithium on arachidonic acid release and metabolism in rat brain does not involve secretory phospholipase A₂ or lipoxygenase/cytochrome P450 pathways," *Brain Research Bulletin*, vol. 63, no. 6, pp. 485–489, 2004.
- [89] E. Ramadan, M. Basselin, J. S. Rao et al., "Lamotrigine blocks NMDA receptor-initiated arachidonic acid signalling in rat brain: implications for its efficacy in bipolar disorder," *International Journal of Neuropsychopharmacology*, vol. 15, no. 7, pp. 931–943, 2012.
- [90] D. A. Lowes, A. M. Almawash, N. R. Webster, V. L. Reid, and H. F. Galley, "Melatonin and structurally similar compounds have differing effects on inflammation and mitochondrial function in endothelial cells under conditions mimicking sepsis," *British Journal of Anaesthesia*, vol. 107, no. 2, pp. 193–201, 2011.
- [91] V. Srinivasan, D. W. Spence, S. R. Pandi-Perumal, G. M. Brown, and D. P. Cardinali, "Melatonin in mitochondrial dysfunction and related disorders," *International Journal of Alzheimer's Disease*, vol. 2011, Article ID 326320, 16 pages, 2011.
- [92] Y. K. Kim, I. B. Suh, H. Kim et al., "The plasma levels of interleukin-12 in schizophrenia, major depression, and bipolar mania: Effects of psychotropic drugs," *Molecular Psychiatry*, vol. 7, no. 10, pp. 1107–1114, 2002.
- [93] Y.-K. Kim, A.-M. Myint, B.-H. Lee et al., "T-helper types 1, 2, and 3 cytokine interactions in symptomatic manic patients," *Psychiatry Research*, vol. 129, no. 3, pp. 267–272, 2004.
- [94] H.-C. Liu, Y.-Y. Yang, Y.-M. Chou, K.-P. Chen, W. W. Shen, and S.-J. Leu, "Immunologic variables in acute mania of bipolar disorder," *Journal of Neuroimmunology*, vol. 150, no. 1-2, pp. 116–122, 2004.
- [95] E. M. Knijff, M. N. Breunis, M. C. van Geest et al., "A relative resistance of T cells to dexamethasone in bipolar disorder," *Bipolar Disorders*, vol. 8, no. 6, pp. 740–750, 2006.
- [96] F. Dickerson, C. Stallings, A. Origoni, J. Boronow, and R. Yolken, "Elevated serum levels of C-reactive protein are associated with mania symptoms in outpatients with bipolar disorder," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 31, no. 4, pp. 952–955, 2007.
- [97] E. M. Knijff, M. N. Breunis, R. W. Kupka et al., "An imbalance in the production of IL-1 β and IL-6 by monocytes of bipolar patients: restoration by lithium treatment," *Bipolar Disorders*, vol. 9, no. 7, pp. 743–753, 2007.
- [98] A. B. Cunha, A. C. Andreazza, F. A. Gomes et al., "Investigation of serum high-sensitive C-reactive protein levels across all mood states in bipolar disorder," *European Archives of Psychiatry and Clinical Neuroscience*, vol. 258, no. 5, pp. 300–304, 2008.
- [99] S. Guloksuz, E. A. Cetin, T. Cetin, G. Deniz, E. T. Oral, and D. J. Nutt, "Cytokine levels in euthymic bipolar patients," *Journal of Affective Disorders*, vol. 126, no. 3, pp. 458–462, 2010.
- [100] E. Brietzke and A. L. Teixeira, "Similar immune profile in bipolar disorder and schizophrenia: Selective increase in soluble tumor necrosis factor receptor I and von Willebrand factor," *Bipolar Disorders*, vol. 12, no. 4, pp. 453–454, 2010.
- [101] I. G. Barbosa, R. B. Huguet, V. A. Mendonça et al., "Increased plasma levels of soluble TNF receptor I in patients with bipolar disorder," *European Archives of Psychiatry and Clinical Neuroscience*, vol. 261, no. 2, pp. 139–143, 2011.

Research Article

Cytomegalovirus Antibody Elevation in Bipolar Disorder: Relation to Elevated Mood States

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The neurobiology of mood states is complicated by exposure to everyday stressors (e.g., psychosocial, ubiquitous environmental infections like CMV), each fluctuating between latency and reactivation. CMV reactivation induces proinflammatory cytokines (e.g., TNF- α) associated with induction of neurotoxic metabolites and the presence of mood states in bipolar disorder (BD). Whether CMV reactivation is associated with bipolar diagnoses (trait) or specific mood states is unclear. We investigated 139 BD type I and 99 healthy controls to determine if concentrations of IgG antibodies to Herpesviridae (e.g., CMV, HSV-1, and HSV-2) were associated with BD-I diagnosis and specific mood states. We found higher CMV antibody concentration in BD-I than in healthy controls ($T_{234} = 3.1$, $P_{\text{uncorr}} = 0.002$; $P_{\text{corr}} = 0.006$) but no difference in HSV-1 ($P > 0.10$) or HSV-2 ($P > 0.10$). Compared to euthymic BD-I volunteers, CMV IgG was higher in BD-I volunteers with elevated moods ($P < 0.03$) but not different in depressed moods ($P > 0.10$). While relationships presented between BD-I diagnosis, mood states, and CMV antibodies are encouraging, they are limited by the study's cross sectional nature. Nevertheless, further testing is warranted to replicate findings and determine whether reactivation of CMV infection exacerbates elevated mood states in BD-I.

1. Introduction

Bipolar disorder (BD) a mood disorder characterized by the presence of elevated, irritable, or mixed mood episodes frequently interspersed with episodes of depression affects approximately 2-3% of the population [1-3]. Despite substantial individual and societal impact, knowledge of the biological processes underlying and driving mood states in BD is limited. Revealing associations between biological factors and both mood traits and states will set a trajectory for understanding the pathophysiology of moods and in developing novel, more efficacious intervention strategies in BD.

Ubiquitous environmental infections (e.g., Herpesviridae including cytomegalovirus; CMV) and associated human

immune responses fluctuate between latency and reactivation in humans, potentially triggered by psychosocial stressors [4-6]. Viruses may facilitate exacerbation of psychiatric disease pathology through various mechanisms, including induction of inflammatory factors (e.g., TNF- α , IL-6, etc.) [7] or via direct interactions with specific illness susceptibility genes. A recent preliminary fMRI study of pediatric bipolar disorder may suggest a mechanism whereby alterations in TNF- α related processes could impact some of the symptoms in BD-I. In this study, Barzman et al. identified correlations between 11 TNF- α related gene expressions and activation within the amygdala or anterior cingulate cortex during the affective Posner task [8]. Evidence from recent studies in BD also shows that TNF- α is higher in BD volunteers compared to healthy control volunteers [9, 10]. Further, as outlined in

a review by Brietzke et al., existing evidence suggests that TNF- α is higher in the midst mood episodes in BD volunteers as compared to healthy control volunteers [10, 11].

Amongst the potential viral candidates, the herpesvirus family has received the most attention. Certain Herpesviridae (e.g., HSV-1) have been associated with clinical features of BD [12], but to date, no studies have determined whether CMV is associated with the presence of either mania or depression in BD. However, evidence from volunteers with schizophrenia, a psychiatric illness sharing certain clinical and biological features with BD (see review by Prossin and colleagues) [13], suggests that *Cytomegalovirus* (CMV) may interact with certain high risk genetic loci to precipitate schizophrenia illness [14–18].

Following the diathesis-stress model of disease [19], exposure to environmental stressors (psychosocial, behavioral, and biological) could potentially increase risk for psychiatric illness, particularly in individuals at high genetic risk for that illness [20, 21]. However, while knowledge of behavioral phenotype in BD has grown [22], facilitating development of more efficacious behavioral interventions (e.g., interpersonal and social rhythm therapy) [23], the biologically based environmental factors contributing to BD remain elusive. Discovery of such factors will facilitate development of novel, personalized, immune-based treatment strategies in this debilitating, life-threatening illness. Here, in cross sectional analyses of volunteers enrolled in a longitudinal study of bipolar disorder, we test our hypotheses that BD volunteers have higher Herpesviridae (e.g., CMV, HSV-1, and HSV-2) IgG concentrations compared to healthy control volunteers and that concentration of these antibodies is associated with common behavioral phenotypes in BD, elevated and/or depressed mood state(s).

2. Materials and Methods

The study was approved by the University of Michigan Investigational Review Board. Written informed consent was obtained from all study participants.

We randomly selected 238 volunteers between 18 and 65 years of age from the Prechter Bipolar Longitudinal Study (139 with BD-I and 99 healthy controls). Volunteers either met DSM IV [24] criteria for BD-I (at least one prior primary manic and/or mixed episode) (with or without comorbid substance use, other psychiatric disorders) or were healthy controls, without mental health diagnoses (on either axis I or axis II) [24]. DSM IV [24] diagnoses were assessed using the Diagnostic Inventory for Genetic Studies (DIGS) [25]. Following the diagnostic interview, all volunteers, provided they do not withdraw consent, remain in the Prechter Bipolar Longitudinal Study regardless of the diagnosis(es) determined. For the current study, we selected 238 volunteers who were actively participating in the Prechter Longitudinal Study. Prechter Bipolar Longitudinal Study volunteers routinely return to the research center for follow-up assessments, including longitudinal diagnostic confirmation and mood assessments. Upon their return to the center, subjects were chosen for the current study based on their availability/consent for blood sampling on a first come first serve basis and within a limited time

frame. All volunteers for the current study, including both healthy control volunteers and BD-I volunteers, were actively participating in the Prechter Bipolar Longitudinal Study.

Additional mood measures including Hamilton Depression Rating Scale (HDRS) [26] and Young Mania Rating Scale (YMRS) [27] were completed during assessments, consistent with time of blood sampling. The YMRS and HDRS were dichotomized into clinically elevated mood (YMRS > 7), clinically depressed mood (HDRS > 7), and clinically mixed mood (YMRS > 7 and HDRS > 7).

Overall, the mean age of volunteers was 36 ± 14 years of age. Study entry was not constrained by either body mass index (BMI) or sex. Based on evidence implicating their potential impact on immune functioning these variables were entered into analyses to test for individual effects of age, sex, or BMI on CMV IgG. Diagnostic breakdown of anthropometric and sociodemographic variables is included in Table 1. Whether or not patients were treated with psychotropic medications (i.e., lithium, lamotrigine, valproate, carbamazepine, atypical antipsychotic, or antidepressant medication) was included in analyses as a dichotomous variable, medication usage.

Whole venous blood was sampled following completion of psychiatric assessments at 1 PM (± 1 hour). Samples were centrifuged for 15 minutes at 4750 rpm and plasma was extracted and stored at -80°C . Serology assessments were performed at the Stanley Neurovirology Laboratory (Johns Hopkins University School of Medicine, Baltimore, MD). CMV IgG antibody concentrations were expressed quantitatively as both continuous (e.g., concentration) and dichotomous (e.g., seropositive, seronegative) measures, each derived via comparisons to standard samples run concurrently in each assay, as previously described [28]. Similar processes were completed for quantification of Herpes Simplex Virus Type 1 (HSV-1) and Herpes Simplex Virus Type 2 (HSV-2).

3. Data Analytic Plan

SPSS Statistics software version 21 (IBM Inc., Chicago, IL) was used to plot the data, rule out the presence of outliers, and perform additional statistical analyses. Medication usage (described above) was used to rule out overt effects of psychotropic medications on CMV IgG. Planned analyses included usage of independent samples T-tests to detect diagnostic differences in viral antibody concentrations (CMV, HSV-1, and HSV-2). Subsequent analyses used separate independent samples T-tests to identify whether CMV antibody concentrations were higher in BD-I volunteers in the midst of a mood episode (i.e., with or without a depressed mood episode, with or without a manic mood episode) while controlling for covariates (age, sex, race, and BMI). Subsequently, we used the Pearson chi-squared test, to show that CMV antibody status was similar in BD-I groups with and without current psychotropic treatment. Separate Spearman correlation analyses (and independent samples T-testing) tested for the presence of linear relationships between CMV antibody concentrations and either age, BMI, sex, or medication treatment, all factors potentially associated with

TABLE 1: Here we provide demographic and anthropometric information on study volunteers.

Diagnosis	Age	Sex		Race						Body mass index (BMI)
	Mean \pm SD (<i>n</i> = 237)	Male (<i>n</i> = 101)	Female (<i>n</i> = 137)	American Alaskan Native	Asian	Black African American	White Caucasian	More than one race	Unknown	Mean \pm SD (<i>n</i> = 130)
Bipolar disorder type I	39 \pm 13 (<i>n</i> = 138)	37% (<i>n</i> = 52)	63% (<i>n</i> = 87)	0.7% (<i>n</i> = 1)	2.9% (<i>n</i> = 4)	3.6% (<i>n</i> = 5)	87.1% (<i>n</i> = 121)	4.3% (<i>n</i> = 6)	1.4% (<i>n</i> = 2)	29 \pm 8 (<i>n</i> = 132)
Healthy control	32 \pm 14 (<i>n</i> = 99)	49% (<i>n</i> = 49)	51% (<i>n</i> = 50)	0% (<i>n</i> = 0)	12.1% (<i>n</i> = 12)	15.2% (<i>n</i> = 15)	68.7% (<i>n</i> = 68)	4.0% (<i>n</i> = 4)	0% (<i>n</i> = 0)	26 \pm 6 (<i>n</i> = 98)

Results are presented as mean \pm standard deviation for body mass index (BMI) and as percentages for other variables within each diagnostic group. These measures include age, sex, race, and body mass index.

TABLE 2: Here we provide clinical information on study volunteers.

Diagnosis	HDRS	YMRS	Medication use					
	Mean \pm SD (<i>n</i> = 235)	Mean \pm SD (<i>n</i> = 230)	AAP's	Antidepressant	Lithium	Valproate	Lamotrigine	Carbamazepine
Bipolar disorder type I	8.1 \pm 7.6 (<i>n</i> = 137)	3.6 \pm 5.6 (<i>n</i> = 134)	45%	50%	36%	20%	24%	5%
Healthy control	0.7 \pm 1.3 (<i>n</i> = 98)	0.1 \pm 0.3 (<i>n</i> = 96)	0%	0%	0%	0%	0%	0%

Results are presented as mean \pm standard deviation (and percentage of volunteers using a particular medication) within each diagnostic group. These measures include Hamilton 17-item Depression Rating Scale (HDRS), Young Mania Rating Scale (YMRS), and medication use including AAP's (atypical antipsychotics), antidepressants, lithium, valproate, lamotrigine, and carbamazepine.

variation in immune activation, to determine whether these factors were likely confounding our results. Data shown are corrected for multiple comparisons where indicated by " P_{corr} " using the Bonferroni technique [29] (uncorrected P -values are simply stated as " $P =$ "). Statistical significance for all analyses was set at $P = 0.05$.

4. Results

In total, plasma samples from 238 study volunteers were assayed for CMV, HSV-1, and HSV-2 antibodies. Of these 238 volunteers, 139 had a diagnosis of BD-I and 99 were healthy controls. Of the 139 BD-I volunteers, sixty-seven BD-I individuals (48%) exhibited evidence of clinically significant mood symptoms (e.g., depressed, elevated, and mixed). Sixty-one BD-I individuals (44%) had clinically significant depression, 22 BD-I individuals (16%) had clinically significant mood elevation, and 13 BD-I individuals (9%) had symptoms consistent with that of a mixed mood state. Mean values of behavioral measures of interest (e.g., YMRS, HDRS) are presented for each diagnostic study group (e.g., BD-I, healthy controls) in Table 2.

Using independent samples T-testing, we found that volunteers with a diagnosis of BD-I have significantly greater CMV IgG concentrations ($T_{234} = 3.1$; $P = 0.002$; $P_{\text{corr}} = 0.006$; mean difference 1.0 ± 0.3) as compared to healthy control volunteers. However, no diagnostic differences were identified with regard to HSV-1 IgG ($T_{236} = 0.15$; $P = 0.89$) and HSV-2 IgG ($T_{236} = 0.14$; $P = 0.89$). Graphical depiction of diagnostic differences in viral antibody concentrations is illustrated in Figure 1; statistical comparisons involving CMV IgG are outlined in Table 3.

Separate independent samples T-tests found that CMV IgG concentrations were higher in those BD-I volunteers in the midst of a clinically elevated mood state ($T_{132} = 2.2$; $P = 0.03$; mean difference 1.3 ± 0.6) but not significantly different in those BD-I volunteers in a clinically depressed mood state ($P > 0.10$) as compared to those study volunteers not in a clinically elevated mood state or clinically depressed mood state, respectively.

Results from individual Spearman correlational testing showed that CMV antibody concentrations were not significantly correlated with either body mass index ($P > 0.10$) or age ($P > 0.10$) in BD-I volunteers. Additionally in BD-I volunteers, using separate independent samples T-tests, we found that (1) CMV antibody concentrations were not significantly different in females as compared to males ($P > 0.10$) and (2) CMV antibody concentrations were not significantly different in volunteers who were being treated with psychotropic medications as compared to volunteers not receiving psychotropic medication treatment ($P > 0.10$).

5. Discussion

This study identified an association between concentrations of plasma CMV IgG antibodies and a diagnosis of BD-I, with BD-I individuals having significantly higher CMV concentrations than healthy control volunteers. Further, chi-squared testing described in Table 3 showed that CMV IgG seropositivity was associated with greater than 5 times increased likelihood of having a diagnosis of BD-I (Table 3). This finding aligns with previous findings of CMV IgG seropositivity in psychiatric disorders, specifically schizophrenia [14–18],

TABLE 3: Here we provide CMV IgG concentrations (reported as mean \pm standard deviation in column 2) for each diagnosis, BD-I and healthy controls.

Diagnosis	CMV IgG concentrations	Diagnostic comparison of CMV Antibody concentrations	CMV IgG seropositivity		Pearson chi-square Testing
	Mean \pm SD		Seronegative	Seropositive	
Healthy control volunteers	2.1 \pm 2.1 ($n = 99$)	$T_{234} = 3.1$ $P = 0.002$	$n = 59$	$n = 40$	Likelihood ratio = 5.2 $P = 0.02$
Bipolar disorder type I	3.0 \pm 2.7 ($n = 139$)		$n = 62$	$n = 77$	

Results of independent samples T-testing are reported in column 3. We identify significantly greater concentration of CMV IgG in BD-I volunteers as compared to healthy control volunteers ($T_{234} = 3.1$, $P_{\text{uncorr}} = 0.002$, and $P_{\text{corr}} = 0.006$). Chi-squared testing confirmed that CMV IgG seropositivity status was associated with 5.2 times greater likelihood of the presence of a diagnosis of BD-I ($P = 0.02$). Neither HSV-1 nor HSV-2 differed significantly between the BD-I and healthy control groups.

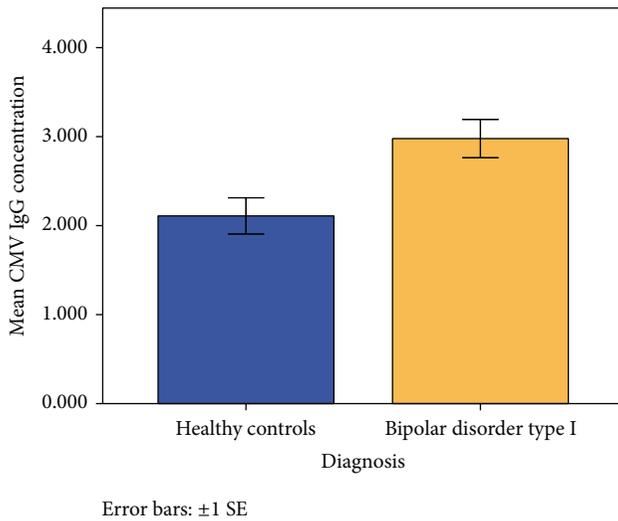


FIGURE 1: Graphical depiction of diagnostic differences in *Cytomegalovirus* (CMV) antibody concentrations. Standardized mean antibody (IgG) concentrations are depicted on the vertical, y -axis and diagnosis on the horizontal x -axis. CMV IgG concentration was higher in BD-I volunteers (shown in maize color) as compared to healthy control volunteers (shown in blue color) ($T_{234} = 3.1$; $P_{\text{uncorr}} = 0.002$; $P_{\text{corr}} = 0.006$). No diagnostic differences were identified with regard to HSV-1 IgG ($T_{236} = 0.15$; $P = 0.89$) and HSV-2 IgG ($T_{236} = 0.14$; $P = 0.89$). Error bars represent ± 1 standard error.

and supports the hypothesis that exposure to environmental/infectious factors like viruses may contribute to the pathophysiology of BD-I. While recent evidence showed that passage of maternal CMV antibodies to the neonate in expecting mothers was not shown to pose significant risk of BD-I in the neonate [30], results we present of associations between CMV antibody concentrations, diagnosis of BD-I, and elevated mood state warrant further investigation and clarification on questions of causality. Approximately 50% of Americans are seropositive for CMV [31]. Initial exposure to infectious agents like CMV induces an immune response [32], memory of the infection persisting in the form of CMV IgG antibodies. Subsequently, CMV persists in a latent state in immature cells [33]. Exposure to psychosocial stress can potentially down-regulate cellular immune responsivity [34–36] reactivating

otherwise latent herpesviruses (e.g., CMV) [37], inducing herpesvirus (e.g., CMV) antibodies [5]. Taken together with the findings we report this evidence suggests that treatment of ubiquitous, asymptomatic herpesvirus infections or targeting their downstream counterparts (e.g., TNF- α) could potentially impact the BD-I illness. However, much further testing on expanded, longitudinal BD-I samples is required to test these hypotheses. Separate evidence does show that elevation of soluble CMV antigens and CMV antibody concentrations is associated with a shift towards CD8 $^{+}$ T-cell production [32] and subsequent induction of CD8 $^{+}$ derived proinflammatory cytokines (e.g., TNF- α , IL-6, and IFN- γ) [7, 38, 39]. Further, enhanced immune activation involving elevated concentrations of these cytokines has been identified in BD volunteers [40] and phasic variation in common clinical features of BD (e.g., depression, mania, suicidality, etc.) has also been associated with particular inflammatory cytokine profiles [41]. Induction of TNF- α has been shown to modulate neurotransmitter metabolism via activation of indoleamine 2,3-dioxygenase (IDO), subsequently reducing neurotransmitter precursors (i.e., tryptophan), and shifting the balance towards production of potentially neurotoxic metabolites (i.e., hydroxykynurenine) [42–45]. The resulting alteration of neurotransmitter metabolism is believed to directly impact central processing of emotionally salient and stressful events, resulting in altered behavioral response to stress [46–51].

In summary, these results augment accumulating evidence suggesting that exposure to Herpesviridae in general (and CMV in particular), its subsequent acquired immune response, and the impact of psychological stressors on immune reactivation may pose a risk of BD-I, potentially via an impact on development or exacerbation of elevated mood states. However, the cross sectional nature of analyses involving CMV in our study limits the extent that causal inferences can be drawn. Potentially, the means by which episodic inflammatory alterations contribute to episodic clinical features in BD could be ascribed to this model but further research in this area is required to discern the exact mechanisms underlying CMV's relationship to clinical mood states in BD-I. Additionally, while we found no significant differences in CMV antibody concentrations when comparing BD-I volunteers receiving pharmacotherapy against those not receiving pharmacotherapy, it remains as a possibility

that interindividual variation in specific psychiatric medication used (and/or specific dosage prescribed) could be confounding the results. Future studies that include designs with an expanded population of BD-I volunteers will be better positioned to test for the presence of medication specific effects on CMV antibody concentrations.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

- [1] K. R. Merikangas, H. S. Akiskal, J. Angst et al., "Lifetime and 12-month prevalence of bipolar spectrum disorder in the national comorbidity survey replication," *Archives of General Psychiatry*, vol. 64, no. 5, pp. 543–552, 2007.
- [2] K. R. Merikangas, R. Jin, J.-P. He et al., "Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative," *Archives of General Psychiatry*, vol. 68, no. 3, pp. 241–251, 2011.
- [3] A. Schmitt, B. Malchow, A. Hasan, and P. Falkai, "The impact of environmental factors in severe psychiatric disorders," *Frontiers in Neuroscience*, no. 8, article 19, 2014.
- [4] S. E. Keller, J. M. Weiss, S. J. Schleifer, N. E. Miller, and M. Stein, "Stress-induced suppression of immunity in adrenalectomized rats," *Science*, vol. 221, no. 4617, pp. 1301–1304, 1983.
- [5] S. Prösch, C. E. C. Wendt, P. Reinke et al., "A novel link between stress and human cytomegalovirus (HCMV) infection: sympathetic hyperactivity stimulates HCMV activation," *Virology*, vol. 272, no. 2, pp. 357–365, 2000.
- [6] S. J. Schleifer, S. E. Keller, M. Camerino, J. C. Thornton, and M. Stein, "Suppression of lymphocyte stimulation following bereavement," *The Journal of the American Medical Association*, vol. 250, no. 3, pp. 374–377, 1983.
- [7] J. Jelinek, I. Adkins, Z. Mikulkova et al., "In vitro activation of CMV-specific human CD8⁺ T cells by adenylate cyclase toxoids delivering pp65 epitopes," *Bone Marrow Transplantation*, vol. 47, no. 2, pp. 243–250, 2012.
- [8] D. Barzman, J. Eliassen, R. McNamara et al., "Correlations of inflammatory gene pathways, corticolimbic functional activities, and aggression in pediatric bipolar disorder: a preliminary study," *Psychiatry Research*, vol. 224, no. 2, pp. 107–111, 2014.
- [9] I. G. Barbosa, C. R. C. Nogueira, N. P. Rocha et al., "Altered intracellular signaling cascades in peripheral blood mononuclear cells from BD patients," *Journal of Psychiatric Research*, vol. 47, no. 12, pp. 1949–1954, 2013.
- [10] S. M. O'Brien, P. Scully, L. V. Scott, and T. G. Dinan, "Cytokine profiles in bipolar affective disorder: focus on acutely ill patients," *Journal of Affective Disorders*, vol. 90, no. 2-3, pp. 263–267, 2006.
- [11] E. Brietzke and F. Kapczinski, "TNF- α as a molecular target in bipolar disorder," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 32, no. 6, pp. 1355–1361, 2008.
- [12] F. B. Dickerson, J. J. Boronow, C. Stallings et al., "Infection with herpes simplex virus type 1 is associated with cognitive deficits in bipolar disorder," *Biological Psychiatry*, vol. 55, no. 6, pp. 588–593, 2004.
- [13] M. M. Prossin A, A. Anand, M. Heitzeg, and J.-K. Zubieta, "Tackling the Kraepelinian Dichotomy: a neuroimaging review," *Psychiatric Annals*, vol. 40, pp. 162–167, 2010.
- [14] J. J. Kim, B. H. Shirts, M. Dayal et al., "Are exposure to cytomegalovirus and genetic variation on chromosome 6p joint risk factors for schizophrenia?" *Annals of Medicine*, vol. 39, no. 2, pp. 145–153, 2007.
- [15] F. M. Leweke, C. W. Gerth, D. Koethe et al., "Antibodies to infectious agents in individuals with recent onset schizophrenia," *European Archives of Psychiatry and Clinical Neuroscience*, vol. 254, no. 1, pp. 4–8, 2004.
- [16] B. H. Shirts, J. J. Kim, S. Reich et al., "Polymorphisms in MICB are associated with human herpes virus seropositivity and schizophrenia risk," *Schizophrenia Research*, vol. 94, no. 1–3, pp. 342–353, 2007.
- [17] B. H. Shirts, K. M. Prasad, M. F. Pogue-Geile, F. Dickerson, R. H. Yolken, and V. L. Nimgaonkar, "Antibodies to cytomegalovirus and Herpes Simplex Virus 1 associated with cognitive function in schizophrenia," *Schizophrenia Research*, vol. 106, no. 2-3, pp. 268–274, 2008.
- [18] E. F. Torrey, M. F. Leweke, M. J. Schwarz et al., "Cytomegalovirus and schizophrenia," *CNS Drugs*, vol. 20, no. 11, pp. 879–885, 2006.
- [19] R. S. Lazarus, "From psychological stress to the emotions: a history of changing outlooks," *Annual Review of Psychology*, vol. 44, no. 1, pp. 1–21, 1993.
- [20] A. Caspi and T. E. Moffitt, "Gene-environment interactions in psychiatry: joining forces with neuroscience," *Nature Reviews Neuroscience*, vol. 7, no. 7, pp. 583–590, 2006.
- [21] T. E. Moffitt, A. Caspi, and M. Rutter, "Strategy for investigating interactions between measured genes and measured environments," *Archives of General Psychiatry*, vol. 62, no. 5, pp. 473–481, 2005.
- [22] C. L. Ehlers, E. Frank, and D. J. Kupfer, "Social zeitgebers and biological rhythms: a unified approach to understanding the etiology of depression," *Archives of General Psychiatry*, vol. 45, no. 10, pp. 948–952, 1988.
- [23] H. A. Swartz, E. Frank, K. O'Toole et al., "Implementing interpersonal and social rhythm therapy for mood disorders across a continuum of care," *Psychiatric Services*, vol. 62, no. 11, pp. 1377–1380, 2011.
- [24] D.-I. Apatfo, *Diagnostic and Statistical Manual of Mental Disorders*, text revision, New York, NY, USA, 4th edition, 2000.
- [25] J. I. Nurnberger Jr., M. C. Blehar, C. A. Kaufmann et al., "Diagnostic interview for genetic studies: rationale, unique features, and training," *Archives of General Psychiatry*, vol. 51, no. 11, pp. 849–859, 1994.
- [26] M. Hamilton, "Development of a rating scale for primary depressive illness," *British Journal of Social and Clinical Psychology*, vol. 6, no. 4, pp. 278–296, 1967.
- [27] R. C. Young, J. T. Biggs, V. E. Ziegler, and D. A. Meyer, "A rating scale for mania: reliability, validity and sensitivity," *The British Journal of Psychiatry*, vol. 133, no. 11, pp. 429–435, 1978.

- [28] F. B. Dickerson, J. J. Boronow, C. R. Stallings, A. E. Origoni, and R. H. Yolken, "Reduction of symptoms by valacyclovir in cytomegalovirus-seropositive individuals with schizophrenia," *The American Journal of Psychiatry*, vol. 160, no. 12, pp. 2234–2236, 2003.
- [29] C. E. Bonferroni, "Teoria statistica delle classi e calcolo delle probabilità," *Pubblicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze*, vol. 8, pp. 3–62, 1936.
- [30] P. B. Mortensen, C. B. Pedersen, J. J. McGrath et al., "Neonatal antibodies to infectious agents and risk of bipolar disorder: a population-based case-control study," *Bipolar Disorders*, vol. 13, no. 7–8, pp. 624–629, 2011.
- [31] S. L. Bate, S. C. Dollard, and M. J. Cannon, "Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988–2004," *Clinical Infectious Diseases*, vol. 50, no. 11, pp. 1439–1447, 2010.
- [32] P. Moss and N. Khan, "CD8⁺ T-cell immunity to cytomegalovirus," *Human Immunology*, vol. 65, no. 5, pp. 456–464, 2004.
- [33] T. Crough and R. Khanna, "Immunobiology of human cytomegalovirus: from bench to bedside," *Clinical Microbiology Reviews*, vol. 22, no. 1, pp. 76–98, 2009.
- [34] M. T. Caserta, T. G. O'Connor, P. A. Wyman et al., "The associations between psychosocial stress and the frequency of illness, and innate and adaptive immune function in children," *Brain, Behavior, and Immunity*, vol. 22, no. 6, pp. 933–940, 2008.
- [35] R. Glaser and J. Kiecolt-Glaser, "How stress damages immune system and health," *Discovery Medicine*, vol. 5, no. 26, pp. 165–169, 2005.
- [36] R. Glaser, B. Rabin, M. Chesney, S. Cohen, and B. Natelson, "Stress-induced immunomodulation: implications for infectious diseases?" *The Journal of the American Medical Association*, vol. 281, no. 24, pp. 2268–2270, 1999.
- [37] R. Glaser, S. B. Friedman, J. Smyth et al., "The differential impact of training stress and final examination stress on herpesvirus latency at the United States Military Academy at West Point," *Brain, Behavior, and Immunity*, vol. 13, no. 3, pp. 240–251, 1999.
- [38] Y. J. Tang-Feldman, G. R. Lochhead, S. R. Lochhead, C. Yu, and C. Pomeroy, "Interleukin-10 repletion suppresses pro-inflammatory cytokines and decreases liver pathology without altering viral replication in murine cytomegalovirus (MCMV)-infected IL-10 knockout mice," *Inflammation Research*, vol. 60, no. 3, pp. 233–243, 2011.
- [39] S. Varani, G. Rossini, A. Mastroianni et al., "High TNF-alpha and IL-8 levels predict low blood dendritic cell counts in primary cytomegalovirus infection," *Journal of Clinical Virology*, vol. 53, no. 4, pp. 360–363, 2012.
- [40] S. Hope, I. Melle, P. Aukrust et al., "Similar immune profile in bipolar disorder and schizophrenia: selective increase in soluble tumor necrosis factor receptor I and von Willebrand factor," *Bipolar Disorders*, vol. 11, no. 7, pp. 726–734, 2009.
- [41] A. Ortiz-Domínguez, M. E. Hernández, C. Berlanga et al., "Immune variations in bipolar disorder: phasic differences," *Bipolar Disorders*, vol. 9, no. 6, pp. 596–602, 2007.
- [42] A. H. Miller, "Mechanisms of cytokine-induced behavioral changes: psychoneuroimmunology at the translational interface," *Brain, Behavior, and Immunity*, vol. 23, no. 2, pp. 149–158, 2009.
- [43] A. H. Miller, V. Maletic, and C. L. Raison, "Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression," *Biological Psychiatry*, vol. 65, no. 9, pp. 732–741, 2009.
- [44] S. Okuda, N. Nishiyama, H. Saito, and H. Katsuki, "Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 22, pp. 12553–12558, 1996.
- [45] S. Okuda, N. Nishiyama, H. Saito, and H. Katsuki, "3-Hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity," *Journal of Neurochemistry*, vol. 70, no. 1, pp. 299–307, 1998.
- [46] L. Brydon, N. A. Harrison, C. Walker, A. Steptoe, and H. D. Critchley, "Peripheral inflammation is associated with altered substantia nigra activity and psychomotor slowing in humans," *Biological Psychiatry*, vol. 63, no. 11, pp. 1022–1029, 2008.
- [47] L. Capuron, G. Pagnoni, M. Demetrashvili et al., "Anterior cingulate activation and error processing during interferon-alpha treatment," *Biological Psychiatry*, vol. 58, no. 3, pp. 190–196, 2005.
- [48] N. A. Harrison, L. Brydon, C. Walker, M. A. Gray, A. Steptoe, and H. D. Critchley, "Inflammation causes mood changes through alterations in subgenual cingulate activity and mesolimbic connectivity," *Biological Psychiatry*, vol. 66, no. 5, pp. 407–414, 2009.
- [49] N. A. Harrison, L. Brydon, C. Walker et al., "Neural origins of human sickness in interoceptive responses to inflammation," *Biological Psychiatry*, vol. 66, no. 5, pp. 415–422, 2009.
- [50] A. R. Prossin, A. E. Koch, P. L. Campbell, M. G. McInnis, S. S. Zalcman, and J.-K. Zubieta, "Association of plasma interleukin-18 levels with emotion regulation and μ -opioid neurotransmitter function in major depression and healthy volunteers," *Biological Psychiatry*, vol. 69, no. 8, pp. 808–812, 2011.
- [51] C. L. Raison, R. Dantzer, K. W. Kelley et al., "CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN- α : relationship to CNS immune responses and depression," *Molecular Psychiatry*, vol. 15, no. 4, pp. 393–403, 2010.

Review Article

The Use of Cannabis as a Predictor of Early Onset of Bipolar Disorder and Suicide Attempts

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Introduction. Bipolar disorder (BD) implies risk of suicide. The age at onset (AAO) of BD carries prognostic significance. Substance abuse may precede the onset of BD and cannabis is the most common illicit drug used. The main goal of this study is to review the association of cannabis use as a risk factor for early onset of BD and for suicide attempts. *Materials and Methods.* PubMed database was searched for articles using key words “bipolar disorder,” “suicide attempts,” “cannabis,” “marijuana,” “early age at onset,” and “early onset.” *Results.* The following percentages in bipolar patients were found: suicide attempts 3.6–42%; suicide attempts and substance use 5–60%; suicide attempts and cannabis use 15–42%. An early AAO was associated with cannabis misuse. The mean age of the first manic episode in individuals with and without BD and cannabis use disorder (CUD) was 19.5 and 25.1 years, respectively. The first depressive episode was at 18.5 and 24.4 years, respectively. Individuals misusing cannabis showed increased risk of suicide. *Discussion.* Cannabis use is associated with increased risk of suicide attempts and with early AAO. However, the effect of cannabis at the AAO and suicide attempts is not clear.

1. Introduction

Bipolar disorder (BD) is associated with poor health outcomes and is responsible for the highest rate of suicide among all mental disorders [1]. Bipolar disorder is often complicated by cooccurring substance use disorders [1]. Cannabis is the most common illicit substance used among individuals with bipolar disorder [2] and up to 38% of the individuals with bipolar disorder misuse it [3]. Cannabis abuse has particularly been reported to be high among young bipolar patients [4], and chronic cannabis use is associated with higher severity of illness and greater noncompliance to treatment among individuals with bipolar disorder [5].

In bipolar disorder, there is evidence that, with many patients, substance abuse precedes the onset of BD, and it has been suggested that affective deregulation may increase the risk of bipolar disorder due to substance abuse [6]. The relationship between substance abuse and age at onset of bipolar disorder is not really well understood [7]. Among individuals with cooccurring CUD, age at onset of bipolar disorder was 6 years lower and the mean number of manic, hypomanic, and depressive episodes per year was greater compared to individuals without CUD [8].

Lev-Ran et al. [9] also reported that cannabis use may decrease the age at onset in both schizophrenia and BD. This is consistent with the view that cannabis use may

unmask a preexisting genetic liability that is partly shared between patients with schizophrenia and bipolar disorder. The reduction was 9 years of the age at onset in bipolar group [9]. Lagerberg et al. [8] found a significant association indicating a dose-response relationship between cannabis use and age at onset, which remained statistically significant after controlling for possible confounders (gender, bipolar subtype, family history of severe mental illness, and alcohol or other substance use disorders). The mean difference in age at onset between the groups with and without cannabis use disorder in that study was 5 years.

It is important to review the definitions of age at onset, since these definitions are controversial in the psychiatric literature. Patients displaying bipolar disorder at a younger age at onset have higher prevalence rates of psychotic symptoms, substance abuse comorbidity, learning disabilities, and episodes of rapid cycling of mood [10, 11]. Moreover, there is an increased risk factor for suicide attempts [12].

Among common mental disorders, BD implies a particular risk of both nonfatal self-harm and completed suicide [13]. The risk of suicide in bipolar patients is 20–30 times higher than that of the general population [14]. The risk is greater among those who have been admitted to inpatient care due to bipolar disorder [15] and especially high in bipolar patients admitted to inpatient care after suicide attempts [16].

The main aims of this study are to review (1) the definition of the age at onset of BD, (2) the association of cannabis use as a risk factor for early onset of BD, and (3) the relationship of cannabis use and suicidal behavior in BD.

2. Materials and Methods

Between June and September 2014, PubMed database was searched for articles using combinations of the following key words: “bipolar disorder,” “suicide,” “suicide attempts,” “cannabis,” “marijuana,” “early age at onset,” and “early onset.” No language or publication time constraints were applied. PubMed database indexes articles published since 1948 up to the present date. Data specifically related to bipolar disorder were chosen. Duplicates and repetitive reviews were excluded. In the cases of similar studies performed by the same group, the study with bigger sample size was included in this review. The “related articles” function of the PubMed database, the reference list of selected articles, conference abstracts, and Google Scholar were also used to identify additional articles. This review follows the PRISMA guidelines.

The entry criteria of the articles included in this review should meet at least one of the following requirements: (1) the selected study should provide data that allowed evaluating the age at onset of bipolar disorder; (2) the paper should present the proportion of bipolar patients who used cannabis; (3) patients included in these studies should have had an early age at onset of BD or suicide attempts.

3. Results

Initially, for the keywords “early onset,” “bipolar disorder,” 1,017 articles were found; for “cannabis” and “bipolar

disorder,” 143 articles; for “suicide attempts” and “bipolar disorder,” 1,069; for “suicide” and “bipolar disorder,” 2,289; for “marijuana” and “bipolar disorder,” 190; and for “early age at onset” and “bipolar disorder,” 626. 59 duplicates or repetitive reviews were excluded. Of all articles reviewed, 77 papers (all of them published) fulfilled the entry criteria and were included, as shown in the Table 1. They were published between 1994 and 2014, 40 of them (51%) in the last 5 years.

3.1. Definition of Early Age at Onset of Bipolar Disorder. The age at which the first bipolar episode occurs is relevant because an early age is associated with poor prognosis [23]. If an early diagnosis of BD is not established, the treatment starts late, compromising its good outcome [34]. Subgroups defined as “early onset” and “very early onset” were associated with greater rates of comorbid anxiety disorders and substance abuse, violent behavior, rapid cycling, and shorter periods of euthymia compared to the “late onset” subgroup [23].

The DSM-IV suggests the age of 21, supported by genetic studies, as the maximum age to be included as the early age at onset of BD [35]. An epidemiological study with 61,392 community adults in 11 countries found that the mean age at onset of BD type I was 18.4 (± 0.7) years, BD type II was 20.0 (± 0.6) years, and subthreshold BD was 21.9 (± 0.4) years [36]. Other studies described different definitions of early age at onset, being <21 years the most common age defined as “early onset,” as shown in Table 2.

Table 2 shows chronological studies with different definitions of early and late ages at onset. The following variables are described: year of publication; sample size; study design; and ages at very early, early, intermediate, and late onset. Sample sizes ranged from 169 to 1,856. Only one article establishes very early age at onset as <13 years. The definition of early age ranged 17.4–21, while late age ranged 18–40.

In a study comparing a sample of early ($n = 58$) and late onset ($n = 39$) bipolar patients, the early onset group had a more severe form of the disorder with more psychotic features, mixed episodes, comorbid panic disorder, and poorer response to lithium. The early onset group was defined as younger than 18 and late onset as older than 40 [10].

Another paper comprised 52 (50 BD type I, 2 BD type II) patients who had an early onset and 38 (30 BD type I, 8 BD type II) a late onset. It was observed that the early onset group is characterized by a higher frequency of psychotic symptoms as compared to patients with late onset [17].

A sample of 320 individuals diagnosed with BD I or II was stratified into subjects with early age (≤ 18 years) and late (>18 years) age at onset of BD. A significant earlier age at onset in subjects with anxiety disorders and rapid cycling course was found. When clinical characteristics between earlier and later onset of BD were compared, subjects with early AAO had more frequent suicidal ideation/attempts, axis I comorbidity, substance use disorders, and rapid cycling course. The odds ratios associated with these variables were 1.4 (suicide ideation), 1.6 (axis I comorbidity), 1.4 (substance abuse), and 2.0 (rapid cycling course) [18].

A study with 368 patients investigated the cut-off in the age at onset in three subgroups (early, intermediate, and late).

TABLE 1: Flowchart—selection of articles (see Figure 1).

Initial search					
“Early age at onset” and “bipolar disorder”	“Early onset” and “bipolar disorder”	“Suicide attempts” and “bipolar disorder”	“Suicide” and “bipolar disorder”	“Marijuana” and “bipolar disorder”	“Cannabis” and “bipolar disorder”
626	1,017	1,069	2,289	190	143
Inclusion of the articles fulfilling the entry criteria* (at least one)					
“Early age at onset” or “early onset” and “bipolar disorder”	“Suicide attempts” or “suicide” and “bipolar disorder”	“Marijuana” or “cannabis” and “bipolar disorder”			
51	44	59			
Exclusion of repetitive and duplicate articles					
17	13	19			
Final analysis					
34	31	40			

* (1) The selected study should provide data that allowed evaluating the age at onset of bipolar disorder.
 (2) The paper should present the proportion of bipolar patients who used cannabis.
 (3) Patients included in these studies should have had an early age at onset of BD or suicide attempts.

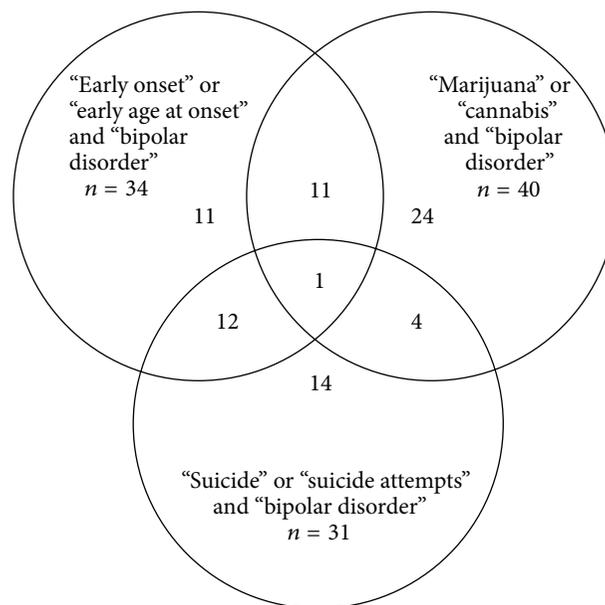


FIGURE 1

The mean age in each group was estimated to be 17.4 (SD = 2.3), 25.1 (SD = 6.2), and 40.4 years (SD = 11.3) [19].

A sample of 1,000 adults with bipolar disorder was divided into three groups: very early (<13 years), early (13–18 years), and late (>18 years) onset of mood symptoms and it resulted in 983 patients whose age at onset could be determined: 272 (27.7%) had very early onset and 370 (37.6%) experienced early onset. Early onset again was associated with higher rates of comorbid anxiety disorders and substance abuse, more recurrences, shorter periods of euthymia, greater likelihood of suicide attempts, and violence, suggesting a more severe course of disease in terms of chronicity and comorbidity [20].

An article reported the age at onset of 211 families with BD type I probands. Part of the probands was analyzed to determine the age at onset distribution. They divided

the analysis into two variables: “early-onset” subgroup as ≤21 years and “late onset” subgroup as >21 years. Clinical features, such as comorbid substance abuse, rapid cycling, suicidality, and increased episode frequency, were correlated with the “early onset” group [21].

History of high depressive recurrence (without history of mania/hypomania) has been proposed as a mood subtype close to bipolar disorders. A study with 224 outpatients diagnosed with Major Depressive Disorder and with 336 outpatients with BD type II was conducted on such putative bipolar validators (early age at onset, high recurrence, mixed depression, and bipolar family history) as early age at onset of first major depressive episode (before 21 years). Early onset was the only variable which identified a major depressive disorder subgroup significantly associated with all bipolar

TABLE 2: Definitions of age at onset of bipolar disorder.

Study	Design	Sample (bipolar patients) ^a	Very early age at onset (in years)	Early age at onset (in years)	Intermediate age at onset (in years)	Late age at onset (in years)
Schürhoff et al., 2000 ^b [10]	Cross-sectional	210	—	<18	—	>40
Schulze et al., 2002 ^b [17]	Cross-sectional	169	—	≤20	—	≥35
Post et al., 2003 ^b [18]	Cross-sectional	320 (202 female; 118 male)	—	≤18	—	>18
Bellivier et al., 2003 ^c [19]	Cross-sectional	368	—	Median age: 17.4	Median age: 25.1	Median age: 40.4
Perlis et al., 2004 ^c [20]	Cross-sectional	1,000	<13	13–18	—	>18
Lin et al., 2006 ^c [21]	Cross-sectional	1,856 (211 probands)	—	≤21 Mean age: 16.6	22–28 Mean age: 26	>28 Mean age: 34.7
Benazzi and Akiskal, 2008 [22]	Cross-sectional	560 ^d	—	<21	—	>21
Hamshere et al., 2009 ^c [23]	Cross-sectional	1,369	—	Limit: <22 mean age: 18.7 ± 3.7	Limit: 25–37 mean age: 28.3 ± 5.5	Limit: >40 mean age: 43.3 ± 9.1
Etain et al., 2012 ^c [3]	Cross-sectional	652	—	<21	—	≥21

(based on Hamshere et al. 2009 [23]).

^aSome studies do not classify the patients whose diagnosis was included.

^bAge of both bipolar disorder (BD) type I and BD type II.

^cAge of BD type I.

^d336 BD type II and 224 unipolar major depressive disorder.

validators. This major depressive disorder subgroup was similar to BD type II in age at onset and bipolar family history and had a high frequency of mixed depression [22].

A sample of 1,369 BD type I patients was divided into three subgroups: early onset as <22 years; intermediate onset as 25–37 years; and late onset as >40 years. The early onset subgroup had more rapid cycling, family history for affective disorders, and episodes of depression and mania when compared to other subgroups [23].

A study with two independent samples from France ($n = 480$) and from the United States ($n = 714$) was carried out with BD type I patients. Early age at onset was defined as <21 years. A correlation of early age at onset, substance use, and suicidal behavior was found [3].

3.2. Cannabis Use as a Risk Factor for Early Onset of BD.

There is no consensus about the criteria of frequency or quantity of cannabis use in BD to establish the definition of abuse, as shown in Table 3. Criteria range from the number of joints per day in the last 12 months [8] to the qualification of “heavy consumers” (several times a day) [9]. The frequency of use was adopted as a parameter in two studies, but with different approaches. While Henquet et al. [24] stratified the cannabis use into daily, weekly, and monthly frequencies, Tijssen et al. [25] classified, in their sample, all patients who had made the use of cannabis five times or more in their lifetime.

Table 3 shows the criteria adopted in several studies to establish the frequency of cannabis use. The following variables are described: year of publication, study design, follow-up period, measures, comparisons, and frequency of cannabis use. Sample sizes ranged from 151 to 4,815.

A study with a sample of 4,815 individuals between 18 and 64 years old was examined, using the Composite International Diagnostic Interview, and it was found that cannabis use can trigger manic symptoms independently of age, ethnicity, neuroticism, sex, educational level, marital status, use of other drugs or alcohol. Frequency of use increases the risk. Patients who used it 3–4 days per week were more likely to manifest manic symptoms than those who used it less frequently [24].

A study in a prospective cohort of 705 adolescents followed up during 8 years analyzed the association between risk factors, such as cannabis use, and the manifestation of manic or depressive symptoms in bipolar patients and found that cannabis use was involved with manic manifestation [25].

A sample with 766 patients between 16 and 65 years old, 676 of them diagnosed with schizophrenia and 90 with BD, was interviewed and cannabis use was linked to early age at onset of schizophrenia and BD [9]. Another study investigated 151 patients with BD types I and II who were in psychiatric treatment and it was observed that excessive cannabis use was related to early onset of BD and that it had been used at an earlier age than the excessive use of alcohol [26].

TABLE 3: Frequency of use of cannabis in bipolar disorder.

Study	Design	Sample (<i>n</i>)	Follow-up (in years)	Measures	Comparisons	Frequency of cannabis use
Henquet et al., 2006 [24]	Cohort	4,815 individuals ^a 18–64 years	3	Composite International Diagnostic Interview (CIDI)	The baseline cannabis use was assessed with the occurrence of mania in the follow-up	Less than once a month; 1–3 days/month; 1–2 days/week; 3–4 days/week and nearly every day.
Tijssen et al., 2010 [25]	Cohort	705 patients 14–24 years	8	Munich-Composite International Diagnostic Interview (M-CIDI)	The onset of manic/depressive symptoms was assessed with the following risk factors (a family history of mood disorders, trauma, substance use, attention-deficit/hyperactivity disorder (ADHD), and temperamental/personality traits)	Lifetime cannabis use was considered in case they reported at baseline that they had used cannabis five times or more
de Hert et al., 2011 [7]	Cross-sectional	766 patients (676 with schizophrenia and 90 with bipolar disorder) 16–65 years	—	Composite International Diagnostic Interview (CIDI), Clinical Global Impression (CGI), and Global Assessment of Functioning (GAF)	A linear regression between the age at onset was done considering the following variables: cannabis use, diagnosis, and gender	Used CIDI (Composite International Diagnostic Interview) for lifetime substance use and classified patients as “heavy users” when consumption was several times a day.
Lagerberg et al., 2011 [26]	Cross-sectional	151 bipolar patients (91 BD I and 60 BD II)	—	Clinical assessments carried out by trained clinical psychologists and psychiatrists	The bivariate analyses revealed significant correlations between age at onset and gender, age, BD type, excessive cannabis use, and sequencing	Patients who met DSM-IV criteria for substance use disorder or had predominant weekly use of cannabis for a period of 4 years from 11–15 years, 16–20 years, 21–27 years, 28–44 years, 45–60 years, and 60 years and more were considered “excessive cannabis use”
LevRan et al., 2013 [9]	Cross-sectional	1,905 bipolar individuals	—	Alcohol use disorder and associated disabilities interview schedule	Rates of CUD in the past 12 months were 7.2%, compared to 1.2% in the general population. Logistic regression models adjusting for sociodemographic variables indicated that cooccurring CUD was at increased risk for nicotine dependence, alcohol and drug use disorders, and antisocial personality disorder compared to those without CUD.	Number of joints consumed with the number of days when cannabis was used in the last 12 months. Frequency was defined as ranging from “almost daily” to “once a year.”

^aThe sample was found to be representative of the Dutch population in terms of gender, marital status and level of urbanisation, with the exception of a slight under-representation of individuals in the age group 18–24 years.

Data from the National Epidemiological Survey of Alcohol and Related Conditions (NESARC Wave 1, 2001-2002) were analyzed and they showed that 1,905 patients were diagnosed with bipolar disorder and 7.2% of these were identified with cannabis use disorder (CUD). The prevalence of CUD in the general population is 1.2%. It was observed that the cooccurrence of BD and CUD was a risk factor for alcohol, nicotine, and other drug dependence [8].

In a study involving approximately 2,000 individuals (471 bipolar individuals and 1,761 controls), bipolar patients were 6.8 times more likely to report cannabis use during their lifetime. Almost 30% of the BD group that had made use of cannabis at least once fulfilled the criteria for CUD of DSM-IV. Individuals with BD and CUD were 1.8 times more likely to have disability due to the disorder than those with the diagnosis of BD that had no history of CUD, even after controlling sociodemographic variables, substance use, and psychiatric covariates. Individuals with BD and CUD had more mixed episodes and a higher probability of suicide attempts than those with BD but without CUD [37].

In a study selecting adult patients from France and the United States that met DSM-IV criteria for BD type I, the age at onset was classified into two subgroups: early age at onset (<21 years) and late age at onset (≥ 21 years). They analyzed the association of clinical and demographic variables with age at onset and polarity. The relationships between “age at onset,” “alcohol/drug misuse,” and “suicidal behavior” are the most important in both samples: early age at onset was associated with suicidal behavior (France: OR = 2.16 95% CI (1.48–3.15) Sensibility = 35%, Specificity = 46%; USA: OR = 2.05 95% CI (1.44–2.92) Sensibility = 27%, Specificity = 54%) and lifetime cannabis misuse (France: OR = 2.60 95% CI (1.51–4.48) Sensibility = 9%, Specificity = 79%; USA: OR = 1.75 95% CI (1.02–3.01) Sensibility = 30%, Specificity = 59%) [3].

A cross-sectional study was conducted on a population-based national representative sample, the National Epidemiological Survey of Alcohol and Related Conditions (NESARC). Individuals with lifetime prevalence of BD ($n = 1,905$) were analyzed regarding sociodemographic characteristics and prevalence of comorbid psychiatric disorder among BD patients with and without CUD (in the last 12 months). They found among BD patients with CUD ($n = 119$) an earlier age at onset of the first manic or hypomanic episode (mean age = 19.5 years) and of the first depressive episode (mean age = 18.5 years) compared to patients without CUD ($n = 1,786$), whose mean ages were 25.1 years and 24.4 years, respectively ($P < 0.0001$). In this study, CUD was associated with earlier onset of bipolar disorder and greater number of depressive and hypomanic/manic episodes [8].

A secondary analysis of data collected by the Netherlands Mental Health Survey and Incidence Study (NEMESIS)—a longitudinal study with three measurements at 1996, 1997, and 1999 (follow-up)—of the Dutch adult population (18–64 years) was conducted. At baseline, 7,076 individuals were interviewed, and, in the last follow-up, 4,848 participants were interviewed. To investigate if cannabis use predicted the first episodes of mood and anxiety disorders, the analyses were made with 3,881 people who had had no lifetime mood disorder and with 3,854 people who had had no lifetime

anxiety disorder. It was found that cannabis was a predictor of subsequent mood episodes, especially bipolar disorder, and that cannabis use was associated with increasing the risk of BD (OR = 7.6; $P < 0.001$) [38].

A paper reported substantial evidence for phenotypic and genetic overlap between schizophrenia and BD. An earlier onset was found in patients that used cannabis (676 schizophrenia patients and 90 BD patients). The reduction was 9 years in the age at onset in the bipolar group [9].

In a sample of 151 BD types I and II, cannabis use was associated with an earlier onset of BD independently of the history of psychosis or polarity of the first episode [26], not only manic or psychotic, as described by Henquet et al. [24] and Öngür et al. [39]. It means that cannabis increases bipolar disorder prevalence in general. Öngür et al. (2009) reported that comorbid lifetime cannabis, but not alcohol, abuse/dependence was associated with a statistically significant 3-year-earlier age at onset of psychosis in schizophrenia ($n = 80$), schizoaffective disorder ($n = 61$), and bipolar disorder with psychotic features ($n = 92$). Patients fulfilled the criteria for cannabis abuse/dependence an additional 3 years before psychosis [39].

In a study with 324 BD types I and II patients, an independent dose-response was shown between cannabis use and age at onset of BD: there was a statistically significant decrease in age at onset with increasing levels of lifetime cannabis use, from 23.2 years (± 9.7) for patients who never used cannabis or used cannabis <10 times during one month lifetime, 20.5 years (± 7.3) for patients who used cannabis >10 times during one month lifetime, and 18.6 years (± 5.0) for patients with a lifetime cannabis use disorder (abuse or dependence) [7].

3.3. Suicidal Behavior and Cannabis Use in Bipolar Disorder. According to the World Health Organization (WHO), about 3,000 people commit suicide every day worldwide—one every 40 seconds. For each suicide, 20 or more attempts are committed. The annual number of suicides is currently around one million and represents about half of all violent deaths recorded in the world [40].

Among psychiatric disorders, BD has the highest risk of suicide, reaching rates 20–30 times higher than in the general population [14]. Approximately 56% of patients with BD who committed suicide had attempted suicide at least once in their life; and 1 in 15 bipolar patients are victims of suicide [41]. The rate that expresses suicide attempts at least once in their life in bipolar patients varies between 25% and 50%; however, 8% to 19% will commit suicide [27].

The number of suicide attempts in bipolar types I and II is a subject of controversy. One study demonstrated that bipolar type II patients have higher rates of suicide risks [41]. Another study shows not statistically significant rates in suicide risk among bipolar patients either with type I or type II in a follow-up of bipolar patients up to 18 months and in a study in Barcelona that analyzes 290 bipolar patients [28].

More suicide attempts are committed by bipolar females than bipolar males (34–19%). However, higher rates of completed suicide are observed among bipolar males. Early onset of psychiatric disorders and personality disorders contributes

TABLE 4: Bipolar disorder and suicide attempts.

Study	Design	Sample (n)	Follow-up time (in years)	Suicides in BD	Suicide attempts in BD and substance use	Suicide attempts in BD and cannabis
Marangell et al., 2006 [27]	Cohort	1,556	2	3.6% (n = 57) suicide attempts (n = 50) or completions (n = 7)	— ^a	— ^a
Valtonen et al., 2006 [28]	Cohort	176	1.5	20% (n = 35) attempts, 1% (n = 2) completions	45% alcohol, 46% smoking	— ^a
Tidemalm et al., 2014 [16]	Cohort	6,086 (male = 2,408 female = 3,678) ^b	After attempted suicide, ranging from 19–30	Male: 4.1% (n = 98) Female: 6.8% (n = 253)	— ^a	— ^a
Hamshere et al., 2009 [23]	Cross-sectional	1,369	— ^a	Early onset (44.3%) (n = 235) Mid-onset (33.7%) (n = 129) Late onset (28.7%) (n = 31) Suicide attempts	— ^a	— ^a
Bellivier et al., 2011 [29]	Prospective observational	2,219	2	29.9%	— ^a	17.3%
Cassidy*, 2011 [30]	Cohort	157	— ^a	37.6%	Nicotine: 66.2% Alcohol: 36.3% Cocaine: 23.6% Benzodiazepine: 5.7% Amphetamine: 7.6% Opiate: 5.1% Hallucinogen: 9.6%	42.7%
Parmentier et al., 2012 [31]	Cross-sectional	652	— ^a	42.9%	— ^a	15.1%
Antypa et al., 2013 [32]	Cohort	3,083	— ^a	4.6%	— ^a	— ^a
Carrà et al., 2014 [33]	Meta-analysis	31,294	— ^a	— ^a	20.1%	— ^a

* Cassidy: the rates of substance use and cannabis use are associated with the total sample. It does not represent necessarily interaction with the rate of suicide attempts.

^aNo data available.

^bIncluding Bipolar Disorder Subtypes 1 and 2, Unspecified type and Schizoaffective Disorder of Bipolar Type.

to the risk of suicide. BD associated with substance use in males doubles the number of suicidal behaviors [16].

The early age at onset of BD and gender, prior suicide attempts, suicide in different mood episodes of bipolar disorder, episode polarity and polarity of first affective episode, and drug and alcohol abuse disorders show a significantly increased risk of suicide attempts [41]. The early onset of this disorder increases the risk of suicide and that early onset is around age 20 [42, 43].

Based on a cohort of 6,086 (60% females) patients followed up annually 2005–2012, it was found that early onset is a significant predictor of suicide attempts only in females. Clinical data was extracted from Swedish National Quality Register for Bipolar Disorder [16]. Early age at onset was also associated with suicide attempts in samples from France and the United States [3].

Another study with a population-based longitudinal cohort sample included 1,542 patients with bipolar depression attending a registry of 388,624 inhabitants of Keelung City,

Taiwan, from 1999 to 2004. This cohort was followed until the end of 2008 and data were from the National Health Insurance Dataset (NHID) and the National Mortality Registry (NMR). The risk of suicide in these patients was doubled in comparison with other depressive patients [44].

Table 4 shows studies associating suicidal behavior and BD. The following variables are described: year of publication; number of individuals; suicide attempts; suicide attempts and substance use; suicide attempts; and cannabis use. Sample sizes ranged from 170 to 31,000. The following percentages in bipolar patients were found: suicide attempts 3.6–42%; suicide attempts and substance use ranging from 5 to 60%; and suicide attempts and cannabis use ranging from 15 to 42%. It is important to notice that higher percentages of suicide attempts were found in the studies with smaller number of participants, which is statistically understandable, but the real number of patients that attempted suicide was significant in all studies taking in credit that all suicide attempts can potentially lead to death.

A sample of 1,556 bipolar patients was examined along 2 years of follow-up. They were participants in the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD), a multicenter study which evaluates longitudinal outcomes in patients with BD. This study analyzed the association between baseline clinical and demographic variables and subsequent suicide attempts and completions. Suicidal ideation, percent of anxiety, depressed and irritable days in past year, history of suicide attempts, age at onset, marital and smoking status, age, and gender were considered for comparison. The sample with complete baseline data was categorized by whether or not participants experienced a suicide attempt or completion over the 2-year follow-up period. A rate of 3.66% (57 patients) attempted or completed suicide. After analysis, only history of suicide (OR = 4.52) and percent of depressed days in the past year (OR = 1.16) were significant. The age at onset of BD (<13 years) presented OR = 1.37 [27].

A prospective study was conducted in the Jorvi Bipolar Study (JoBS) screening 1,630 patients, of whom 546 obtained a positive MDQ screen or suspicion of BD. 490 patients of this sample could be interviewed; however, 201 had the diagnosis of BD confirmed by the Structured Clinical Interview for DSM-IV Disorders (SCID-I) and 10 patients refused to participate. This research aimed to investigate the risk for suicide attempts in psychiatric inpatients and outpatients with BD, and it found that during the 18-month follow-up, 20% of patients (35/176) attempted suicide. In a Cox regression model, baseline previous suicide attempts, hopelessness, depressive phase at index episode, and younger age at intake were independent risk factors for suicide attempts during follow-up, whereas factors such as bipolar I or II or comorbidity did not reach statistical significance [28].

Recent affective episodes predicted attempted suicide during follow-up in male (OR = 3.63) and in female (OR = 2.81) patients as well as previous suicide attempts (male: OR = 3.93; female: OR = 4.24) and recent psychiatric inpatient care (male: OR = 3.57; female: OR = 2.68). Those with many lifetime depressive episodes were more likely to attempt suicide. Comorbid substance use disorder was a predictor in male, while many lifetime mixed episodes, early onset of mental disorder, personality disorder, and social problems related to the primary group were predictors in women [16].

A study was conducted with a group of 1,369 bipolar patients, divided into three subgroups: the first was represented by patients who had had an early age at onset, the second an intermediate age at onset of BD, and the third a late onset, producing a sample of 1,225 individuals (144 with borderline values were excluded). Results showed that 44.3% of the first group attempted suicide while in the second and third groups, respectively, the rate was 33.7 and 28.7%. The early onset group in comparison with the others had a greater frequency of suicide attempts [23].

In a prospective study with a sample of 2,219 bipolar patients who provided data about their lifetime history, 663 (29.9%) had made at least one suicide attempt. Factors that were associated with a history of suicide attempts included the following variables: female gender, a history of alcohol abuse, a history of substance abuse, *early onset*, longer

disorder duration, greater depressive symptom severity, current benzodiazepine use, higher overall symptom severity, and poor compliance. Of the 663 patients with suicide attempts, 17.3% of them had lifetime cannabis abuse. Of the sample without suicidal behavior (1,556), 10.7% had lifetime cannabis abuse [29].

A study conducted with a bipolar cohort of 87 males and 70 females aimed to detect factors that may be predictive for suicide attempt. Among 157 patients, 59 of them had a history of at least one suicide attempt. White race, family history of completed suicide, and history of cocaine abuse/dependence were predictive of suicide attempt histories [30].

Clinical and dimensional characteristics in bipolar patients were analyzed through diagnostic interview and questionnaires. In a sample of 652 patients, 280 (42.9%) suicide attempts were detected. Some variables were associated with a lifetime history of suicidal behavior like being a woman, a history of head injury, tobacco misuse, early age at onset, high number of depressive episodes, positive history of rapid cycling, alcohol misuse, and social phobia. Data was analyzed comparing two different groups: bipolar patients with and without suicidal behavior (BD + SB and BD – SB) and an earlier age at onset was associated with suicidal behavior (23.1 ± 9.1 in BP + SB versus 27.0 ± 11.1 in BD – SD). Cannabis misuse was indicated in 41 (15.1%) of 280 (42.9%) bipolar patients that made a suicide attempt [31].

In a cohort of 3,083 bipolar patients, 140 (4.6%) had a suicide event (8 died by suicide and 132 attempted suicide). The strongest predictor of a suicide event was a history of suicide attempt, in line with prior literature. Additional predictors were younger age, a high total score on the personality disorder questionnaire, and a high percentage of days spent depressed in the year prior to study entry [32].

A meta-analysis was conducted in order to relate comorbidities and suicide attempts in patients with BD. Twenty-nine of 222 studies assessed for eligibility met the inclusion criteria, comprising a total of 31,294 individuals with BD, of whom 6,308 (20.1%) had documented suicide attempts. There were consistent findings across the studies included. As compared to controls, subjects with BD and comorbid alcohol and other substance use disorder were more likely to attempt suicide [33].

The association between suicide and substance abuse in bipolar patients can be demonstrated by substantial evidence of suicidal behavior in patients with comorbidities for substance use disorders. This relationship can be sustained on the basis of data stating that cannabis use affects negatively the course of bipolar disorder. The effects of substance use on suicidal behavior were registered by a large epidemiological study in 1,643 bipolar patients among 43,093 general-population respondents who were interviewed in the 2001-2002 by the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) [45].

The use of cannabis reduces the age at onset of BD; it may also increase manic episodes and their durations and may increase the risk of suicide in patients with bipolar depression. A study involving 101 patients concluded that the first exposure to cannabis represented strong evidence of an earlier age at onset of first depressive episode [46].

Cannabis use in patients with bipolar disorder increases the incidence of psychotic symptoms [47] and suicide attempts [33] and still decreases the response to treatment by lithium compared to patients who do not use this substance [48].

The abusive use of cannabis may act as a predictor of early onset of bipolar disorder and both conditions can interfere with increasing the risk of suicide in patients with BD [29]. Greater suicidal behavior in bipolar patients who experienced a reduction in the age at onset in the context of cannabis use was also demonstrated [37].

A group of 125 bipolar patients with psychotic features was analyzed and it was demonstrated that bipolar disorder occurred earlier in a subgroup that had substance abuse associated with other comorbidities from axis I [49].

A meta-analysis of 29 studies evaluated suicide attempts and substance use (including cannabis) specifically in BD population. It comprised 31,294 individuals with BD of which 6,308 (20.1%) had suicide attempts. A consistent association of alcohol and drug use with suicide attempts was found. Cannabis use disorders were evaluated in 4 studies comprising 3,439 individuals with specific information on BD, suicide attempts, and CUD (559 with BD and lifetime cannabis use disorders). Individuals misusing cannabis showed an increased risk of suicide (OR = 1.44) when compared with their noncomorbid counterparts [33].

4. Discussion

4.1. Age at Onset of Bipolar Disorder. The definition of early age at onset of BD is not clear; however, it is associated with poor prognosis of BD [23]. If an early diagnosis of BD is not established, the treatment starts late, compromising a good outcome [34]. Nine studies between 2000 and 2012 were evaluated and a wide variation in the meaning of “early age at onset” was found (6–22 years), as shown in Table 2.

The definition of the age at onset of bipolar disorder is important because there is robust evidence suggesting that the age at onset distribution of individuals affected with bipolar disorder is composed of at least two age distributions (early and late onset) [10, 17, 18, 22, 43], three divided into early, intermediate, and late [19, 21, 23] and only one into very early, early, and late onset [20]. The three normal distributions (early, intermediate, and late onset) may reflect genetic heterogeneity within bipolar I disorder [23].

Results may vary in the literature relating to age at onset because different criteria were used to establish the definition of age at onset. Hamshere et al. [23] incorporated the duration of the disorder at the time of the interview and gender as covariates, while Benazzi and Akiskal [22] used age at assessment-time, gender, bipolar type II, and mania/hypomania family history. Perlis et al. [20] also included the duration of the disorder and finally Lin et al. [21] included the variables for age of contact and gender.

The definition of very early age at onset is even more troublesome because the first symptoms of mood variation that could be diagnosed as the first bipolar episode are difficult to diagnose by MINI and other instruments [20].

The relationship between early age at onset and the severity of bipolar disorder is well established. Early age at onset is associated with a greater number of rapid cycling [18, 21, 23], mixed episodes [10], and psychotic episodes [10, 17] and panic disorder [10], anxiety disorder [18, 20], substance use disorder [3, 18, 20, 21], and major depression [22], even worse response to lithium [10] and suicidal behavior [3, 18, 20, 21].

4.2. Early Age at Onset of Bipolar Disorder and Cannabis Use. Cannabis use is a risk factor for early age at onset of BD. Substance use disorder is associated with a worse course of BD. Cannabis is the most common illicit substance used among individuals with BD (7.2% in BD versus 1.2% in general population). The use of marijuana seems to be a predictor of mood episodes in BD patients and it reduces the age at onset in 6–9 years [8, 9].

Cannabis use disorder triggers earlier episodes of BD when compared with alcohol use disorder [50]. Especially in cases of cannabis abuse, the relationship between abuse and age at onset is not well understood. It happens because substance use disorder can be a prodromal symptom of mood disorder and can be both a consequence and a cause of BD [30]. The interaction of childhood stressor events and cannabis use appears to be the potential factor for a vicious cycle [51, 52]. Another interesting point was that patients with lifetime cannabis and alcohol abuse/dependence had a later onset compared with those who had only cannabis abuse/dependence [39].

4.3. Cannabis Use and Suicide Behavior in Bipolar Disorder. There is an association between early age at onset of BD and suicide attempts. Among psychiatric disorders, BD has the highest risk of suicide [14]. Early age at onset is associated with suicide attempts [3] and is considered a predictor, especially in females [16]. It is still necessary to elucidate whether early age at onset in BD is an independent risk factor for suicide attempts or whether it depends on other factors, such as disorder severity, rapid cycling, more psychiatric comorbidity, abuse in childhood, family history of mood disorder, differences of gender, and BD subtypes [53, 54]. The difference in suicide rates between bipolar types I and II is controversial. One study suggests higher suicide rates in BD type II group [41]. But when gender was compared, females showed higher rate of suicide attempts, while males presented higher rates of completed suicide [16]. There is not enough data to differentiate between suicide rates in bipolar types. BD type II was associated with higher rates of suicide attempts [41], while other studies have shown no significant differences between BD types I and II [28].

Cannabis use in patients with BD increases the risk of suicide attempts [33]. The findings of an association between cannabis use, early age at onset, and suicide attempts may be taken as a support of the view that cannabis negatively affects the course of the disorder [46]. Substance use disorder may be genetically associated with bipolar disorder. Recent research confirms the importance of the genetics of the bipolar disorder, although the involvement of no specific

chromosome region or gene has been specifically confirmed [55–58].

There is literature evidence that BD and alcohol dependence are highly genetically influenced. The pathophysiology of this interaction is not completely elucidated and thus points to the importance of researching common biomarkers of both disorders for a better understanding of the course of the disorder. It may be hypothesized that cannabis use and BD may share a common genetic background.

4.4. Neurophysiological Changes Associated with Cannabis Use. The neuropathological bases of comorbidity between bipolar disorder and cannabis use disorder are a challenge because there are few studies addressing the pathophysiology of these two disorders. Areas of medial temporal cortex and prefrontal and subcortical regions with structural and functional abnormalities are associated with emotional and motivational processing in adolescents with BD [59] and substance use disorder [60]. Thus, neurophysiological dysfunctions may overlap in some individuals predisposed to the development of both disorders: BD and substance use disorder [61].

Only one study addressed the relationship between bipolar patients and the expression of CB1-R and did not find a direct relation to neither increase nor decrease the density in bipolar patients. However, it was observed that bipolar patients who were taking first-generation antipsychotics had their levels of CB1-R immunoreactive glial cells reduced [37]. Type 1 cannabinoid receptor, CB1-R, is a G protein-coupled receptor and located mainly in the central and peripheral nervous system. It is activated by the compound THC found in cannabis. CB1-R is related to search behavior in patients with cannabis abuse. This is due to influence on the mesolimbic pathway, especially in the nucleus accumbens region [62]. Another research found that polymorphism of AKT1 gene may be related to the use of cannabis and to the developing of psychosis, but not specifically for bipolar patients [63].

Bipolar adolescents with cooccurring cannabis use disorder had structural differences in frontal and temporal cortical regions and the right caudate nucleus, which is extended and is related to emotional and motivational regulation [61]. In addition, patients who use cannabis and tobacco had lower activation of the right hippocampus when compared with controls [64]. In patients with cooccurrence of BD and cannabis use disorder an increase in gray matter volume in the right caudate and precentral gyrus and increased gray matter density in the occipital, middle right fusiform, and cerebellar vermis were observed, while there was a reduction in gray matter volume in the left fusiform [61].

Abnormalities in the subcortical region have been linked to dysfunction in the reward system in patients with substance use disorder. The activity of the caudate is related to desire [65] and the fusiform gyrus, which is altered in bipolar patients with cannabis use disorder, is associated with craving and drug seeking behavior [66]. The results of studies attempting to correlate structural and functional changes in the brains of patients with BD and cannabis use disorder found some limitations because they were not able to

differentiate preexisting structural brain abnormalities from the consequences of repeated exposure to abused substances [67]. Furthermore, a preliminary study with a small sample of patients ($n = 10$) found no significant changes in the brain of patients who were frequently using cannabis [68].

Several studies have demonstrated that BD is more prevalent in individuals who have experienced early-life events stressors. Neuroendocrine, autonomic, immune, and oxidative responses are triggered, once a state of chronic inflammation is elicited, altering cellular mediators of plasticity and energy metabolism, besides the deleterious programming of the hypothalamic-pituitary-adrenal (HPA) axis [50]. van Leeuwen et al. [51] proposed opposite effects on the hypothalamic-pituitary-adrenal (HPA) axis when childhood abuse and cannabis use cooccur. That is why cannabis seems to decrease the programming of HPA while childhood trauma is linked as a stressor to HPA, triggering a factor for long-term hyperactivation of HPA [52].

The catechol-O-methyltransferase has been studied because it is involved in the metabolism of catecholamines (dopamine, adrenaline, and noradrenaline). Massat et al. [69] observed the influence of catechol-O-methyltransferase variants on major depression and BD, particularly in early onset subjects. Experimental studies support a theory which shows an interaction between cannabis and catechol-O-methyltransferase as a mechanism for cognitive abnormalities [9].

A reduced expression of polyamines was observed in bipolar patients with completed suicide. The polymorphism of the gene responsible for enzyme SAT1 is related to dysfunction in the catabolism of polyamines [70]. Bipolar patients who had been hospitalized with or without suicidal ideation had a reduced amount of SAT1 when compared with those who had not been hospitalized [71].

4.5. Limitations. This study has some limitations. There is sparse literature on the issue involving cannabis use, early age at onset of BD, and suicide attempts. The definition of early age at onset is not consensual; therefore studies may not be entirely comparable. Some studies report data on the bipolar group as a whole, not specifying the relationship of cannabis use, early age at onset, and suicide attempts. Other studies report data involving the early age at onset in bipolar and schizophrenia making it harder to distinguish to which group of patients the study is referring. A definition of what constitutes a prodromal phase or an index episode in many studies is lacking. Information on whether there is only cannabis use/abuse or other psychiatric comorbidities in terms of drug use/abuse is missing in many studies. The definition of cannabis use/abuse is subject of controversy. There are arbitrary criteria for specifying use and abuse. Finally, some studies published data only on BD type I and others on BD type II and both (BD I and II) and, in some of them, it is not specified whether the sample refers to BD and schizophrenia [72–75].

However, in this review, as in other studies, the relationship between abuse/dependence and age at onset differentiated on the basis of psychiatric diagnosis or gender

aspects has not been investigated, although some of these associations are potentially plausible.

5. Conclusion

In conclusion, the age at onset of bipolar disorder was reviewed due to its prognostic value, making it clear that the first episode should have an early diagnosis. The evidence of cannabis use as a risk factor to early onset of bipolar disorder highlights the necessity to be attentive to the first bipolar episode so that treatment can be started promptly. The use of cannabis is an important factor that may trigger early onset of BD and, by itself, is associated with higher rates of suicidal behavior in BD. However, it is not clear whether the effect of cannabis at the age at onset and suicide attempts are independent of each other or not.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

- [1] K. Gao, B. K. Tolliver, D. E. Kemp et al., "Differential interactions between comorbid anxiety disorders and substance use disorder in rapid cycling bipolar I or II disorder," *Journal of Affective Disorders*, vol. 110, no. 1-2, pp. 167-173, 2008.
- [2] M. A. Cerullo and S. M. Strakowski, "The prevalence and significance of substance use disorders in bipolar type I and II disorder," *Substance Abuse: Treatment, Prevention, and Policy*, vol. 2, article 29, 2007.
- [3] B. Etain, M. Lajnef, F. Bellivier et al., "Clinical expression of bipolar disorder type I as a function of age and polarity at onset: convergent findings in samples from France and the United States," *Journal of Clinical Psychiatry*, vol. 73, no. 4, pp. e561-e566, 2012.
- [4] B. Dell'Osso, M. Buoli, S. Bortolussi, G. Camuri, V. Vecchi, and A. C. Altamura, "Patterns of Axis I comorbidity in relation to age in patients with Bipolar Disorder: a cross-sectional analysis," *Journal of Affective Disorders*, vol. 130, no. 1-2, pp. 318-322, 2011.
- [5] I. van Rossum, M. Boomsma, D. Tenback, C. Reed, and J. van Os, "Does cannabis use affect treatment outcome in bipolar disorder? A longitudinal analysis," *Journal of Nervous and Mental Disease*, vol. 197, no. 1, pp. 35-40, 2009.
- [6] S. M. Strakowski, K. W. Sax, S. L. McElroy, P. E. Keck Jr., J. M. Hawkins, and S. A. West, "Course of psychiatric and substance abuse syndromes co-occurring with bipolar disorder after a first psychiatric hospitalization," *Journal of Clinical Psychiatry*, vol. 59, no. 9, pp. 465-471, 1998.
- [7] M. de Hert, M. Wampers, T. Jendricko et al., "Effects of cannabis use on age at onset in schizophrenia and bipolar disorder," *Schizophrenia Research*, vol. 126, no. 1-3, pp. 270-276, 2011.
- [8] T. V. Lagerberg, L. R. Kvitland, S. R. Aminoff et al., "Indications of a dose-response relationship between cannabis use and age at onset in bipolar disorder," *Psychiatry Research*, vol. 215, no. 1, pp. 101-104, 2014.
- [9] S. Lev-Ran, B. le Foll, K. McKenzie, T. P. George, and J. Rehm, "Bipolar disorder and co-occurring cannabis use disorders: characteristics, co-morbidities and clinical correlates," *Psychiatry Research*, vol. 209, no. 3, pp. 459-465, 2013.
- [10] F. Schürhoff, F. Bellivier, R. Jouvent et al., "Early and late onset bipolar disorders: two different forms of manic-depressive illness?" *Journal of Affective Disorders*, vol. 58, no. 3, pp. 215-221, 2000.
- [11] J. D. Lish, S. Dime-Meenan, P. C. Whybrow, R. A. Price, and R. M. A. Hirschfeld, "The National Depressive and Manic-depressive Association (DMDA) survey of bipolar members," *Journal of Affective Disorders*, vol. 31, no. 4, pp. 281-294, 1994.
- [12] K. Hawton, L. Sutton, C. Haw, J. Sinclair, and L. Harriss, "Suicide and attempted suicide in bipolar disorder: a systematic review of risk factors," *Journal of Clinical Psychiatry*, vol. 66, no. 6, pp. 693-704, 2005.
- [13] I. M. Anderson, P. M. Haddad, and J. Scott, "Bipolar disorder," *British Medical Journal*, vol. 345, Article ID e8508, 2012.
- [14] M. Pompili, X. Gonda, G. Serafini et al., "Epidemiology of suicide in bipolar disorders: a systematic review of the literature," *Bipolar disorders*, vol. 15, no. 5, pp. 457-490, 2013.
- [15] U. Ösby, L. Brandt, N. Correia, A. Ekblom, and P. Sparén, "Excess mortality in bipolar and unipolar disorder in Sweden," *Archives of General Psychiatry*, vol. 58, no. 9, pp. 844-850, 2001.
- [16] D. Tidemalm, A. Haglund, A. Karanti, M. Landén, and B. Runeson, "Attempted suicide in bipolar disorder: risk factors in a cohort of 6086 patients," *PLoS ONE*, vol. 9, no. 4, Article ID e94097, 2014.
- [17] T. G. Schulze, D. J. Müller, H. Krauss et al., "Further evidence for age of onset being an indicator for severity in bipolar disorder," *Journal of Affective Disorders*, vol. 68, no. 2-3, pp. 343-345, 2002.
- [18] R. M. Post, G. S. Leverich, R. W. Kupka et al., "Early age at onset as a risk factor for poor outcome of bipolar disorder," *Journal of Psychiatric Research*, vol. 37, no. 4, pp. 297-303, 2003.
- [19] F. Bellivier, J. L. Golmard, M. Rietschel et al., "Age at onset in bipolar I affective disorder: further evidence for three subgroups," *The American Journal of Psychiatry*, vol. 160, no. 5, pp. 999-1001, 2003.
- [20] R. H. Perlis, S. Miyahara, L. B. Marangell et al., "Long-term implications of early onset in bipolar disorder: data from the first 1000 participants in the systematic treatment enhancement program for bipolar disorder (STEP-BD)," *Biological Psychiatry*, vol. 55, no. 9, pp. 875-881, 2004.
- [21] P.-I. Lin, M. G. McInnis, J. B. Potash et al., "Clinical correlates and familial aggregation of age at onset in bipolar disorder," *American Journal of Psychiatry*, vol. 163, no. 2, pp. 240-246, 2006.
- [22] F. Benazzi and H. S. Akiskal, "How best to identify a bipolar-related subtype among major depressive patients without spontaneous hypomania: superiority of age at onset criterion over recurrence and polarity?" *Journal of Affective Disorders*, vol. 107, no. 1-3, pp. 77-88, 2008.
- [23] M. L. Hamshere, K. Gordon-Smith, L. Forty et al., "Age-at-onset in bipolar-I disorder: mixture analysis of 1369 cases identifies three distinct clinical sub-groups," *Journal of Affective Disorders*, vol. 116, no. 1-2, pp. 23-29, 2009.
- [24] C. Henquet, L. Krabbendam, R. de Graaf, M. ten Have, and J. van Os, "Cannabis use and expression of mania in the general population," *Journal of Affective Disorders*, vol. 95, no. 1-3, pp. 103-110, 2006.
- [25] M. J. A. Tjissen, J. van Os, H. U. Wittchen, R. Lieb, K. Beesdo, and M. Wichers, "Risk factors predicting onset and persistence of subthreshold expression of bipolar psychopathology among youth from the community," *Acta Psychiatrica Scandinavica*, vol. 122, no. 3, pp. 255-266, 2010.

- [26] T. V. Lagerberg, K. Sundet, S. R. Aminoff et al., "Excessive cannabis use is associated with earlier age at onset in bipolar disorder," *European Archives of Psychiatry and Clinical Neuroscience*, vol. 261, no. 6, pp. 397–405, 2011.
- [27] L. B. Marangell, M. S. Bauer, E. B. Dennehy et al., "Prospective predictors of suicide and suicide attempts in 1,556 patients with bipolar disorders followed for up to 2 years," *Bipolar Disorders*, vol. 8, no. 5, part 2, pp. 566–575, 2006.
- [28] H. M. Valtonen, K. Suominen, O. Mantere, S. Leppämäki, P. Arvilommi, and E. T. Isometsä, "Prospective study of risk factors for attempted suicide among patients with bipolar disorder," *Bipolar Disorders*, vol. 8, no. 5, pp. 576–585, 2006.
- [29] F. Bellivier, L. Yon, A. Luquiens et al., "Suicidal attempts in bipolar disorder: results from an observational study (EMBLEM)," *Bipolar Disorders*, vol. 13, no. 4, pp. 377–386, 2011.
- [30] F. Cassidy, "Risk factors of attempted suicide in bipolar disorder," *Suicide and Life-Threatening Behavior*, vol. 41, no. 1, pp. 6–11, 2011.
- [31] C. Parmentier, B. Etain, L. Yon et al., "Clinical and dimensional characteristics of euthymic bipolar patients with or without suicidal behavior," *European Psychiatry*, vol. 27, no. 8, pp. 570–576, 2012.
- [32] N. Antypa, M. Antonioli, and A. Serretti, "Clinical, psychological and environmental predictors of prospective suicide events in patients with Bipolar Disorder," *Journal of Psychiatric Research*, vol. 47, no. 11, pp. 1800–1808, 2013.
- [33] G. Carrà, F. Bartoli, C. Crocamo et al., "Attempted suicide in people with co-occurring bipolar and substance use disorders: systematic review and meta-analysis," *Journal of Affective Disorders*, vol. 167, pp. 125–135, 2014.
- [34] B. Birmaher, D. Axelson, M. Strober et al., "Comparison of manic and depressive symptoms between children and adolescents with bipolar spectrum disorders," *Bipolar Disorders*, vol. 11, no. 1, pp. 52–62, 2009.
- [35] J. B. Potash, J. Toolan, J. Steele et al., "The bipolar disorder phenome database: a resource for genetic studies," *The American Journal of Psychiatry*, vol. 164, no. 8, pp. 1229–1237, 2007.
- [36] K. R. Merikangas, R. Jin, J.-P. He et al., "Prevalence and correlates of bipolar spectrum disorder in the World Mental Health Survey Initiative," *Archives of General Psychiatry*, vol. 68, no. 3, pp. 241–251, 2011.
- [37] A. Agrawal, J. I. Nurnberger, and M. T. Lynskey, "Cannabis involvement in individuals with bipolar disorder," *Psychiatry Research*, vol. 185, no. 3, pp. 459–461, 2011.
- [38] M. van Laar, S. van Dorsselaer, K. Monshouwer, and R. de Graaf, "Does cannabis use predict the first incidence of mood and anxiety disorders in the adult population?" *Addiction*, vol. 102, no. 8, pp. 1251–1260, 2007.
- [39] D. Öngür, L. Lin, and B. M. Cohen, "Clinical characteristics influencing age at onset in psychotic disorders," *Comprehensive Psychiatry*, vol. 50, no. 1, pp. 13–19, 2009.
- [40] World Health Organization, *Preventing Suicide: A Global Imperative*, WHO, Geneva, Switzerland, 2008, http://www.who.int/iris/bitstream/10665/131056/1/9789241564779_eng.pdf.
- [41] Z. Rihmer and K. Kiss, "Bipolar disorders and suicidal behaviour," *Bipolar Disorders*, vol. 4, no. supplement 1, pp. 21–25, 2002.
- [42] J. Y. Song, H. Y. Yu, S. H. Kim et al., "Assessment of risk factors related to suicide attempts in patients with bipolar disorder," *Journal of Nervous and Mental Disease*, vol. 200, no. 11, pp. 978–984, 2012.
- [43] F. Angst, H. H. Stassen, P. J. Clayton, and J. Angst, "Mortality of patients with mood disorders: follow-up over 34–38 years," *Journal of Affective Disorders*, vol. 68, no. 2–3, pp. 167–181, 2002.
- [44] J.-C. Chang, H.-H. Chen, A. M.-F. Yen, S. L.-S. Chen, and C.-S. Lee, "Survival of bipolar depression, other type of depression and comorbid ailments: ten-year longitudinal follow-up of 10,922 Taiwanese patients with depressive disorders (KCIS no. PSY1)," *Journal of Psychiatric Research*, vol. 46, no. 11, pp. 1442–1448, 2012.
- [45] M. A. Oquendo, D. Currier, S.-M. Liu, D. S. Hasin, B. F. Grant, and C. Blanco, "Increased risk for suicidal behavior in comorbid bipolar disorder and alcohol use disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC)," *Journal of Clinical Psychiatry*, vol. 71, no. 7, pp. 902–909, 2010.
- [46] L. R. Kvitland, I. Melle, S. R. Aminoff, T. V. Lagerberg, O. A. Andreassen, and P. A. Ringen, "Cannabis use in first-treatment bipolar I disorder: relations to clinical characteristics," *Early Intervention in Psychiatry*, 2014.
- [47] G. Katz, R. Durst, E. Shufman, R. Bar-Hamburger, and L. Grunhaus, "Cannabis abuse and severity of psychotic and affective disorders in Israeli psychiatric inpatients," *Comprehensive Psychiatry*, vol. 51, no. 1, pp. 37–41, 2010.
- [48] J. F. Goldberg, J. L. Garno, A. C. Leon, J. H. Kocsis, and L. Portera, "A history of substance abuse complicates remission from acute mania in bipolar disorder," *Journal of Clinical Psychiatry*, vol. 60, no. 11, pp. 733–740, 1999.
- [49] S. Pini, L. Dell'Osso, C. Mastrocinque et al., "Axis I comorbidity in bipolar disorder with psychotic features," *British Journal of Psychiatry*, vol. 175, pp. 467–471, 1999.
- [50] A. J. Walker, Y. Kim, J. Blair Price et al., "Stress, inflammation, and cellular vulnerability during early stages of affective disorders: biomarker strategies and opportunities for prevention and intervention," *Frontiers in Psychiatry*, vol. 5, article 34, 2014.
- [51] A. P. van Leeuwen, H. E. Creemers, K. Greaves-Lord, F. C. Verhulst, J. Ormel, and A. C. Huizink, "Hypothalamic-pituitary-adrenal axis reactivity to social stress and adolescent cannabis use: the TRAILS study," *Addiction*, vol. 106, no. 8, pp. 1484–1492, 2011.
- [52] C. Heim, D. J. Newport, T. Mletzko, A. H. Miller, and C. B. Nemeroff, "The link between childhood trauma and depression: insights from HPA axis studies in humans," *Psychoneuroendocrinology*, vol. 33, no. 6, pp. 693–710, 2008.
- [53] M. Leboyer, C. Henry, M.-L. Paillere-Martinot, and F. Bellivier, "Age at onset in bipolar affective disorders: a review," *Bipolar Disorders*, vol. 7, no. 2, pp. 111–118, 2005.
- [54] J. L. Garno, J. F. Goldberg, P. M. Ramirez, and B. A. Ritzler, "Impact of childhood abuse on the clinical course of bipolar disorder," *British Journal of Psychiatry*, vol. 186, pp. 121–125, 2005.
- [55] N. Craddock and P. Sklar, "Genetics of bipolar disorder: successful start to a long journey," *Trends in Genetics*, vol. 25, no. 2, pp. 99–105, 2009.
- [56] A. Farmer, A. Elkin, and P. McGuffin, "The genetics of bipolar affective disorder," *Current Opinion in Psychiatry*, vol. 20, no. 1, pp. 8–12, 2007.
- [57] A. Serretti and L. Mandelli, "The genetics of bipolar disorder: genome 'hot regions,' genes, new potential candidates and future directions," *Molecular Psychiatry*, vol. 13, no. 8, pp. 742–771, 2008.
- [58] Psychiatric GWAS Consortium Coordinating Committee, S. Cichon, N. Craddock et al., "Genomewide association studies:

- history, rationale, and prospects for psychiatric disorders,” *The American Journal of Psychiatry*, vol. 166, no. 5, pp. 540–556, 2009.
- [59] M. P. DelBello, C. M. Adler, and S. M. Strakowski, “The neurophysiology of childhood and adolescent bipolar disorder,” *CNS Spectrums*, vol. 11, no. 4, pp. 298–311, 2006.
- [60] S. E. Hyman, R. C. Malenka, and E. J. Nestler, “Neural mechanisms of addiction: the role of reward-related learning and memory,” *Annual Review of Neuroscience*, vol. 29, pp. 565–598, 2006.
- [61] K. Jarvis, M. P. DelBello, N. Mills, I. Elman, S. M. Strakowski, and C. M. Adler, “Neuroanatomic comparison of bipolar adolescents with and without cannabis use disorders,” *Journal of Child and Adolescent Psychopharmacology*, vol. 18, no. 6, pp. 557–563, 2008.
- [62] M. Melis and M. Pistis, “Hub and switches: endocannabinoid signalling in midbrain dopamine neurons,” *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 367, no. 1607, pp. 3276–3285, 2012.
- [63] R. Uher, “Gene-environment interactions in severe mental illness,” *Frontiers in Psychiatry*, vol. 5, article 48, 2014.
- [64] L. K. Jacobsen, W. E. Mencl, M. Westerveld, and K. R. Pugh, “Impact of cannabis use on brain function in adolescents,” *Annals of the New York Academy of Sciences*, vol. 1021, pp. 384–390, 2004.
- [65] N. S. Miller and R. J. Goldsmith, “Craving for alcohol and drugs in animals and humans: biology and behavior,” *Journal of Addictive Diseases*, vol. 20, no. 3, pp. 87–104, 2001.
- [66] I. K. Lyoo, M. H. Pollack, M. M. Silveri et al., “Prefrontal and temporal gray matter density decreases in opiate dependence,” *Psychopharmacology*, vol. 184, no. 2, pp. 139–144, 2006.
- [67] T. E. Schlaepfer, E. Lancaster, R. Heidebreder et al., “Decreased frontal white-matter volume in chronic substance abuse,” *International Journal of Neuropsychopharmacology*, vol. 9, no. 2, pp. 147–153, 2006.
- [68] L. E. Delisi, H. C. Bertisch, K. U. Szulc et al., “A preliminary DTI study showing no brain structural change associated with adolescent cannabis use,” *Harm Reduction Journal*, vol. 3, article 17, 2006.
- [69] I. Massat, N. A. Kocabas, C. Crisafulli et al., “COMT and age at onset in mood disorders: a replication and extension study,” *Neuroscience Letters*, vol. 498, no. 3, pp. 218–221, 2011.
- [70] L. M. Fiori, B. Wanner, V. Jomphe et al., “Association of polyaminergic loci with anxiety, mood disorders, and attempted suicide,” *PLoS ONE*, vol. 5, no. 11, Article ID e15146, 2010.
- [71] H. Le-Niculescu, D. F. Levey, M. Ayalew et al., “Discovery and validation of blood biomarkers for suicidality,” *Molecular Psychiatry*, vol. 18, no. 12, pp. 1249–1264, 2013.
- [72] P. Lichtenstein, B. H. Yip, C. Björk et al., “Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study,” *The Lancet*, vol. 373, no. 9659, pp. 234–239, 2009.
- [73] V. Moskvina, N. Craddock, P. Holmans et al., “Gene-wide analyses of genome-wide association data sets: Evidence for multiple common risk alleles for schizophrenia and bipolar disorder and for overlap in genetic risk,” *Molecular Psychiatry*, vol. 14, no. 3, pp. 252–260, 2009.
- [74] S. M. Purcell, N. R. Wray, International Schizophrenia Consortium et al., “Common polygenic variation contributes to risk of schizophrenia and bipolar disorder,” *Nature*, vol. 460, no. 7256, pp. 748–752, 2009.
- [75] J. X. van Snellenberg and T. de Candia, “Meta-analytic evidence for familial coaggregation of schizophrenia and bipolar disorder,” *Archives of General Psychiatry*, vol. 66, no. 7, pp. 748–755, 2009.

Review Article

Disruption in the Blood-Brain Barrier: The Missing Link between Brain and Body Inflammation in Bipolar Disorder?

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The blood-brain barrier (BBB) regulates the transport of micro- and macromolecules between the peripheral blood and the central nervous system (CNS) in order to maintain optimal levels of essential nutrients and neurotransmitters in the brain. In addition, the BBB plays a critical role protecting the CNS against neurotoxins. There has been growing evidence that BBB disruption is associated with brain inflammatory conditions such as Alzheimer's disease and multiple sclerosis. Considering the increasing role of inflammation and oxidative stress in the pathophysiology of bipolar disorder (BD), here we propose a novel model wherein transient or persistent disruption of BBB integrity is associated with decreased CNS protection and increased permeability of proinflammatory (e.g., cytokines, reactive oxygen species) substances from the peripheral blood into the brain. These events would trigger the activation of microglial cells and promote localized damage to oligodendrocytes and the myelin sheath, ultimately compromising myelination and the integrity of neural circuits. The potential implications for research in this area and directions for future studies are discussed.

1. Physiology of the Blood-Brain Barrier

1.1. Structure of the BBB. The characterization of blood-brain barrier (BBB) began in 1885 with Paul Ehrlich's reports that various water-soluble dyes failed to stain the brain and spinal cord upon injection into the circulatory system, which he attributed to the lower affinity for the dye by the CNS [1, 2]. Later in 1898, Biedl and Kraus demonstrated that only injection of bile acids directly into the brain caused symptoms including seizures and coma, but not when injected into the circulatory system [2]. In 1900, Lewandowsky demonstrated a similar effect using potassium ferrocyanide and attempted to describe this with the term *bluthirnschranke* (blood-brain barrier) [1]. Further experimentation by Goldmann, a student of Ehrlich, demonstrated that trypan blue when injected into the cerebrospinal fluid (CSF) stained CNS tissue, contradicting Ehrlich's dye affinity hypothesis and lending support to the notion that there is a barrier between the

circulatory system and the CNS [2]. Then in 1967, with newly available electron microscopy technology, Reese and Karnovsky demonstrated at the ultrastructural level that horseradish peroxidase (HRP) was unable to enter the CNS due to the presence of tight junctions (TJ) [3]. This showed the continuous nature of the BBB in the CNS and led Reese and Karnovsky to conclude that the BBB existed at the level of the endothelial cells.

Acting as a diffusion barrier, the BBB is composed primarily of brain endothelial cells, astrocyte end-feet, pericytes, perivascular macrophages, and a basal membrane. Its barrier is a result of a tightly sealed monolayer of endothelial cells with TJ and adherens junctions (AJ) forming the seal between cells at junctional complexes. The basal membrane and astrocyte end-feet contribute to BBB function and integrity by regulating the expression of specific TJ proteins and other BBB transporters. Essentially, the TJ are the result of ostensible fusion between the outer lipid bilayers of neighbouring

endothelial cells. Claudin, occludin, and junction adhesion molecules primarily form the composition of TJ, which serve to limit permeability between cells and to increase the barrier's electrical resistance. As a class of transmembrane proteins, two claudin extracellular loops undergo homophilic binding to loops from claudins on adjacent endothelial cells, forming the primary seal of the TJ. Distinct claudins isoforms regulate the diffusion of different sizes of molecules. To date, claudins 3, 5, and 12 are thought to be incorporated in the BBB [4, 5], while the presence of claudin-1 is still in debate [6]. For instance, claudin-5 knockout mice display abnormal endothelial cell TJ, increased BBB permeability to small molecules (<800 Da), and die shortly after birth [5]. Another transmembrane protein, occludin, is also implicated in the foundation of TJ. Similar to claudins, two occludin extracellular loops homophilically bind to occluding loops on a neighbouring cell, abetting in the formation of the TJ. In an occludin construct lacking the N-terminus and extracellular domains, an efficient permeability barrier failed to take shape with unblocked diffusion of several small markers and the presence of gaps, thus establishing the underlying significance of occludin proteins in the formation of TJ. Belonging to the immunoglobulin superfamily, junctional adhesion molecules (JAMs) with their single transmembrane domain are thought to contribute to the sealing capacity of TJ. However, the exact role of JAMs in the function of the BBB is still not fully understood. Adherens junctions (AJ) are typically found to be intermixed with TJ in the BBB. AJ are composed of the membrane protein cadherin whose extracellular domain homophilically binds cadherin on adjacent cells while the cytosolic domain is bound to catenins, which in turn are bound to the actin cytoskeleton of the cell, effectively joining neighbouring cells.

Unlike in the BBB where the barrier is localized at the level of the endothelial cells, the blood-cerebrospinal fluid (CSF) barrier is established by choroid plexus epithelial cells [7]. The choroid plexus is connected by apical TJ and consists of a capillary network, which is enclosed, in a single layer of epithelium cells [7, 8]. The choroid plexus epithelial cells limit paracellular diffusion and contain a secretory function producing the CSF. While the BBB may be the predominant site of transport for O₂, glucose, and amino acids, the blood-CSF barrier plays a critical role in maintaining brain Ca²⁺ homeostasis [9]. The choroid plexus is also responsible for the entry of certain hormones into the CSF and also secretes insulin like growth factor-II (IGF-II) into the CSF [10]. The blood-CSF barrier also boasts of other active transport systems which aid in the efflux of certain solutes including iodide, thiocyanate and penicillin, and the neurotransmitter metabolites homovanillic acid and 5-hydroxyindoleacetic acid [11].

1.2. Functions of the BBB. The BBB limits the passage of large and hydrophilic solutes, while allowing small lipophilic molecules (O₂, CO₂, and hormones) to freely diffuse following concentration gradients. The BBB possesses specific transporters which are used to move complex nutrients such as glucose and amino acids into the brain. The BBB can also use receptor-mediated endocytosis to transport certain

proteins such as insulin, leptin, and iron transferrin into the brain [12, 13].

1.2.1. Regulation of Ion and Neurotransmitter Systems. The BBB plays a critical role not only in regulating the transport of macro- and micromolecules as mentioned above but also in the management of ion and neurotransmitter levels in the CNS and is the primary defence against neurotoxins. For instance, neuronal function and synaptic signalling relies on a stable environment containing optimal concentrations of specific ions such as potassium [K⁺]. In spite of a higher and fluctuating [K⁺] in the plasma akin to ~4.5 mM, the BBB helps maintain [K⁺] at ~2.4–2.9 mM in the CNS. Other major ions and systems regulated by the BBB include calcium (Ca²⁺), magnesium (Mg²⁺), and pH levels. The BBB also plays a major role in maintaining physiological levels of certain neurotransmitters, such as glutamate via excitatory amino acid transporters (EAATs), in the CNS. Additionally, the betaine/GABA transporter 1 (BGT1, SLC6A12), present in the brain microvessels, may play a role in the regulation of γ -aminobutyric acid (GABA) in the CNS [14, 15]. Notably, this compartmentalization of central and peripheral neurotransmitter pools by the BBB is important in the minimization of “cross talk” between these separate systems.

1.2.2. Neurotoxins, Macromolecules, and Essential Nutrients. Taking into account that in the adult CNS steady neurodegeneration greatly overshadows neurogenesis [16], the sheltering of the CNS from endogenous and foreign toxins is of paramount importance. The TJ of the BBB provide an effective and stable barrier from potential toxins circulating in the peripheral blood, while a family of ATP-binding cassette (ABC) transporters in the BBB actively pump such toxins out of the brain. Total protein content in the CNS is inherently lower than plasma levels given the highly selective permeability of the BBB. Consequently, many plasma macromolecules such as albumin, prothrombin, and plasminogen, which can cause irreversible damage to nervous tissue resulting in apoptosis, are excluded. Furthermore, specific transporter systems such as the glucose transporter 1 (GLUT1) which is exclusive to the BBB and monocarboxylate transporter 1 (MCT1) facilitate the transport of glucose and monocarboxylates (i.e., lactate), respectively, as fuel for the brain [17]. The L1 and y⁺ systems, present ubiquitously in the BBB, provide transport for all essential amino acids into the CNS [18]. Five sodium dependent systems, ASC, A, LNAA, EAAT, and N, facilitate the efflux of nonessential AA (ASC, A), essential AA (LNAA), the excitatory acidic AA (EAAT), and nitrogen-rich AA (N) from the brain [4]. Larger neuroactive peptides and proteins including enkephalins [19], arginine-vasopressin (AVP) [20], and luteinizing-hormone releasing hormone (LHRH) can generally not pass the BBB and thus rely upon highly specific transporter systems to move from blood to brain and vice versa. Peptide transport system 1 (PTS-1) and PTS-2 mediate the efflux of enkephalins and AVP, respectively [21], from the brain while PTS-4 facilitates bidirectional transport of LHRH [4]. Other large proteins such as leptin [22], insulin and insulin-like growth factor [12], low-density lipoproteins

(LDL) [23], and immunoglobulin-G (IgG) [24] also rely on receptor-mediated transport systems to cross the BBB.

In sum, the BBB serves two main functions: (i) establishment and maintenance of a specific and stable fluid environment to meet the rigorous needs of the CNS and (ii) protection of the CNS from potentially damaging material originating from both within and outside the confines of the body. The slightly imperfect nature of the BBB allows for the free diffusion of certain small essential water-soluble nutrients, while other complex nutrients rely on highly selective transport systems to enter the brain. Therefore, considering the central role of the BBB in protecting the CNS against neurotoxic compounds, there has been growing interest in the understanding of the BBB function in neuropsychiatric disorders.

1.3. Neurovascular Unit. The neurovascular unit (NVU) was initially defined as “interactions between circulating blood elements and the blood vessel wall, extracellular matrix, glia, and neurons” [25] but has recently developed to incorporate other structures including pericytes and microglia (detailed anatomy and organization is reviewed in [26, 27]). Due to the amalgamation of these structures, the NVU is considered the site of the coupling of neuronal activity and cerebral blood flow [28, 29]. The various components of the NVU are intricately linked to one another, and this relationship is facilitated by adhesion molecules (including cadherins and integrins) and gap junctions [30–32], which in conjunction with ion channels aid in the movement of various ions such as calcium and potassium, and also other neuromodulators (ATP) [33, 34]. The interlink between neuronal and vascular components is genetically tied as during early embryogenesis neural progenitor cells (originating from neural tube) and vascular progenitor cells (originating from neural plate) are positioned in close proximity [35–37]. Due to their position relative to one another, both neural and vascular cells are exposed to similar factors and both respond to vascular endothelial growth factor (VEGF) and nerve growth factor (NGF) [38, 39]. The various components of the NVU all play a distinct and specific role in maintaining the functionality of the NVU [30, 40]; however the exact role of each component is still yet to be elucidated [37, 41].

Exploring the relationship between neurological conditions and NVU dysfunction is still in its infancy; however indirect and epidemiological data does suggest a role for NVU dysfunction in psychiatric conditions such as major depressive disorder (MDD). A study examining endothelial dysfunction via the relative uptake ratio (RUR) of blood flow in the brachial artery following hyperemic challenge found a significantly lower RUR in patients with MDD or minor depressive disorder as compared to healthy controls, implying impaired vascular endothelial function [42]. Another study exploring apoptotic activity in the endothelium (% of apoptotic nuclei in human umbilical vein endothelial cells) found a significantly greater amount of apoptotic nuclei in patients with MDD when compared to healthy controls [43]. Epidemiological studies also point towards a

role for vascular endothelial impairment in MDD. A meta-analysis encompassing 16,221 studies found an increased risk for MDD in those with major vascular diseases including diabetes, cardiovascular disease, and stroke [44].

2. Models of BBB Disruption in Neuropsychiatric Disorders

2.1. BBB in Alzheimer's Disease and Multiple Sclerosis

2.1.1. Alzheimer's Disease. Alzheimer's disease (AD) is characterized by a progressive decline in cognitive function with an onset of >65 years old in most cases. Biologically, AD has been associated with defects in the neurovascular system, accumulation of amyloid- β ($A\beta$; neurotoxin) on and around blood vessels as well as in the brain parenchyma, and the presence of neurofibrillar tangles (NT) [4, 45, 46] and hyperphosphorylated tau [47]. The role of $A\beta$ in AD is the most studied and well understood. Notably, it has been recently shown that peripheral circulating $A\beta$ is transported into the brain via the receptor for advanced glycation end products (RAGE) [48]. Normally expressed in relatively low levels, the expression of RAGE in the BBB is greatly amplified in response to the accumulation of RAGE ligands including $A\beta$ [48, 49]. This $A\beta$ /RAGE interaction in the BBB may lead to increased transportation of circulating $A\beta$ into the CNS, resulting in a NF- κ B mediated activation of endothelial cells and the release of proinflammatory cytokines. It has been demonstrated that binding of $A\beta$ /RAGE at the luminal membrane of the BBB can destroy RAGE expressing neurons through oxidative damage. The clearance of $A\beta$ from the brain is facilitated by lipoprotein receptor-related protein 1 (LRP1). Numerous studies using both animal models and human patients with AD show that $A\beta$ clearance is impaired in these cases [49–53]. For instance, LRP1 functions to transport $A\beta$ into the periphery vascular system whereupon soluble LRP1 (sLRP1) facilitates the total systematic clearance of $A\beta$ from the body via the kidney and liver. The role of LRP2 is not well understood but is hypothesized to utilize apolipoprotein J (APOJ) to facilitate the transfer of $A\beta$ out of the brain [54]. Moreover, the ATP-binding cassette (ABC) family of transporters have also been implicated in $A\beta$ clearance. ABCB1 (P-glycoprotein, P-gp), the product of the MDRI gene, is the best known and best studied of these transporters. Most commonly found in the BBB, several *in vitro* and *in vivo* studies have found ABCB1 to clear $A\beta$ from the albuminal to the luminal side of the membrane [55]. In MDRI transfected pig kidney epithelial cells, the transport of $A\beta_{40}$ and $A\beta_{42}$ was significantly decreased in the presence of cyclosporine A (ABCB1 inhibitor) [56]. Additionally, following the injection of labelled $A\beta_{40}$ and $A\beta_{42}$ into ABCB1 knockout mice, the clearance rate of $A\beta$ was found to be half that of the wild type [57]. In addition to faulty BBB clearing mechanisms in the pathology of AD, recent evidence using APP23 transgenic mice overexpressing mutant human APP, the precursor of $A\beta$, suggests that the BBB may be susceptible to peripherally induced inflammation [46]. For instance, Takeda et al. administered a peripheral LPS

injection to APP transgenic mice and observed a greater increase in inflammatory levels in the brain interstitial fluid, which was accompanied by abnormalities in food intake, social behaviour, and basal activity [46]. In summary, various models of AD suggest that BBB dysfunction is associated with abnormal A β clearance and increased permeability and subsequent influx of proinflammatory substances into the CNS.

2.1.2. Multiple Sclerosis. Multiple sclerosis (MS), a brain disorder characterized by extensive damage to the myelin sheath, presents a wide host of symptoms including but not limited to numbness and weakness in limbs, visual impairments, electric shock sensations, tingling and/or pain across the body, and cognitive impairment. While the exact cause of MS remains unknown, there is still a debate whether or not MS is an autoimmune disease, as classically held, or if it is in reality a neurodegenerative disorder [58, 59]. With respect to the autoimmune aspect of MS, the BBB is responsible for the regulation of immune cell transport and inflammatory pathway mediator activity from the periphery into the CNS. Under physiological conditions, few leukocytes are present in the CNS but in response to injury and/or disease peripheral leukocytes are thought to enter the cerebral spinal fluid (CSF), the parenchymal perivascular space, and the subarachnoid space [2, 18, 19]. In the experimental autoimmune encephalomyelitis (EAE) model of MS, it has been shown that aggressive CD4+ T lymphocytes accumulate in the brain via the BBB and blood-CSF barrier [60–62]. A subset of these T lymphocytes have been reported to exert immunosurveillance in the CNS while another subset is implicated in the destruction of neurons. The regulation and transport of immune cells and other mediators across the BBB and blood-CSF barrier are thus thought to be implicated in the pathophysiology of MS. An imaging study using dynamic contrast-enhanced MRI (DCE-MRI) noted an increase in BBB permeability, as measured by K^{trans}, in the periventricular normal appearing white matter (NAWM) in patients with MS [63]. Notably, immunomodulatory treatment (with β -interferon or glatiramer acetate) aided in the gradual decrease of BBB permeability following a relapse episode. Considering that β -interferon has been shown to stabilize the barrier on brain capillary endothelial cells *in vitro* [64], this study provided strong evidence that abnormalities in the BBB function may be associated with the neurobiology of MS. Notably, a recent *in vitro* study that exposed human brain microvascular endothelial cells (BMECs) to serum from patients with relapse-remitting MS (RRMS) found that serum from patients with RRMS lowered claudin-5, an integral TJ protein expression, and decreased transendothelial electrical resistance [65]. Together, these clinical and preclinical studies indicate that an increase in BBB permeability may occur soon after the flare-ups observed in MS. In addition, preliminary yet encouraging data suggest that successful anti-inflammatory treatment may speed up the rate of closing of the BBB.

2.1.3. The Role of Matrix Metalloproteinase-9 on BBB Function. Matrix metalloproteinases (MMPs) encompass a large family of proteases which are typically produced in a latent form and upon activation by inflammatory stimuli regulate pathophysiological pathways including the regulation of growth factors, death receptors, and various other signalling molecules [66, 67]. The effects of MMPs are diverse and depend on a host of factors such as location, time, and surrounding environment and thus some MMPs can engage in opposite functions at different points in time. For instance, MMPs have been implicated in angiogenesis, neurogenesis, axon growth, tissue repair, myelinogenesis, and apoptotic protection [68–70]. Notably, the promoter region of MMP9 includes a binding region for activator protein-1 (AP1) and NF- κ B, both of which are involved in key inflammatory pathways and thus linking neuroinflammation and MMP9 [67]. Upon the induction of the neuroinflammatory pathway, MMP9 along with MMP2 and MMP3 can facilitate the proteolysis of the basal lamina, TJ, and extracellular matrix resulting in increased BBB permeability [71, 72]. Inhibitors of MMPs have been shown to restore BBB integrity [73]. In individuals experiencing an exacerbation of MS, MMP9 was found to be elevated in the CSF [74] and treatment with prednisolone was found to restore BBB integrity resulting in a decrease of MMP9 levels in the CSF [75]. Furthermore, in an EAE model of MS in which demyelination is associated with neuroinflammation, treatment with the MMP inhibitor GM-6001 halted the progression of EAE in mice [76].

Accumulation of A β endogenously induces the secretion of MMPs in microglia and astrocytes as a part of the neuroinflammatory pathway [67, 77]. Plasma MMP9 levels are elevated in patients with AD [78]. PCR and immunohistochemistry data show accumulation of a latent/inactive form of MMP9 in the hippocampus of patients with AD [79], which is postulated to be associated with less degradation of A β plaques in the brain. In addition, A β -induced cognitive impairment and neurotoxicity were significantly alleviated in MMP9 homozygous K/O mice and with administration of MMP inhibitors [80]. Together, these studies indicate an important role of MMP9 in AD and MS via BBB dysfunction.

2.2. BBB in Schizophrenia. The role of BBB dysfunction in psychiatric conditions has been far less studied. Some studies have investigated “blood-CSF barrier dysfunction” as measured by CSF-to-serum albumin ratio. Evidence of increased CSF-to-serum albumin ratio has been reported in individuals with schizophrenia (SCZ) [81–83], bipolar disorder (BD) [84], and a mixed sample of inpatients with mood and SCZ spectrum disorders [85]. Given that albumin is not synthesized in the CSF, all albumins present in the CSF originated from the peripheral blood compartment. Thus, these findings of elevated CSF-to-serum albumin in mood and SCZ subjects have been interpreted as potential blood-CSF or BBB dysfunction. A recent controversial study [86] proposed a link between BBB dysfunction and SCZ based on two indirect findings: (a) worse scores in the Cambridge Neurological Inventory in SCZ subjects who were positive for anti-NMDA receptor autoantibodies and had past history of birth complications or head trauma (used as proxies of

BBB disruption) and (b) behavioural changes in ApoE $-/-$ mice (known to display BBB deficiency) after injection of Ig fractions from NMDAR-autoantibodies (NMDAR-AB) seropositive (IgM, IgG, and IgA) subjects compared to serum from control subjects. However, this study has been criticized [87] by (i) using retrospective data to determine birth complications and history of head trauma and assuming that these retrospective events disturbed BBB integrity; (ii) by providing no confocal microscopy images pertaining to their NMDA receptor immunostaining in the presence of NMDAR-AB, thus calling into question their immunostaining results by pointing to other studies [88, 89] which utilized anti-NMDR encephalitis antibodies to visualize NMDAR internalization with confocal microscopy and could not draw the same conclusions; and (iii) by suggesting that the study needed to prove that the injection of patients' IgG reached the brain, bound to NMDAR, and altered receptor levels and functions before drawing strong conclusions using the ApoE $-/-$ mice data.

Therefore, while the study of BBB in psychiatric disorders is still in its infancy, there is converging data showing that SCZ and BD are associated with increased CSF-to-serum albumin ratio.

3. Why BBB Disruption May Be Associated with Bipolar Disorder?

Like most major neuropsychiatric disorders, BD has also been heavily linked with inflammatory processes. In fact, increased neuroinflammation is thought to mediate, at least in part, the cognitive decline as well as the abnormalities observed in gray and white matter content in individuals with BD. In addition, several cohort studies have now demonstrated that BD is associated with excessive mortality rates [90–92]. Compared to the general population, individuals with BD die on average 9 years younger [93], but, more importantly, these striking elevated mortality rates are primarily due to death from natural causes including cardiovascular, respiratory, diabetes, and infectious diseases, all of which have been associated with increased inflammation [93–95]. Below, we propose a novel model where disruption in the BBB is associated with less protection and subsequently more influx of inflammatory material from the periphery to the brain of individuals with BD.

3.1. Inflammation and Oxidative Stress in Bipolar Disorder. Several lines of evidence indicate that BD is associated with increased inflammation and oxidative stress. For instance, the monocyte-T cell theory of mood disorders implicates the inflammatory response system (IRS) as a primary contributor to the neurobiology of BD [96]. This theory is supported in part by evidence of increased levels of proinflammatory cytokines including IL-1, IL-6, and TNF- α in plasma [97, 98], abnormal expression of proinflammatory genes in circulating monocytes [99], and evidence that psychotropics can modulate the immune system [98–101]. Activation of the immune system is linked with neuroinflammation through activation of microglia which is a central player in neuroinflammatory

pathways [97]. A recent PET imaging study using [^{11}C]-(*R*)-PK11195 found greater [^{11}C]-(*R*)-PK11195 binding potential in the right hippocampus and a similar nonsignificant trend in the left hippocampus of bipolar type I subjects, suggesting increased microglial activity and neuroinflammation in these brain areas. Notably, oxidative damage to RNA [102] and decreased expression of growth associated proteins [103], both believed to be involved in neuroinflammation [104], have been observed in postmortem hippocampal samples from BD subjects. Disruption of mitochondria, responsible for the regulation of apoptosis and intracellular calcium levels, has been increasingly implicated as a contributing factor in the oxidative stress facet of BD perhaps through decreased activity of mitochondrial complex I [105]. Moreover, studies conducted in the peripheral blood have consistently found increased markers of oxidative damage to lipids, RNA, and DNA in BD [106, 107].

3.2. Oligodendrocyte and Myelin Damage in Bipolar Disorder. Oligodendrocytes facilitate the formation and stability of neural circuits by insulating axons with myelin sheath. In the last several years, there has been increasing attention to changes in white matter and oligodendrocyte structure/function in BD. For instance, oligodendrocyte-specific mRNA markers including OLIG2, SOX10, GALC, MAG, PLP1, CLDN11, MOG, ERBB3, and TF were found to be downregulated in the brain of individuals with BD [108]. Uranova et al. used electron microscopy to analyze ultrastructural alterations in oligodendrocytes in the prefrontal cortex of individuals with BD [109]. The oligodendrocyte cells in BD were found to be surrounded by astroglial cells and displayed strong signs of apoptosis and necrosis. In this qualitative study, apoptosis was characterized by nuclear chromatin aggregation, cell shrinkage, and the preservation of organelles while necrosis was characterized by chromatin condensation, cell swelling, and membrane lysis of organelles. Previously, this group described a decrease in oligodendrocyte density in layer VI of BD patients (31%) [110], further implicating oligodendrocyte disruption in the pathophysiology of BD. Furthermore, several imaging, genetic, and postmortem tissue analyses have shown myelin abnormalities in BD subjects [108, 111–114], establishing a link between oligodendrocyte dysfunction and myelin damage in BD.

3.3. Implication of Inflammation and Oxidative Stress in the Treatment of Bipolar Disorder. One of the key questions in BD research has been the extent to which available treatments may reverse/prevent inflammation and oxidative stress. While an extensive review of the effects of pharmacological and nonpharmacological treatments on inflammation and oxidative stress is beyond the objective of the present paper, there is growing evidence that mood stabilizing and antidepressant agents possess anti-inflammatory and antioxidant properties (as reviewed in [115, 116]). Lithium, the hallmark treatment of BD, was shown to aid in the defence against oxidative stress by upregulating mitochondrial complexes I and II [117]. Relevant to the notion that lithium can protect against ROS-induced damage, previous studies

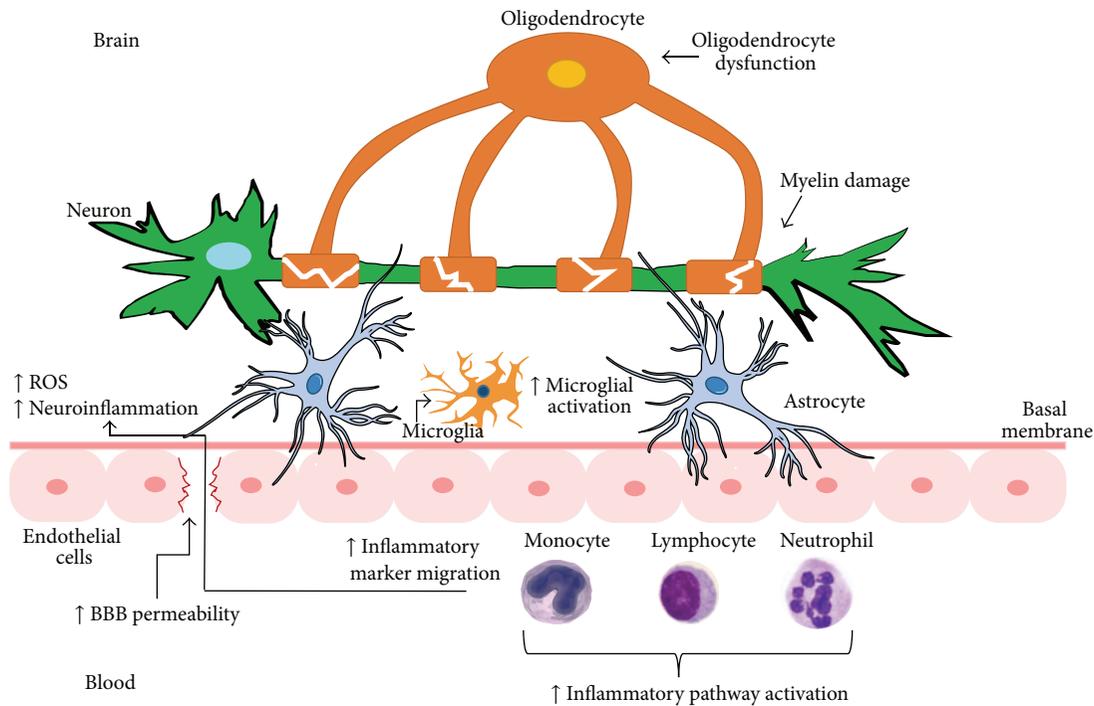


FIGURE 1: Proposed model of blood-brain barrier (BBB) disruption in bipolar disorder. Increased BBB permeability through the endothelial cells (pink) and basal membrane (dark pink) may facilitate increased migration of inflammatory molecules into the brain. Activation of microglial cells (light orange) and an increase in reactive oxygen species (ROS) would amplify neuroinflammatory processes and ultimately induce damage in the myelin sheath, either directly via lipid/protein oxidation or indirectly via oligodendrocyte dysfunction (dark orange).

have shown that oxidative stress can effect BBB permeability, particularly by affecting the integral TJ protein occludin [118, 119]. Administration of tempol, a ROS scavenger, to λ -carrageenan-induced peripheral inflammatory pain (CIP) rats attenuated (14)C-sucrose and (3)H-codeine uptake in the brain and provided protection to occludin, thus preserving BBB integrity [120]. Future studies investigating the ability of lithium to protect against BBB disruption are warranted.

Lithium also downregulates the arachidonic acid-prostaglandins (PGs) pathway [121, 122] which has been implicated with neuroinflammation [123, 124]. More specifically, chronic lithium treatment resulted in decreased AA to PGs turnover, decreased activity of cyclooxygenase-2 (COX-2), the enzyme responsible for converting AA to PGs, and PG-E₂ concentration in rat brain [125]. Another pre-clinical study showed that lithium treatment significantly increased levels of 17-hydroxy-docosahexaenoic acid [126], which possesses known anti-inflammatory properties [127, 128]. Furthermore, several *in vitro* and *in vivo* studies have shown that lithium treatment results in the attenuation of proinflammatory cytokines including TNF- α [129–131], IL-1 β [132–134], IL-6 [135–137], and interferon- γ (INF- γ) [138–140] while increasing the secretion of the anti-inflammatory cytokines IL-2 [141–143] and IL-10 [134, 140, 144]. With respect to oligodendrocyte function, lithium treatment has been shown to increase oligodendrocyte proliferation and increase myelination of optic nerves in mice [145].

In summary, there is overwhelming data pointing towards inflammatory and oxidative stress modulation by lithium and other psychotropic agents. Given that inflammation and oxidative stress have been associated with disruption in the BBB integrity, a natural next step for future studies is to test whether lithium and/or other mood stabilizing agents used in the treatment of BD may protect against BBB damage.

3.4. A Novel Model of BBB Disruption in Bipolar Disorder.

Decades of research has implicated increased peripheral inflammation and oxidative stress, as well as oligodendrocyte and white matter changes in the pathophysiology of BD. This is in line with a number of cohort studies showing increased mortality rates due to general medical conditions associated with inflammation and oxidative stress. Further evidence is provided by studies showing that first-line treatments for BD, such as lithium, can modulate inflammatory and oxidative stress pathways. More recently, imaging and postmortem studies have provided evidence of increased neuroinflammation in BD through excessive microglial activation. Considering the close anatomical proximity of microglia, oligodendrocytes, and astrocytes to the BBB, and the increasing attention of BBB disruption in other neuropsychiatric conditions, such as AD, MS, and SCZ, we propose a novel model of BBB dysfunction in BD wherein transient or persistent loss of BBB integrity is associated with decreased CNS protection and increased permeability of proinflammatory (e.g., cytokines, reactive oxygen species) substances from the peripheral

blood into the brain. This will trigger the activation of microglial cells and promote localized damage to oligodendrocytes and the myelin sheath, thereby compromising myelination and neural circuit integrity (Figure 1).

While we could not identify a study that directly examined the BBB integrity in BD, a recent study found increased levels of MMP9, which increases BBB permeability during proinflammatory states (see Section 2.1.3), in bipolar depression [146]. In addition, both manic and depressive episodes are associated with increased levels of proinflammatory cytokines [147] and, therefore, it is conceivable that BD subjects may experience a transient increase in the BBB permeability during each major mood episode. Also, it is well established that most drugs of abuse disrupt the BBB integrity [148, 149]. Given the exceeding rates of drug abuse in individuals with BD, it is also likely that excessive drug use can contribute to the disruption in BBB permeability in a substantial proportion of individuals with BD. This is in line with an elegant twin study showing that peripheral proinflammatory states in BD are primarily the result of environmental as opposed to genetic factors [150].

3.5. Future Directions. It is imperative to test this model by further analyzing the role of the BBB in BD. Currently, at least a couple of brain imaging techniques are available to test the hypothesis of disrupted BBB structure or function directly in individuals with BD. One possibility would be the use of dynamic contrast-enhanced MRI (DCE-MRI) as a method for studying BBB disruption *in vivo* [151]. Another available technique is the use of [¹¹C]-verapamil to study the function of the P-glycoprotein (Pgp) transporter at the blood-brain barrier (BBB) with PET [152]. Finally, the use of *in vivo* and *in vitro* preclinical models may be particularly useful to test whether lithium and other medications commonly used in the treatment of BD can reverse and/or prevent BBB damage. If a link between BD and BBB disruption is established, this would not only advance the knowledge on the neurobiology of BD but also open numerous possibilities to investigate new treatment pathways (e.g., MMP inhibitors [153], ROS scavengers [120]) for this devastating major mental illness.

Conflict of Interests

The authors report no conflict of interests regarding the content of this paper.

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References

- [1] D. Ribatti, B. Nico, E. Crivellato, and M. Artico, "Development of the blood-brain barrier: a historical point of view," *Anatomical Record—Part B New Anatomist*, vol. 289, no. 1, pp. 3–8, 2006.
- [2] A. D. Wong, M. Ye, A. F. Levy, J. D. Rothstein, D. E. Bergles, and P. C. Searson, "The blood-brain barrier: an engineering perspective," *Frontiers in Neuroengineering*, vol. 6, article 7, 2013.
- [3] M. W. Bradbury, "The blood-brain barrier," *Experimental Physiology*, vol. 78, no. 4, pp. 453–472, 1993.
- [4] B. V. Zlokovic, "The blood-brain barrier in health and chronic neurodegenerative disorders," *Neuron*, vol. 57, no. 2, pp. 178–201, 2008.
- [5] T. Nitta, M. Hata, S. Gotoh et al., "Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice," *The Journal of Cell Biology*, vol. 161, no. 3, pp. 653–660, 2003.
- [6] S.-W. Lee, W. J. Kim, Y. K. Choi et al., "SSECKS regulates angiogenesis and tight junction formation in blood-brain barrier," *Nature Medicine*, vol. 9, no. 7, pp. 900–906, 2003.
- [7] B. Engelhardt and L. Sorokin, "The blood-brain and the blood-cerebrospinal fluid barriers: function and dysfunction," *Seminars in Immunopathology*, vol. 31, no. 4, pp. 497–511, 2009.
- [8] C. E. Johanson, E. G. Stopa, and P. N. McMillan, "The blood-cerebrospinal fluid barrier: structure and functional significance," *Methods in Molecular Biology*, vol. 686, pp. 101–131, 2011.
- [9] J. Laterra, R. Keep, L. A. Betz, and G. W. Goldstein, *Blood—Cerebrospinal Fluid Barrier*, Lippincott-Raven, 1999.
- [10] C. Nilsson, M. Lindvall-Axelsson, and C. Owman, "Neuroendocrine regulatory mechanisms in the choroid plexus-cerebrospinal fluid system," *Brain Research Reviews*, vol. 17, no. 2, pp. 109–138, 1992.
- [11] A. V. Lorenzo, "Factors governing the composition of the cerebrospinal fluid," *Experimental Eye Research*, vol. 25, supplement, pp. 205–228, 1977.
- [12] W. M. Pardridge, "Molecular biology of the blood-brain barrier," *Molecular Biotechnology*, vol. 30, no. 1, pp. 57–69, 2005.
- [13] W. M. Pardridge, J. Eisenberg, and J. Yang, "Human blood-brain barrier transferrin receptor," *Metabolism*, vol. 36, no. 9, pp. 892–895, 1987.
- [14] R. Shawahna, Y. Uchida, X. Declèves et al., "Transcriptomic and quantitative proteomic analysis of transporters and drug metabolizing enzymes in freshly isolated human brain microvessels," *Molecular Pharmaceutics*, vol. 8, no. 4, pp. 1332–1341, 2011.
- [15] Y. Uchida, S. Ohtsuki, Y. Katsukura et al., "Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors," *Journal of Neurochemistry*, vol. 117, no. 2, pp. 333–345, 2011.
- [16] E. Gould and C. G. Gross, "Neurogenesis in adult mammals: some progress and problems," *Journal of Neuroscience*, vol. 22, no. 3, pp. 619–623, 2002.
- [17] I. A. Simpson, A. Carruthers, and S. J. Vannucci, "Supply and demand in cerebral energy metabolism: the role of nutrient transporters," *Journal of Cerebral Blood Flow and Metabolism*, vol. 27, no. 11, pp. 1766–1791, 2007.
- [18] R. A. Hawkins, R. L. O’Kane, I. A. Simpson, and J. R. Viña, "Structure of the blood-brain barrier and its role in the transport of amino acids," *Journal of Nutrition*, vol. 136, no. 1, supplement, pp. 218S–226S, 2006.
- [19] B. V. Zlokovic, M. N. Lipovac, D. J. Begley, H. Davson, and L. Rakić, "Transport of leucine-enkephalin across the blood-brain barrier in the perfused guinea pig brain," *Journal of Neurochemistry*, vol. 49, no. 1, pp. 310–315, 1987.
- [20] B. V. Zlokovic, S. Hyman, J. G. McComb, M. N. Lipovac, G. Tang, and H. Davson, "Kinetics of arginine-vasopressin uptake at the blood-brain barrier," *Biochimica et Biophysica Acta*, vol. 1025, no. 2, pp. 191–198, 1990.
- [21] W. A. Banks, "The CNS as a target for peptides and peptide-based drugs," *Expert Opinion on Drug Delivery*, vol. 3, no. 6, pp. 707–712, 2006.

- [22] B. V. Zlokovic, S. Jovanovic, W. Miao, S. Samara, S. Verma, and C. L. Farrell, "Differential regulation of leptin transport by the choroid plexus and blood-brain barrier and high affinity transport systems for entry into hypothalamus and across the blood-cerebrospinal fluid barrier," *Endocrinology*, vol. 141, no. 4, pp. 1434–1441, 2000.
- [23] S. Meresse, C. Delbart, J.-C. Fruchart, and R. Cecchelli, "Low-density lipoprotein receptor on endothelium of brain capillaries," *Journal of Neurochemistry*, vol. 53, no. 2, pp. 340–345, 1989.
- [24] R. Deane, A. Sagare, K. Hamm et al., "IgG-assisted age-dependent clearance of Alzheimer's amyloid β peptide by the blood-brain barrier neonatal Fc receptor," *Journal of Neuroscience*, vol. 25, no. 50, pp. 11495–11503, 2005.
- [25] J. Grotta, M. Moskowitz, T. Jacobs et al., *Report of the Stroke Progress Review Group*, National Institute of Neurological Disorders and Stroke, 2002, http://www.ninds.nih.gov/about_ninds/groups/stroke_prg/strokeprgreport-4-23-02.pdf.
- [26] N. J. Abbott, L. Rönnbäck, and E. Hansson, "Astrocyte-endothelial interactions at the blood-brain barrier," *Nature Reviews Neuroscience*, vol. 7, no. 1, pp. 41–53, 2006.
- [27] E. A. Neuwelt, B. Bauer, C. Fahlke et al., "Engaging neuroscience to advance translational research in brain barrier biology," *Nature Reviews Neuroscience*, vol. 12, no. 3, pp. 169–182, 2011.
- [28] N. J. Abbott and A. Friedman, "Overview and introduction: the blood-brain barrier in health and disease," *Epilepsia*, vol. 53, supplement 6, pp. 1–6, 2012.
- [29] S. Najjar, D. M. Pearlman, S. Hirsch et al., "Brain biopsy findings link major depressive disorder to neuroinflammation, oxidative stress, and neurovascular dysfunction: a case report," *Biological Psychiatry*, vol. 75, no. 12, pp. e23–e26, 2014.
- [30] M. Simard, G. Arcuino, T. Takano, Q. S. Liu, and M. Nedergaard, "Signaling at the gliovascular interface," *The Journal of Neuroscience*, vol. 23, no. 27, pp. 9254–9262, 2003.
- [31] G. J. del Zoppo, "The neurovascular unit, matrix proteases, and innate inflammation," *Annals of the New York Academy of Sciences*, vol. 1207, pp. 46–49, 2010.
- [32] C. R. Figley and P. W. Stroman, "The role(s) of astrocytes and astrocyte activity in neurometabolism, neurovascular coupling, and the production of functional neuroimaging signals," *European Journal of Neuroscience*, vol. 33, no. 4, pp. 577–588, 2011.
- [33] G. R. J. Gordon, S. J. Mulligan, and B. A. MacVicar, "Astrocyte control of the cerebrovasculature," *Glia*, vol. 55, no. 12, pp. 1214–1221, 2007.
- [34] J. A. Filosa, "Vascular tone and neurovascular coupling: considerations toward an improved *in vitro* model," *Frontiers in Neuroenergetics*, vol. 2, article 16, 2010.
- [35] R. Kraemer and B. L. Hempstead, "Neurotrophins: novel mediators of angiogenesis," *Frontiers in Bioscience*, vol. 8, pp. s1181–s1186, 2003.
- [36] N. L. Ward and J. C. Lamanna, "The neurovascular unit and its growth factors: coordinated response in the vascular and nervous systems," *Neurological Research*, vol. 26, no. 8, pp. 870–883, 2004.
- [37] V. Muoio, P. B. Persson, and M. M. Sendeski, "The neurovascular unit—concept review," *Acta Physiologica*, vol. 210, no. 4, pp. 790–798, 2014.
- [38] S. Soker, H.-Q. Miao, M. Nomi, S. Takashima, and M. Klagsbrun, "VEGF165 mediates formation of complexes containing VEGFR-2 and neuropilin-1 that enhance VEGF165-receptor binding," *Journal of Cellular Biochemistry*, vol. 85, no. 2, pp. 357–368, 2002.
- [39] A. Sanchez, D. Tripathy, J. Luo, X. Yin, J. Martinez, and P. Grammas, "Neurovascular unit and the effects of dosage in VEGF toxicity: role for oxidative stress and thrombin," *Journal of Alzheimer's Disease*, vol. 34, no. 1, pp. 281–291, 2013.
- [40] R. D. Fields and B. Stevens-Graham, "Neuroscience: new insights into neuron-glia communication," *Science*, vol. 298, no. 5593, pp. 556–562, 2002.
- [41] P. Kowiański, G. Lietzau, A. Steliga, M. Waśkow, and J. Moryś, "The astrocytic contribution to neurovascular coupling—still more questions than answers?" *Neuroscience Research*, vol. 75, no. 3, pp. 171–183, 2013.
- [42] K. L. Lavoie, R. Pelletier, A. Arsenault, J. Dupuis, and S. L. Bacon, "Association between clinical depression and endothelial function measured by forearm hyperemic reactivity," *Psychosomatic Medicine*, vol. 72, no. 1, pp. 20–26, 2010.
- [43] P. Politi, N. Brondino, and E. Emanuele, "Increased proapoptotic serum activity in patients with chronic mood disorders," *Archives of Medical Research*, vol. 39, no. 2, pp. 242–245, 2008.
- [44] V. Valkanova and K. P. Ebmeier, "Vascular risk factors and depression in later life: a systematic review and meta-analysis," *Biological Psychiatry*, vol. 73, no. 5, pp. 406–413, 2013.
- [45] B. V. Zlokovic, "Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders," *Nature Reviews Neuroscience*, vol. 12, no. 12, pp. X723–738, 2011.
- [46] S. Takeda, N. Sato, and R. Morishita, "Systemic inflammation, blood-brain barrier vulnerability and cognitive/non-cognitive symptoms in Alzheimer disease: relevance to pathogenesis and therapy," *Frontiers in Aging Neuroscience*, vol. 6, article 171, 2014.
- [47] E. C. Phillips, C. L. Croft, K. Kurbatskaya et al., "Astrocytes and neuroinflammation in Alzheimer's disease," *Biochemical Society Transactions*, vol. 42, no. 5, pp. 1321–1325, 2014.
- [48] R. Deane, S. D. Yan, R. K. Subramanyam et al., "RAGE mediates amyloid- β peptide transport across the blood-brain barrier and accumulation in brain," *Nature Medicine*, vol. 9, no. 7, pp. 907–913, 2003.
- [49] J. E. Donahue, S. L. Flaherty, C. E. Johanson et al., "RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease," *Acta Neuropathologica*, vol. 112, no. 4, pp. 405–415, 2006.
- [50] R. Deane, Z. Wu, A. Sagare et al., "LRP/amyloid β -peptide interaction mediates differential brain efflux of A β isoforms," *Neuron*, vol. 43, no. 3, pp. 333–344, 2004.
- [51] R. D. Bell, R. Deane, N. Chow et al., "SRF and myocardin regulate LRP-mediated amyloid- β clearance in brain vascular cells," *Nature Cell Biology*, vol. 11, no. 2, pp. 143–153, 2009.
- [52] M. A. Erickson, P. E. Hartvigson, Y. Morofuji, J. B. Owen, D. A. Butterfield, and W. A. Banks, "Lipopolysaccharide impairs amyloid beta efflux from brain: altered vascular sequestration, cerebrospinal fluid reabsorption, peripheral clearance and transporter function at the blood-brain barrier," *Journal of Neuroinflammation*, vol. 9, article 150, 2012.
- [53] M. Shibata, S. Yamada, S. Ram Kumar et al., "Clearance of Alzheimer's amyloid- β 1-40 peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier," *Journal of Clinical Investigation*, vol. 106, no. 12, pp. 1489–1499, 2000.
- [54] R. D. Bell, A. P. Sagare, A. E. Friedman et al., "Transport pathways for clearance of human Alzheimer's amyloid β -peptide and apolipoproteins E and J in the mouse central nervous system," *Journal of Cerebral Blood Flow and Metabolism*, vol. 27, no. 5, pp. 909–918, 2007.
- [55] A. H. Abuznait and A. Kaddoumi, "Role of ABC transporters in the pathogenesis of Alzheimer's disease," *ACS Chemical Neuroscience*, vol. 3, no. 11, pp. 820–831, 2012.

- [56] D. Kuhnke, G. Jedlitschky, M. Grube et al., "MDRI-P-glycoprotein (ABCB1) mediates transport of Alzheimer's amyloid- β peptides—implications for the mechanisms of A β clearance at the blood-brain barrier," *Brain Pathology*, vol. 17, no. 4, pp. 347–353, 2007.
- [57] J. R. Cirrito, R. Deane, A. M. Fagan et al., "P-glycoprotein deficiency at the blood-brain barrier increases amyloid- β deposition in an Alzheimer disease mouse model," *Journal of Clinical Investigation*, vol. 115, no. 11, pp. 3285–3290, 2005.
- [58] H. Lassmann, "Multiple sclerosis: is there neurodegeneration independent from inflammation?" *Journal of the Neurological Sciences*, vol. 259, no. 1-2, pp. 3–6, 2007.
- [59] B. D. Trapp and K.-A. Nave, "Multiple sclerosis: an immune or neurodegenerative disorder?" *Annual Review of Neuroscience*, vol. 31, pp. 247–269, 2008.
- [60] R. M. Ransohoff, P. Kivisäkk, and G. Kidd, "Three or more routes for leukocyte migration into the central nervous system," *Nature Reviews Immunology*, vol. 3, no. 7, pp. 569–581, 2003.
- [61] B. Engelhardt and R. M. Ransohoff, "The ins and outs of T-lymphocyte trafficking to the CNS: anatomical sites and molecular mechanisms," *Trends in Immunology*, vol. 26, no. 9, pp. 485–495, 2005.
- [62] S. Man, E. E. Ubogu, and R. M. Ransohoff, "Inflammatory cell migration into the central nervous system: a few new twists on an old tale," *Brain Pathology*, vol. 17, no. 2, pp. 243–250, 2007.
- [63] S. P. Cramer, H. Simonsen, J. L. Frederiksen, E. Rostrup, and H. B. W. Larsson, "Abnormal blood-brain barrier permeability in normal appearing white matter in multiple sclerosis investigated by MRI," *NeuroImage: Clinical*, vol. 4, pp. 182–189, 2014.
- [64] J. Kraus, K. Voigt, A. M. Schuller et al., "Interferon- β stabilizes barrier characteristics of the blood-brain barrier in four different species in vitro," *Multiple Sclerosis*, vol. 14, no. 6, pp. 843–852, 2008.
- [65] F. Shimizu, A. Tasaki, Y. Sano et al., "Sera from remitting and secondary progressive multiple sclerosis patients disrupt the blood-brain barrier," *PLoS ONE*, vol. 9, no. 3, Article ID e92872, 2014.
- [66] B. Cauwe, P. E. van den Steen, and G. Opdenakker, "The biochemical, biological, and pathological kaleidoscope of cell surface substrates processed by matrix metalloproteinases," *Critical Reviews in Biochemistry and Molecular Biology*, vol. 42, no. 3, pp. 113–185, 2007.
- [67] G. A. Rosenberg, "Matrix metalloproteinases and their multiple roles in neurodegenerative diseases," *The Lancet Neurology*, vol. 8, no. 2, pp. 205–216, 2009.
- [68] V. W. Yong, C. Power, P. Forsyth, and D. R. Edwards, "Metalloproteinases in biology and pathology of the nervous system," *Nature Reviews Neuroscience*, vol. 2, no. 7, pp. 502–511, 2001.
- [69] J. Gardner and A. Ghorpade, "Tissue inhibitor of metalloproteinase (TIMP)-1: the TIMPed balance of matrix metalloproteinases in the central nervous system," *Journal of Neuroscience Research*, vol. 74, no. 6, pp. 801–806, 2003.
- [70] E. Stomrud, M. Björkqvist, S. Janciauskiene, L. Minthon, and O. Hansson, "Alterations of matrix metalloproteinases in the healthy elderly with increased risk of prodromal Alzheimer's disease," *Alzheimer's Research & Therapy*, vol. 2, no. 3, article 20, 2010.
- [71] G. F. Hamann, Y. Okada, R. Fitridge, G. J. del Zoppo, and J. T. Povlishock, "Microvascular basal lamina antigens disappear during cerebral ischemia and reperfusion," *Stroke*, vol. 26, no. 11, pp. 2120–2126, 1995.
- [72] J. H. Heo, J. Lucero, T. Abumiya, J. A. Koziol, B. R. Copeland, and G. J. Del Zoppo, "Matrix metalloproteinases increase very early during experimental focal cerebral ischemia," *Journal of Cerebral Blood Flow and Metabolism*, vol. 19, no. 6, pp. 624–633, 1999.
- [73] Y. Gasche, J.-C. Copin, T. Sugawara, M. Fujimura, and P. H. Chan, "Matrix metalloproteinase inhibition prevents oxidative stress-associated blood-brain barrier disruption after transient focal cerebral ischemia," *Journal of Cerebral Blood Flow and Metabolism*, vol. 21, no. 12, pp. 1393–1400, 2001.
- [74] K. Gijbels, S. Masure, H. Carton, and G. Opdenakker, "Gelatinase in the cerebrospinal fluid of patients with multiple sclerosis and other inflammatory neurological disorders," *Journal of Neuroimmunology*, vol. 41, no. 1, pp. 29–34, 1992.
- [75] G. A. Rosenberg, J. E. Dencoff, N. Correa Jr., M. Reiners, and C. C. Ford, "Effect of steroids on CSF matrix metalloproteinases in multiple sclerosis: relation to blood-brain barrier injury," *Neurology*, vol. 46, no. 6, pp. 1626–1632, 1996.
- [76] K. Gijbels, R. E. Galardy, and L. Steinman, "Reversal of experimental autoimmune encephalomyelitis with a hydroxamate inhibitor of matrix metalloproteinases," *The Journal of Clinical Investigation*, vol. 94, no. 6, pp. 2177–2182, 1994.
- [77] C. Reitz, F. J. A. van Rooij, H. D. Soares et al., "Matrix metalloproteinase 3 haplotypes and plasma amyloid beta levels: the Rotterdam Study," *Neurobiology of Aging*, vol. 31, no. 4, pp. 715–718, 2010.
- [78] S. Lorenzl, D. S. Albers, N. Relkin et al., "Increased plasma levels of matrix metalloproteinase-9 in patients with Alzheimer's disease," *Neurochemistry International*, vol. 43, no. 3, pp. 191–196, 2003.
- [79] J. R. Backstrom, G. P. Lim, M. J. Cullen, and Z. A. Tökés, "Matrix metalloproteinase-9 (MMP-9) is synthesized in neurons of the human hippocampus and is capable of degrading the amyloid-beta peptide (1-40)," *Journal of Neuroscience*, vol. 16, no. 24, pp. 7910–7919, 1996.
- [80] H. Mizoguchi, K. Takuma, E. Fukuzaki et al., "Matrix metalloproteinase-9 inhibition improves amyloid β -mediated cognitive impairment and neurotoxicity in mice," *Journal of Pharmacology and Experimental Therapeutics*, vol. 331, no. 1, pp. 14–22, 2009.
- [81] D. G. Kirch, C. A. Kaufmann, N. M. Papadopoulos, B. Martin, and D. R. Weinberger, "Abnormal cerebrospinal fluid protein indices in schizophrenia," *Biological Psychiatry*, vol. 20, no. 10, pp. 1039–1046, 1985.
- [82] K. Bauer and J. Kornhuber, "Blood-cerebrospinal fluid barrier in schizophrenic patients," *European Archives of Psychiatry and Neurological Sciences*, vol. 236, no. 5, pp. 257–259, 1987.
- [83] E. G. Severance, K. L. Gressitt, A. Alaedini et al., "IgG dynamics of dietary antigens point to cerebrospinal fluid barrier or flow dysfunction in first-episode schizophrenia," *Brain, Behavior, and Immunity*, vol. 44, pp. 148–158, 2015.
- [84] H. Zetterberg, J. Jakobsson, M. Redsäter et al., "Blood-cerebrospinal fluid barrier dysfunction in patients with bipolar disorder in relation to antipsychotic treatment," *Psychiatry Research*, vol. 217, no. 3, pp. 143–146, 2014.
- [85] K. Bechter, H. Reiber, S. Herzog, D. Fuchs, H. Tumani, and H. G. Maxeiner, "Cerebrospinal fluid analysis in affective and schizophrenic spectrum disorders: identification of subgroups with immune responses and blood-CSF barrier dysfunction," *Journal of Psychiatric Research*, vol. 44, no. 5, pp. 321–330, 2010.

- [86] C. Hammer, B. Stepniak, A. Schneider et al., "Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity," *Molecular Psychiatry*, vol. 19, pp. 1143–1149, 2013.
- [87] M. J. Titulaer and J. Dalmau, "Antibodies to NMDA receptor, blood-brain barrier disruption and schizophrenia: a theory with unproven links," *Molecular Psychiatry*, vol. 19, article 1054, 2014.
- [88] E. G. Hughes, X. Peng, A. J. Gleichman et al., "Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis," *The Journal of Neuroscience*, vol. 30, no. 17, pp. 5866–5875, 2010.
- [89] E. Moscato, X. Peng, T. Parsons, J. Dalmau, and R. Balice-Gordon, "No title," *Journal of Neuroimmunology*, vol. 253, no. 145, 2012, Abstrat 408.
- [90] C. M. Ramsey, A. P. Spiraa, R. Mojtabai, W. W. Eaton, K. Roth, and H. B. Leeb, "Lifetime manic spectrum episodes and all-cause mortality: 26-year follow-up of the NIMH Epidemiologic Catchment Area Study," *Journal of Affective Disorders*, vol. 151, no. 1, pp. 337–342, 2013.
- [91] J. G. Fiedorowicz, D. A. Solomon, J. Endicott et al., "Manic/hypomanic symptom burden and cardiovascular mortality in bipolar disorder," *Psychosomatic Medicine*, vol. 71, no. 6, pp. 598–606, 2009.
- [92] F. Angst, H. H. Stassen, P. J. Clayton, and J. Angst, "Mortality of patients with mood disorders: follow-up over 34–38 years," *Journal of Affective Disorders*, vol. 68, no. 2-3, pp. 167–181, 2002.
- [93] C. Crump, K. Sundquist, M. A. Winkleby, and J. Sundquist, "Comorbidities and mortality in bipolar disorder: a Swedish national cohort study," *JAMA Psychiatry*, vol. 70, no. 9, pp. 931–939, 2013.
- [94] D. J. Kupfer, "The increasing medical burden in bipolar disorder," *Journal of the American Medical Association*, vol. 293, no. 20, pp. 2528–2530, 2005.
- [95] U. Hoang, R. Stewart, and M. J. Goldacre, "Mortality after hospital discharge for people with schizophrenia or bipolar disorder: retrospective study of linked English hospital episode statistics, 1999–2006," *British Medical Journal*, vol. 343, no. 7824, Article ID d5422, 2011.
- [96] M. Maes, R. Smith, and S. Scharpe, "The monocyte-T-lymphocyte hypothesis of major depression," *Psychoneuroendocrinology*, vol. 20, no. 2, pp. 111–116, 1995.
- [97] L. Stertz, P. V. S. Magalhães, and F. Kapczinski, "Is bipolar disorder an inflammatory condition? The relevance of microglial activation," *Current Opinion in Psychiatry*, vol. 26, no. 1, pp. 19–26, 2013.
- [98] B. C. M. B. Haarman, R. F. Riemersma-van der Lek, J. C. de Groot et al., "Neuroinflammation in bipolar disorder—a [¹¹C]-(R)-PK11195 positron emission tomography study," *Brain, Behavior, and Immunity*, vol. 40, pp. 219–225, 2014.
- [99] R. C. Padmos, M. H. J. Hillegers, E. M. Knijff et al., "A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes," *Archives of General Psychiatry*, vol. 65, no. 4, pp. 395–407, 2008.
- [100] Ł. Drzyzga, E. Obuchowicz, A. Marciniowska, and Z. S. Herman, "Cytokines in schizophrenia and the effects of antipsychotic drugs," *Brain, Behavior, and Immunity*, vol. 20, no. 6, pp. 532–545, 2006.
- [101] B. B. C. M. Haarman, R. F. R.-V. der Lek, H. Burger et al., "Relationship between clinical features and inflammation-related monocyte gene expression in bipolar disorder—towards a better understanding of psychoimmunological interactions," *Bipolar Disorders*, vol. 16, no. 2, pp. 137–150, 2014.
- [102] Y. Che, J.-F. Wang, L. Shao, and L. T. Young, "Oxidative damage to RNA but not DNA in the hippocampus of patients with major mental illness," *Journal of Psychiatry and Neuroscience*, vol. 35, no. 5, pp. 296–302, 2010.
- [103] S. Y. Tian, J.-F. Wang, Y. B. Bezchlibnyk, and L. T. Young, "Immunoreactivity of 43 kDa growth-associated protein is decreased in post mortem hippocampus of bipolar disorder and schizophrenia," *Neuroscience Letters*, vol. 411, no. 2, pp. 123–127, 2007.
- [104] N. Kato, K. Nemoto, H. Arino, and K. Fujikawa, "Influence of peripheral inflammation on growth-associated phosphoprotein (GAP-43) expression in dorsal root ganglia and on nerve recovery after crush injury," *Neuroscience Research*, vol. 45, no. 3, pp. 297–303, 2003.
- [105] G. Scola, H. K. Kim, L. T. Young, and A. C. Andreazza, "A fresh look at complex i in microarray data: clues to understanding disease-specific mitochondrial alterations in bipolar disorder," *Biological Psychiatry*, vol. 73, no. 2, pp. e4–e5, 2013.
- [106] A. C. Andreazza, M. Kauer-Sant'Anna, B. N. Frey et al., "Oxidative stress markers in bipolar disorder: a meta-analysis," *Journal of Affective Disorders*, vol. 111, no. 2-3, pp. 135–144, 2008.
- [107] N. C. Brown, A. C. Andreazza, and L. T. Young, "An updated meta-analysis of oxidative stress markers in bipolar disorder," *Psychiatry Research*, vol. 218, no. 1-2, pp. 61–68, 2014.
- [108] C. Konradi, S. E. Sullivan, and H. B. Clay, "Mitochondria, oligodendrocytes and inflammation in bipolar disorder: evidence from transcriptome studies points to intriguing parallels with multiple sclerosis," *Neurobiology of Disease*, vol. 45, no. 1, pp. 37–47, 2012.
- [109] N. Uranova, D. Orlovskaya, O. Vikhreva et al., "Electron microscopy of oligodendroglia in severe mental illness," *Brain Research Bulletin*, vol. 55, no. 5, pp. 597–610, 2001.
- [110] D. D. Orlovskaya, V. M. Vostrikov, V. I. Rachmanova, and N. A. Uranova, "Decreased numerical density of oligodendroglial cells in postmortem prefrontal cortex in schizophrenia, bipolar affective disorder and major depression," *Schizophrenia Research*, vol. 41, no. 1, pp. 105–106, 2000.
- [111] S. Heng, A. W. Song, and K. Sim, "White matter abnormalities in bipolar disorder: insights from diffusion tensor imaging studies," *Journal of Neural Transmission*, vol. 117, no. 5, pp. 639–654, 2010.
- [112] B. P. Sokolov, "Oligodendroglial abnormalities in schizophrenia, mood disorders and substance abuse. Comorbidity, shared traits, or molecular phenocopies?" *The International Journal of Neuropsychopharmacology*, vol. 10, no. 4, pp. 547–555, 2007.
- [113] C. Aston, L. Jiang, and B. P. Sokolov, "Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder," *Molecular Psychiatry*, vol. 10, no. 3, pp. 309–322, 2005.
- [114] N. R. Herring and C. Konradi, "Myelin, copper, and the cuprizone model of schizophrenia," *Frontiers in Bioscience*, vol. 3, no. 1, pp. 23–40, 2011.
- [115] B. S. Diniz, R. Machado-Vieira, and O. V. Forlenza, "Lithium and neuroprotection: translational evidence and implications for the treatment of neuropsychiatric disorders," *Neuropsychiatric Disease and Treatment*, vol. 9, pp. 493–500, 2013.
- [116] G. A. Behr, J. C. F. Moreira, and B. N. Frey, "Preclinical and clinical evidence of antioxidant effects of antidepressant agents: implications for the pathophysiology of major depressive disorder," *Oxidative Medicine and Cellular Longevity*, vol. 2012, Article ID 609421, 13 pages, 2012.

- [117] I. C. Maurer, P. Schippel, and H.-P. Volz, "Lithium-induced enhancement of mitochondrial oxidative phosphorylation in human brain tissue," *Bipolar Disorders*, vol. 11, no. 5, pp. 515–522, 2009.
- [118] P. M. Cummins, "Occludin: one protein, many forms," *Molecular and Cellular Biology*, vol. 32, no. 2, pp. 242–250, 2012.
- [119] L. González-Mariscal, R. Tapia, and D. Chamorro, "Crosstalk of tight junction components with signaling pathways," *Biochimica et Biophysica Acta*, vol. 1778, no. 3, pp. 729–756, 2008.
- [120] J. J. Lochhead, G. Mccaffrey, L. Sanchez-Covarrubias et al., "Tempol modulates changes in xenobiotic permeability and occludin oligomeric assemblies at the blood-brain barrier during inflammatory pain," *American Journal of Physiology: Heart and Circulatory Physiology*, vol. 302, no. 3, pp. H582–H593, 2012.
- [121] D. L. Murphy, C. Donnelly, and J. Moskowitz, "Inhibition by lithium of prostaglandin E1 and norepinephrine effects on cyclic adenosine monophosphate production in human platelets," *Clinical Pharmacology and Therapeutics*, vol. 14, no. 5, pp. 810–814, 1973.
- [122] M. Karmazyn, M. S. Manku, and D. F. Horrobin, "Changes of vascular reactivity induced by low vasopressin concentrations: interactions with cortisol and lithium and possible involvement of prostaglandins," *Endocrinology*, vol. 102, no. 4, pp. 1230–1236, 1978.
- [123] M. C. Chang, E. Grange, O. Rabin, J. M. Bell, D. D. Allen, and S. I. Rapoport, "Lithium decreases turnover of arachidonate in several brain phospholipids," *Neuroscience Letters*, vol. 220, no. 3, pp. 171–174, 1996.
- [124] J. S. Rao, H.-J. Lee, S. I. Rapoport, and R. P. Bazinet, "Mode of action of mood stabilizers: is the arachidonic acid cascade a common target?" *Molecular Psychiatry*, vol. 13, no. 6, pp. 585–596, 2008.
- [125] F. Bosetti, J. Rintala, R. Seemann et al., "Chronic lithium downregulates cyclooxygenase-2 activity and prostaglandin E₂ concentration in rat brain," *Molecular Psychiatry*, vol. 7, no. 8, pp. 845–850, 2002.
- [126] M. Basselin, H.-W. Kim, M. Chen et al., "Lithium modifies brain arachidonic and docosahexaenoic metabolism in rat lipopolysaccharide model of neuroinflammation," *Journal of Lipid Research*, vol. 51, no. 5, pp. 1049–1056, 2010.
- [127] S. Hong, K. Gronert, P. R. Devchand, R.-L. Moussignac, and C. N. Serhan, "Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells: autacoids in anti-inflammation," *The Journal of Biological Chemistry*, vol. 278, no. 17, pp. 14677–14687, 2003.
- [128] A. González-Pérez, A. Planagumà, K. Gronert et al., "Docosahexaenoic acid (DHA) blunts liver injury by conversion to protective lipid mediators: protectin D1 and 17S-hydroxy-DHA," *The FASEB Journal*, vol. 20, no. 14, pp. 2537–2539, 2006.
- [129] W.-F. Tan, X.-Z. Cao, J.-K. Wang, H.-W. Lv, B.-Y. Wu, and H. Ma, "Protective effects of lithium treatment for spatial memory deficits induced by tau hyperphosphorylation in splenectomized rats," *Clinical and Experimental Pharmacology and Physiology*, vol. 37, no. 10, pp. 1010–1015, 2010.
- [130] H.-M. Wang, T. Zhang, Q. Li, J.-K. Huang, R.-F. Chen, and X.-J. Sun, "Inhibition of glycogen synthase kinase-3 β by lithium chloride suppresses 6-hydroxydopamine-induced inflammatory response in primary cultured astrocytes," *Neurochemistry International*, vol. 63, no. 5, pp. 345–353, 2013.
- [131] Y. Wang, W. C. Huang, C. Y. Wang et al., "Inhibiting glycogen synthase kinase-3 reduces endotoxaemic acute renal failure by down-regulating inflammation and renal cell apoptosis," *British Journal of Pharmacology*, vol. 157, no. 6, pp. 1004–1013, 2009.
- [132] H. F. Green and Y. M. Nolan, "GSK-3 mediates the release of IL-1 β , TNF- α and IL-10 from cortical glia," *Neurochemistry International*, vol. 61, no. 5, pp. 666–671, 2012.
- [133] H. Himmerich, S. Bartsch, H. Hamer et al., "Impact of mood stabilizers and antiepileptic drugs on cytokine production in vitro," *Journal of Psychiatric Research*, vol. 47, no. 11, pp. 1751–1759, 2013.
- [134] T. F. Tay, M. Maharan, S. L. Too, M. S. Hasidah, G. Ismail, and N. Embi, "Glycogen synthase kinase-3 β inhibition improved survivability of mice infected with *Burkholderia pseudomallei*," *Tropical Biomedicine*, vol. 29, no. 4, pp. 551–567, 2012.
- [135] K. Chen, Y. Wu, M. Zhu et al., "Lithium chloride promotes host resistance against *Pseudomonas aeruginosa* keratitis," *Molecular Vision*, vol. 19, pp. 1502–1514, 2013.
- [136] P. Zhang, J. Katz, and S. M. Michalek, "Glycogen synthase kinase-3 β (GSK3 β) inhibition suppresses the inflammatory response to Francisella infection and protects against tularemia in mice," *Molecular Immunology*, vol. 46, no. 4, pp. 677–687, 2009.
- [137] E. Beurel and R. S. Jope, "Glycogen synthase kinase-3 promotes the synergistic action of interferon- γ on lipopolysaccharide-induced IL-6 production in RAW264.7 cells," *Cellular Signalling*, vol. 21, no. 6, pp. 978–985, 2009.
- [138] F. Boufidou, C. Nikolaou, B. Alevizos, I. A. Liappas, and G. N. Christodoulou, "Cytokine production in bipolar affective disorder patients under lithium treatment," *Journal of Affective Disorders*, vol. 82, no. 2, pp. 309–313, 2004.
- [139] A. L. Rowse, R. Naves, K. S. Cashman et al., "Lithium controls central nervous system autoimmunity through modulation of IFN- γ signaling," *PLoS ONE*, vol. 7, no. 12, Article ID e52658, 2012.
- [140] S. Agrawal, S. Gollapudi, S. Gupta, and A. Agrawal, "Dendritic cells from the elderly display an intrinsic defect in the production of IL-10 in response to Lithium Chloride," *Experimental Gerontology*, vol. 48, no. 11, pp. 1285–1292, 2013.
- [141] M. H. Rapaport, L. Guylai, and P. Whybrow, "Immune parameters in rapid cycling bipolar patients before and after lithium treatment," *Journal of Psychiatric Research*, vol. 33, no. 4, pp. 335–340, 1999.
- [142] E. J. Kucharz, S. J. Sierakowski, and J. S. Goodwin, "Lithium *in vitro* enhances interleukin-2 production by T cells from patients with systemic lupus erythematosus," *Immunopharmacology and Immunotoxicology*, vol. 15, no. 5, pp. 515–523, 1993.
- [143] M. B. Szein, G. L. Simon, D. M. Parenti et al., "In vitro effects of thymosin and lithium on lymphoproliferative responses of normal donors and HIV seropositive male homosexuals with AIDS-related complex," *Clinical Immunology and Immunopathology*, vol. 44, no. 1, pp. 51–62, 1987.
- [144] F. Ballanger, I. Tenaud, C. Volteau, A. Khammari, and B. Dréno, "Anti-inflammatory effects of lithium gluconate on keratinocytes: a possible explanation for efficiency in seborrhoeic dermatitis," *Archives of Dermatological Research*, vol. 300, no. 5, pp. 215–223, 2008.
- [145] K. Azim and A. M. Butt, "GSK3 β negatively regulates oligodendrocyte differentiation and myelination in vivo," *Glia*, vol. 59, no. 4, pp. 540–553, 2011.
- [146] J. K. Rybakowski, A. Remlinger-Molenda, A. Czech-Kucharska, M. Wojcicka, M. Michalak, and J. Losy, "Increased serum matrix metalloproteinase-9 (MMP-9) levels in young patients during

- bipolar depression,” *Journal of Affective Disorders*, vol. 146, no. 2, pp. 286–289, 2013.
- [147] B. I. Goldstein, D. E. Kemp, J. K. Soczynska, and R. S. McIntyre, “Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature,” *The Journal of Clinical Psychiatry*, vol. 70, no. 8, pp. 1078–1090, 2009.
- [148] S. M. Kousik, T. C. Napier, and P. M. Carvey, “The effects of psychostimulant drugs on blood brain barrier function and neuroinflammation,” *Frontiers in Pharmacology*, vol. 3, article 121, 2012.
- [149] E. O’Shea, A. Urrutia, A. R. Green, and M. I. Colado, “Current preclinical studies on neuroinflammation and changes in blood-brain barrier integrity by MDMA and methamphetamine,” *Neuropharmacology*, vol. 87, pp. 125–134, 2014.
- [150] R. C. Padmos, G. C. M. Van Baal, R. Vonk et al., “Genetic and environmental influences on pro-inflammatory monocytes in bipolar disorder: a twin study,” *Archives of General Psychiatry*, vol. 66, no. 9, pp. 957–965, 2009.
- [151] A. K. Heye, R. D. Culling, M. D. C. Valdés Hernández, M. J. Thrippleton, and J. M. Wardlaw, “Assessment of blood-brain barrier disruption using dynamic contrast-enhanced MRI. A systematic review,” *NeuroImage: Clinical*, vol. 6, pp. 262–274, 2014.
- [152] N. H. Hendrikse and W. Vaalburg, “Imaging of P glycoprotein function in vivo with PET” *Novartis Foundation Symposium*, vol. 243, pp. 137–148, 180–185, 2002.
- [153] E. Candelario-Jalil, Y. Yang, and G. A. Rosenberg, “Diverse roles of matrix metalloproteinases and tissue inhibitors of metalloproteinases in neuroinflammation and cerebral ischemia,” *Neuroscience*, vol. 158, no. 3, pp. 983–994, 2009.

Research Article

Correlation between Peripheral Levels of Brain-Derived Neurotrophic Factor and Hippocampal Volume in Children and Adolescents with Bipolar Disorder

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Pediatric bipolar disorder (PBD) is a serious mental disorder that affects the development and emotional growth of affected patients. The brain derived neurotrophic factor (BDNF) is recognized as one of the possible markers of the framework and its evolution. Abnormalities in BDNF signaling in the hippocampus could explain the cognitive decline seen in patients with TB. Our aim with this study was to evaluate possible changes in hippocampal volume in children and adolescents with BD and associate them to serum BDNF. Subjects included 30 patients aged seven to seventeen years from the ProCAB (Program for Children and Adolescents with Bipolar Disorder). We observed mean right and left hippocampal volumes of 41910.55 and 41747.96 mm³, respectively. No statistically significant correlations between peripheral BDNF levels and hippocampal volumes were found. We believe that the lack of correlation observed in this study is due to the short time of evolution of BD in children and adolescents. Besides studies with larger sample sizes to confirm the present findings and longitudinal assessments, addressing brain development versus a control group and including drug-naive patients in different mood states may help clarify the role of BDNF in the brain changes consequent upon BD.

1. Introduction

Bipolar disorder (BD) is a severe mental disorder characterized by mood swings during which a person has distinct periods of impairing elevated (mania) or decreased (depression) mood and energy [1]. It occurs in approximately 0.4 to 1.6%

of adults and in 1% in children and adolescents [2, 3]. In the early-age onset presentation (pediatric bipolar disorder, PBD), difficulties in interpersonal relationships, academic functioning, and negative outcomes such as multiple hospitalizations and high rates of suicide attempts are observed [4, 5].

Despite the devastating effects of BD on child development, little is known about the causes of this disorder. Its etiology is probably multifactorial, including biological and environmental factors [6]. Studies in adults with BD suggest that neurotrophins, particularly brain-derived neurotrophic factor (BDNF), inflammatory markers, and oxidative stress may be related to the etiology of this disorder [7, 8]. BDNF is the most abundant neurotrophin in the brain, and it has been implicated in neuronal processes such as neurogenesis, neuronal survival, dendritic growth, and synaptic plasticity [9]. It has been suggested that neuronal viability might be affected by neurotrophins persistent reduction [10]. Kauer-Sant'Anna and colleagues found BDNF levels were lower in patients who had multiple episodes of the disorder, which led to the hypothesis that episode-related reduction of neurotrophins could explain some of the structural changes in the brain observed in bipolar patients [11]. Besides that, BDNF is highly expressed in the cortex and hippocampus, areas of the brain known to regulate complex functions such as memory and emotion.

Structural and functional neuroimaging studies of pediatric BD generally converge with adult studies in implicating frontolimbic structures [12] and smaller sizes of amygdala [13] and hippocampus [14, 15], and a significant negative correlation between the volume of the right hippocampus of adolescents with BD and disease duration has been reported. Although these findings are preliminary due to relatively small sample sizes, hippocampal commitment is consistent across several investigations.

Altogether, these findings suggest that abnormalities of BDNF signaling in the hippocampus could be an explanation to the cognitive deficits observed in PBD and brain alterations present in adults after multiple episodes [10, 16]. The most consistent associations between PBD and cognitive deficits were reported for impairments in working memory, verbal memory, attention, executive function, response flexibility, reversal learning, processing speed, set shifting, and visuospatial memory [17].

Due to the involvement of BDNF in BD and its abundance and influence on neurogenesis in the hippocampus, we evaluated the correlation between peripheral levels of BDNF and hippocampal volumetric measurements in children and adolescents with BD. Furthermore, based on previous studies showing [1] early cognitive deficits in PBD, we evaluated the working memory of patients with PBD. We also hypothesized that patients with longer disease duration would present lower serum BDNF levels, poorer neurocognitive performance, and reduced hippocampal volumes.

2. Methods

This was a cross-sectional study. Children and adolescents with bipolar disorder I, bipolar disorder II, or bipolar disorder NOS evaluated in the ProCAB (Pediatric Bipolar Disorder Outpatient Program) of the Hospital de Clínicas de Porto Alegre were invited to participate. Enrollment was performed from 2012 to 2013. Inclusion criteria were as follows: ages

7–17 years; both genders; bipolar diagnosis I, bipolar diagnosis II, or bipolar diagnosis NOS according to DSM-IV. Of note, since the most representative sample of pediatric bipolar disorder is the COBY study (Course and Outcome of Bipolar Youth), our definition BD-NOS followed the same criteria, that is, at least 4 episodes of 4-hour lasting mood changes clearly differing from the usual for the subject [18]. Exclusion criteria were as follows: presence of schizophrenia, pervasive developmental disorder, active substance abuse, and contraindications to MRI.

2.1. Diagnostic Assessment. All patients underwent a three-step procedure for diagnosis ascertainment.

First, a child and adolescent psychiatrist performed BD symptom (DSM-IV and DSM5 criteria) and family history of mental disorders screening with parents and children together. A total of 127 subjects were assessed, and 95 (75%) were excluded due to not presenting BD-I, BD-II, or BD-NOS. Two patients started the assessment but did not complete it.

When the diagnosis of BD was suspected, patients and parents went through a semistructured interview with the schedule for affective disorders and schizophrenia for school-age children, present and lifetime version (K-SADS-PL), conducted by a research assistant.

Finally, a clinical evaluation was conducted by a second child and adolescent psychiatrist that received all information from previous queries. An important observation is that a clinical meeting with all the professionals involved in the assessment was conducted to define diagnosis, comorbidity, and the treatment plan.

Patients diagnosed with BD-I, BD-II, or BD-NOS underwent neuropsychological assessment, blood sampling for BDNF level determination, and MRI. Young Mania Rating Scale (YMRS) and Children's Depression Rating Scale (CDRS) were also applied for the purpose of measuring manic and depressive symptoms at the evaluation time.

2.2. Neuropsychological or Neurocognitive Assessment. Full scale IQ was determined using the vocabulary and block design subsets of the Wechsler Intelligence Scale for Children-Third Version (WISC-III) and the Digit Span of the WISC-III (total score and inverse order). According to previous studies, Digit Span Inverse Order is more directly correlated with working memory [19].

2.3. Neuroimaging Assessment

2.3.1. Images Acquisition. All patients underwent a 1.5 T Philips Achieva magnetic resonance imaging (MRI) using an eight-channel head coil. The structural images were acquired using a sagittal 3D T1 weighted magnetization-prepared rapid gradient-echo (MPRAGE) sequence (repetition time = 8.7 ms, echo time = 4.0 ms, inversion time = 1000 ms, and flip angle = 8°). Possible head movements were minimized by placing foam pads inside the head coil. The volumetric segmentation and measurement were performed with the FreeSurfer image analysis suite, which

is documented and freely available for download online (<http://surfer.nmr.mgh.harvard.edu/>).

2.3.2. Processing of Structural Images. The image processing was carried out with support from the National Supercomputing Center (CESUP), Federal University of Rio Grande do Sul [20]. FreeSurfer v 5.3 was installed in the cluster with Novell SUSE Linux Enterprise Server 11-SP1 operating system. Each data set subject was allocated to one processing core and included in the submission queue processing script.

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite, which is documented and freely available for download online (<http://surfer.nmr.mgh.harvard.edu/>). Briefly, this processing includes motion correction and averaging of multiple volumetric T1 weighted images (when more than one is available), removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, segmentation of the subcortical white matter, and deep gray matter volumetric structures (including hippocampus, amygdala, caudate, putamen, and ventricles) [21, 22]. Automatic segmentation method was used to measure hippocampal volume. Shortly, a neuroanatomical label is assigned to every voxel in the brain, comparing to an atlas whose encoding is based on class and location classification. This procedure is based on modeling the segmentation as a nonstationary anisotropic Markov random field (MRF), in which the probability of a label is modulated by the probability of its neighbors, with the probabilities computed separately at each position in an atlas, for each pair of tissue classes and for each of the six cardinal directions [21].

2.4. BDNF Serum Levels Determination. BDNF serum levels were measured with sandwich-ELISA, using a commercial kit according to the manufacturer's instructions (Millipore, USA). Briefly, microtiter plates (96-well flat-bottom) were coated for 24 h at 4°C with the samples diluted 1:100 in sample diluent and standard curve ranging from 7.8 to 500 pg of BDNF. Plates were then washed four times with wash buffer followed by the addition of biotinylated mouse anti-human BDNF monoclonal antibody (diluted 1:1000 in sample diluent), which was incubated for 3 h at room temperature. After washing, a second incubation with streptavidin-horseradish peroxidase conjugate solution (diluted 1:1000) for 1 h at room temperature was carried out. After addition of substrate and stop solution, the amount of BDNF was determined (absorbance set in 450 nm). The standard curve demonstrates a direct relationship between optical density (OD) and BDNF concentration. Total protein was measured by Bradford's method (samples diluted 1:200) using bovine serum albumin (BSA) as a standard.

2.5. Data Analysis. Data analysis was performed using the Spearman correlation due to the fact that the dependent variables (hippocampal volume and peripheral levels of BDNF) had asymmetric distribution. Correlations between hippocampal volume, BDNF levels, working memory, and

TABLE 1: Demographic data ($N = 27$).

	Mean	SD
Age (years)	13.89	2.98
Age of onset (years)	9.18	3.79
Disease duration (years)	4.74	3.38
YMRS	7.51	8.86
CDRS	28.11	11.31
IQ	105.77	14.05

SD: standard deviation; YMRS: Young Mania Rating Scale; CDRS: Children's Depression Rating Scale.

IQ: intelligence quotient.

duration of disorder were also performed. All the analyses were performed considering raw volumes for the hippocampus and also for a corrected value according to total intracranial volume, due to the high variation in our age range.

SPSS 20 for Windows was used for statistical analyses. All statistical tests were two-tailed with a set at 0.05.

2.6. Ethical Issues. Children, adolescents, and their parents were properly informed about the goal of the project and accepted participating in the protocol and use of data anonymously for publications. This project was approved by the Ethics Committee in Research of the Hospital de Clínicas de Porto Alegre. A statement of informed consent was provided by the parent or guardian and verbal assent by the patient.

3. Results

During the conduction of this study, 127 patients were assessed, and 75% were excluded due to not presenting BD-I, BD-II, or BD-NOS. Two patients started the assessment but did not complete it. From the 30 patients available for the protocol, twenty-seven patients completed the entire evaluation. Three patients did not undergo MRI: one patient got pregnant, and two patients were not able due to the use of dental braces. Demographic/clinical data of the subjects are described in Table 1. The final sample was composed of 14 male and 13 female subjects, and their mean age was 13.8 years. The age of onset of bipolar disorder among patients ranged from 3 to 15 years. Intelligence quotient (IQ) in the subjects ranged around 105.77 ± 14.05 . The majority of subjects presented bipolar disorder type I (77.7%), 3.7% bipolar disorder type II, and 18.6% BD-NOS. Disease duration varied from zero to 14 years (average: 4.74; SD: 3.38). From the 27 patients, 33.3% had comorbid ADHD, 18.5% had comorbid anxiety disorders, and 18.5% had formal diagnosis of ODD, as described in Table 2.

Most subjects (77.7%) were taking a number of psychiatric medications: lithium or valproate monotherapy ($n = 4$; 14.8%); atypical antipsychotics monotherapy ($n = 4$, 14.8%); combined lithium plus anticonvulsants/antipsychotics ($n = 6$, 22.2%); combined anticonvulsants/antidepressants ($n = 1$, 3.7%); multiple combination therapy ($n = 5$, 18.5%); concomitant use of stimulants ($n = 7$, 25.9%). The mean

TABLE 2: Diagnostic data ($n = 27$).

	Subjects (%)
BD-I	21 (77.7%)
BD-II	1 (3.7%)
BD-NOS	5 (18.6%)
ADHD	11 (41%)
Anxiety disorders	5 (18.6%)
ODD	5 (18.5%)

TABLE 3: MRI/working memory results ($N = 27$).

	Average	Standard deviation
Right hippoc. vol ^a (mm ³)	4191.05	416.30
Left hippoc. vol ^b (mm ³)	4174.79	506.74
Total hippoc. vol ^c (mm ³)	8365.85	901.58
BDNF (pg/ μ g protein)	19.58	6.33
Digit Span TS ^d	6.66	1.83
Digit Span IO ^e	4.48	1.90

^aRight hippocampus volume; ^bleft hippocampus volume; ^ctotal hippocampus volume; all were determined with automatic segmentation method.

^dDigit Span Total Score; ^eDigit Span Inverse Order.

number of psychoactive medications for children in this group was 1.74.

Table 3 shows right hippocampus volume (RHV) and left hippocampus volume (LHV), respectively, 4191.05 (mm³) (SD = 416.30) and 4174.79 mm³ (SD = 506.74), as determined by automatic segmentation method. Total intracranial volume means were 1512863.14 mm³ (SD = 151579.76). Peripheral BDNF levels varied around 19.58 \pm 6.33 (pg/ μ g protein). The average Digit Span Total Score was 6.66. The average Digit Span Inverse Order was 4.48.

3.1. Primary Analysis. Table 4 shows correlation coefficients of right hippocampus volume, left hippocampus volume, and total hippocampus volume with BDNF. Those were, consecutively, 0.15, 0.03, and 0.10, with significance levels of 0.46, 0.89, and 0.62, respectively. After correction for intracranial total volume, those values were, consecutively, .090, -.125, and .007, with significance levels of .663, .544, and .972 (Table 5).

Correlation coefficients between RHV, LHV, and THV and the Digit Span Inverse Order were 0.02, 0.10, and 0.05, respectively, and are also presented in Table 4. No correlation was significant. We did not observe any correlations between BDNF levels and disorder duration, as well as in working memory as measured by the Digit Span Inverse Order test (Table 6). Adjustment for intracranial total volume has not shown a statistically significant correlation.

4. Discussion

In our evaluation of hippocampus volume and peripheral levels in children and adolescents with bipolar disorder, no statistically significant correlations were detected. The same

occurred with respect to working memory and disease duration. We emphasize that post hoc analyses were conducted, evaluating peripheral BDNF levels and hippocampus volume in patients with higher and lower disease duration. But still no correlations were found.

Although studies in adults have been able to show the relationship between peripheral BDNF levels and hippocampus volume, we hypothesized that the lack of correlation found in this study may represent the short time of evolution of BD in children and adolescents. Usually in adult BD studies, reduced BDNF levels are found in chronic or late-stage individuals with BD, in comparison with patients in early stages of the illness [11]. The same occurs in neuroimaging studies in bipolar disorder, meaning that the effects of systemic toxicity, cognitive and functional impairment, and biological changes seen in BD tend to be cumulative and much more prominent after multiple episodes [23]. Thus, these changes may not yet be found in patients with few years of the disease, as occurs in children and adolescents with BD.

Even diseases that are proven to exert a strong influence on hippocampus morphology and structure, such as epilepsy, may still not reveal changes in neuroimaging exams when in children. For instance, studies of newly diagnosed epilepsy typically fail to find many patients with clear structural subcortical changes at the onset. Zhang et al. compared hippocampus volumes in children with temporal lobe epilepsy (TLE) and healthy controls using magnetic resonance imaging. They have not found hippocampus volume reduction in diagnosed definite/probable TLE children [24]. While there is evidence from adult studies and studies in chronic epilepsy patients that hippocampus atrophy may be a progressive lesion, there is little information regarding hippocampus abnormalities early in the course of epilepsy in patients, particularly in children [25–27].

Our study was limited, in part, by the fact that we used a convenience sample, due to the short time we had available. Therefore, our sample only offered statistical power higher than 80% to detect correlation coefficients higher than 0.5 using two-sided hypothesis tests with a significance level of 0.05. In this way, the possibility of type 2 error cannot be ruled out. Another limitation was the lack of a control group for comparison of BDNF levels, which is a suggestion to future researches. The inclusion of a control group would allow the observation of developmental differences not associated with BD. However, this is the first study addressing brain volumetrics and peripheral biomarkers in PBD, and our exploratory analyses suggest neurobiological underpinnings of BD in children and adolescents may differ from this same disorder in another developmental stage (adult life). Besides that, most of our subjects were taking psychotropic medications at the time of the assessment and were euthymic. This is not uncommon given the ethical issues inherent in discontinuing medication in children with severe psychiatric illnesses. Previous investigations demonstrated that antimanic and antidepressant agents may influence the effects of BDNF on hippocampus, and that could induce morphological changes in subcortical area in BD, which could also lead to recovery of cognitive function. Due to the cross-sectional design of our study, we cannot state that

TABLE 4: Correlations between levels of BDNF, hippocampal volume, and neuropsychological measures.

	RHV ^a		LHV ^b		THV ^c	
	ρ (rho)	<i>P</i>	ρ (rho)	<i>P</i>	ρ (rho)	<i>P</i>
Disease duration	.107	.595	.251	.207	.217	.276
BDNF	.148	.461	.027	.892	.100	.621
IQ	-.009	.963	.065	.749	.045	.825
Digit Span TS ^f	-.098	.587	.102	.743	.014	.985
Digit Span IO ^g	-.021	.918	.097	.632	.045	.825

^aRight hippocampus volume; ^bleft hippocampus volume; ^ctotal hippocampus volume; ^dcorrelation coefficient; ^esignificance; ^fDigit Span Total Score; ^gDigit Span Inverse Order.

TABLE 5: Correlations between levels of BDNF and hippocampal volume controlling for intracranial volume.

	RHV ^a		LHV ^b		THV ^c	
	ρ (rho)	<i>P</i>	ρ (rho)	<i>P</i>	ρ (rho)	<i>P</i>
BDNF	.090	.663	-.125	.544	.007	.972

^aRight hippocampus volume; ^bleft hippocampus volume; ^ctotal hippocampus volume.

TABLE 6: Correlation among BDNF versus disease time, IQ, and digit span.

	BDNF	
	ρ (rho)	<i>P</i> value
Disease time	.069	.731
IQ	.069	.732
Digit span	-.187	.351

medication use may have influenced the results. Also, we ran additional analyses comparing results for patients who were on and off medication, and no significant differences have arisen [28–31].

Another limitation of our study was the use of automatic segmentation method to measure and compare hippocampal volumes. Manual and automatic segmentation methods have been compared in some studies [32–34]. The findings mostly validate the use of automatic methods for segmentation of brain structures, confirming FreeSurfer's potential to determine hippocampal volumes in large-scale studies, even though there was a systematic volume difference between FreeSurfer and manual results. Dewey et al. compared FreeSurfer and individual brain atlases using statistical parametric mapping (IBASPM) to autoassisted manual tracings [35]. They evaluated FreeSurfer to be effective for subcortical volumetry but recommend visual inspection of segmentation output along with manual correction to ensure validity of the data. Wenger et al. compared automatic versus manual segmentation in including 44 younger participants (20–30 years) and 47 older participants (60–70 years) and results reveal high stability coefficients over time for both manual and FreeSurfer segmentations [36]. With FreeSurfer, correlations over time were significantly lower in the older than in the younger age group, which was not the case with manual segmentation. Absolute agreements between the two measures, however, were considerably lower, as FreeSurfer

estimated volumes to be higher. This volume difference was larger in the young than in the old. FreeSurfer detected a significant age difference in hippocampal volume, whereas manual tracing did not. However, manual tracing resulted in a significant difference between left and right Hc, whereas FreeSurfer segmented both sides in a more similar manner. They concluded that, in the younger age group, FreeSurfer can be regarded as a reliable and valid method for assessing differences in hippocampal volume, with at least as high reliability as manual tracing.

The study of possible correlations between serum levels of BDNF, working memory, and hippocampal volumetric changes in patients with BD through neuroimaging can make important contributions to the understanding of the neurobiology of these disorders, such as how and when the course of the disorder would play its effects on brain development. Our findings suggest that, in early ages, brain volumetric alterations may not be associated with the BDNF peripheral levels and cognitive dysfunction. This information raises the possibility that, in children and adolescents, BDNF level changes must not be a priority when attempting to prevent some of the future atrophic modifications.

Despite the lack of significant statistical correlation observed in this study, this is the first study in pediatric bipolar disorder correlating hippocampus volume and BDNF, and no association between these factors was observed. Replication for result confirmation is crucial, as well as interpretation of these findings in the light of a developmental context. Studies with larger samples and longitudinal studies evaluating normal and disrupted brain development, which include a control group and patients in different mood episodes, may be able to clarify the role of BDNF in brain changes caused by bipolar disorder.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

- [1] American Psychiatric Association, *DSM: Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, 1994.
- [2] B. Geller and J. Luby, "Child and adolescent bipolar disorder: a review of the past 10 years," *Journal of the American Academy of Child and Adolescent Psychiatry*, vol. 36, no. 9, pp. 1168–1176, 1997.
- [3] R. H. Perlis, S. Miyahara, L. B. Marangell et al., "Long-term implications of early onset in bipolar disorder: data from the first 1000 participants in the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD)," *Biological Psychiatry*, vol. 55, no. 9, pp. 875–881, 2004.
- [4] H. S. Akiskal, J. Downs, P. Jordan, S. Watson, D. Daugherty, and D. B. Pruit, "Affective disorders in referred children and younger siblings of manic-depressives. Mode of onset and prospective course," *Archives of General Psychiatry*, vol. 42, no. 10, pp. 996–1003, 1985.
- [5] G. A. Carlson and K. L. Kelly, "Manic symptoms in psychiatrically hospitalized children—what do they mean?" *Journal of Affective Disorders*, vol. 51, no. 2, pp. 123–135, 1998.
- [6] H. Kaplan, B. Sadock, and J. Grebb, *Compêndio de Psiquiatria: Ciência do Comportamento e Psiquiatria Clínica*, 9th edition, 1997.
- [7] M. Berk, F. Kapczinski, A. C. Andreazza et al., "Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors," *Neuroscience and Biobehavioral Reviews*, vol. 35, no. 3, pp. 804–817, 2011.
- [8] V. V. Dias, S. Brissos, B. N. Frey, A. C. Andreazza, C. Cardoso, and F. Kapczinski, "Cognitive function and serum levels of brain-derived neurotrophic factor in patients with bipolar disorder," *Bipolar Disorders*, vol. 11, no. 6, pp. 663–671, 2009.
- [9] R. M. Post, "Role of BDNF in bipolar and unipolar disorder: clinical and theoretical implications," *Journal of Psychiatric Research*, vol. 41, no. 12, pp. 979–990, 2007.
- [10] G. Shaltiel, G. Chen, and H. K. Manji, "Neurotrophic signaling cascades in the pathophysiology and treatment of bipolar disorder," *Current Opinion in Pharmacology*, vol. 7, no. 1, pp. 22–26, 2007.
- [11] M. Kauer-Sant'Anna, F. Kapczinski, A. C. Andreazza et al., "Brain-derived neurotrophic factor and inflammatory markers in patients with early- vs. late-stage bipolar disorder," *International Journal of Neuropsychopharmacology*, vol. 12, no. 4, pp. 447–458, 2009.
- [12] S. C. Caetano, R. L. Olvera, D. Glahn, M. Fonseca, S. Pliszka, and J. C. Soares, "Fronto-limbic brain abnormalities in juvenile onset bipolar disorder," *Biological Psychiatry*, vol. 58, no. 7, pp. 525–531, 2005.
- [13] J. C. Pfeifer, J. Welge, S. M. Strakowski, C. M. Adler, and M. P. DelBello, "Meta-analysis of amygdala volumes in children and adolescents with bipolar disorder," *Journal of the American Academy of Child and Adolescent Psychiatry*, vol. 47, no. 11, pp. 1289–1298, 2008.
- [14] J. A. Frazier, S. Chiu, J. L. Breeze et al., "Structural brain magnetic resonance imaging of limbic and thalamic volumes in pediatric bipolar disorder," *The American Journal of Psychiatry*, vol. 162, no. 7, pp. 1256–1265, 2005.
- [15] H. P. Blumberg, J. Kaufman, A. Martin et al., "Amygdala and hippocampal volumes in adolescents and adults with bipolar disorder," *Archives of General Psychiatry*, vol. 60, no. 12, pp. 1201–1208, 2003.
- [16] D. Suri and V. A. Vaidya, "Glucocorticoid regulation of brain-derived neurotrophic factor: relevance to hippocampal structural and functional plasticity," *Neuroscience*, vol. 239, pp. 196–213, 2013.
- [17] K. Horn, V. Roessner, and M. Holtmann, "Neurocognitive performance in children and adolescents with bipolar disorder: a review," *European Child and Adolescent Psychiatry*, vol. 20, no. 9, pp. 433–450, 2011.
- [18] Course and Outcome of Bipolar Illness in Youth (COBY), <http://www.nimh.nih.gov/news/science-news/2006/largest-study-to-date-on-pediatric-bipolar-disorder-describes-disease-characteristics-and-short-term-outcomes.shtml>.
- [19] M. D. Lezak, D. B. Howieson, and D. W. Loring, *Neuropsychological Assessment*, Oxford University Press, New York, NY, USA, 4th edition, 2004.
- [20] CESUP NSC, <http://www.cesup.ufrgs.br>.
- [21] B. Fischl, D. H. Salat, E. Busa et al., "Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain," *Neuron*, vol. 33, no. 3, pp. 341–355, 2002.
- [22] B. Fischl, D. H. Salat, A. J. W. van der Kouwe et al., "Sequence-independent segmentation of magnetic resonance images," *NeuroImage*, vol. 23, supplement 1, pp. S69–S84, 2004.
- [23] C. S. Gama, M. Kunz, P. V. S. Magalhães, and F. Kapczinski, "Staging and neuroprogression in bipolar disorder: a systematic review of the literature," *Revista Brasileira de Psiquiatria*, vol. 35, no. 1, pp. 70–74, 2013.
- [24] Y.-Z. Zhang, W.-H. Li, Y. Gao, Y.-H. Li, J. Wu, and W.-B. Li, "Hippocampal volume in children with temporal lobe epilepsy compared to healthy children: a magnetic resonance imaging study," *Neurology India*, vol. 60, no. 1, pp. 29–35, 2012.
- [25] A. T. Berg, H. R. Pardoe, R. K. Fulbright, S. U. Schuele, and G. D. Jackson, "Hippocampal size anomalies in a community-based cohort with childhood-onset epilepsy," *Neurology*, vol. 76, no. 16, pp. 1415–1421, 2011.
- [26] H. Goldberg, A. Weinstock, N. Bergsland et al., "MRI segmentation analysis in temporal lobe and idiopathic generalized epilepsy," *BMC Neurology*, vol. 14, no. 1, article 131, 2014.
- [27] C. A. Guimarães, L. Bonilha, R. C. Franzon, L. M. Li, F. Cendes, and M. M. Guerreiro, "Distribution of regional gray matter abnormalities in a pediatric population with temporal lobe epilepsy and correlation with neuropsychological performance," *Epilepsy and Behavior*, vol. 11, no. 4, pp. 558–566, 2007.
- [28] B. N. Frey, A. C. Andreazza, K. M. M. Ceresér et al., "Effects of mood stabilizers on hippocampus BDNF levels in an animal model of mania," *Life Sciences*, vol. 79, no. 3, pp. 281–286, 2006.
- [29] M. D. Hammonds and S. S. Shim, "Effects of 4-week treatment with lithium and olanzapine on levels of brain-derived neurotrophic factor, B-Cell CLL/Lymphoma 2 and phosphorylated cyclic adenosine monophosphate response element-binding protein in the sub-regions of the hippocampus," *Basic and Clinical Pharmacology and Toxicology*, vol. 105, no. 2, pp. 113–119, 2009.
- [30] L. G. Chepenik, C. Fredericks, X. Papademetris et al., "Effects of the brain-derived neurotrophic growth factor Val66Met variation on hippocampus morphology in bipolar disorder," *Neuropsychopharmacology*, vol. 34, no. 4, pp. 944–951, 2009.
- [31] K. Yucel, M. C. McKinnon, V. H. Taylor et al., "Bilateral hippocampal volume increases after long-term lithium treatment in patients with bipolar disorder: a Longitudinal MRI study," *Psychopharmacology*, vol. 195, no. 3, pp. 357–367, 2007.

- [32] N. Cherbuin, K. J. Anstey, C. Réglade-Meslin, and P. S. Sachdev, "In vivo hippocampal measurement and memory: a comparison of manual tracing and automated segmentation in a large community-based sample," *PLoS ONE*, vol. 4, no. 4, Article ID e5265, 2009.
- [33] R. A. Morey, C. M. Petty, Y. Xu et al., "A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes," *NeuroImage*, vol. 45, no. 3, pp. 855–866, 2009.
- [34] L. Shen, A. J. Saykin, S. Kim et al., "Comparison of manual and automated determination of hippocampal volumes in MCI and early AD," *Brain Imaging and Behavior*, vol. 4, no. 1, pp. 86–95, 2010.
- [35] J. Dewey, G. Hana, T. Russell et al., "Reliability and validity of MRI-based automated volumetry software relative to auto-assisted manual measurement of subcortical structures in HIV-infected patients from a multisite study," *NeuroImage*, vol. 51, no. 4, pp. 1334–1344, 2010.
- [36] E. Wenger, J. Mårtensson, H. Noack et al., "Comparing manual and automatic segmentation of hippocampal volumes: reliability and validity issues in younger and older brains," *Human Brain Mapping*, vol. 35, no. 8, pp. 4236–4248, 2014.

Research Article

Normal Metabolic Levels in Prefrontal Cortex in Euthymic Bipolar I Patients with and without Suicide Attempts

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Introduction/Objective. Evidence suggests that the prefrontal cortex has been implicated in the pathophysiology of bipolar disorder (BD), but few neurochemical studies have evaluated this region in bipolar patients and there is no information from BD suicide attempters using Proton Magnetic Resonance Spectroscopy (H^+ MRS). The objective was to evaluate the metabolic function of the medial orbital frontal cortex in euthymic BD type I suicide and nonsuicide attempters compared to healthy subjects by H^+ MRS. *Methods.* 40 euthymic bipolar I outpatients, 19 without and 21 with history of suicide attempt, and 22 healthy subjects were interviewed using the Structured Clinical Interview with the DSM-IV axis I, the Hamilton Depression Rating Scale, the Young Mania Rating Scale, and the Barratt Impulsiveness Scale-11 and underwent H^+ MRS. *Results.* We did not find any metabolic abnormality in medial orbital frontal regions of suicide and nonsuicide BD patients and BD patients as a group compared to healthy subjects. *Conclusions.* The combined chronic use of psychotropic drugs with neuroprotective or neurotrophic effects leading to a euthymic state for longer periods of time may improve neurometabolic function, at least measured by H^+ MRS, even in suicide attempters. Besides, these results may implicate mood dependent alterations in brain metabolic activity. However, more studies with larger sample sizes of this heterogeneous disorder are warranted to clarify these data.

1. Introduction

Bipolar disorder (BD) is a prevalent and chronic mental illness that is associated with high rates of suicidal behavior. The risk for suicide among BD patients is up to 20–30 times greater than that for the general population [1]. The cause of these high rates is unknown, but some studies have suggested a possible relationship between suicidal behavior with impulsivity [2, 3], and others have demonstrated an association of impulsivity and the orbital frontal cortex (OFC) dysfunction [4, 5].

In fact, evidence of an association between suicidal behavior and structural and functional abnormalities of the brain is mounting, and most of these regions involved in suicidal behavior are part of emotional regulation neuronal circuits, including amygdala, hippocampus, thalamus, and olfactocentric paralimbic cortices (insular, temporopolar and orbital frontal cortices) [6]. In this context, particularly, the OFC has strong connections with the amygdala, thalamus, and basal; in addition, the OFC mediate the individual's affect, impulse control, and recognition of reinforcing stimuli. In keeping with this line of reasoning, some authors have

demonstrated an association between OFC dysfunction, decision-making impairment, and suicidal behavior; in a first study, they used the Iowa Gambling Task (IGT) and showed that violent suicide attempters make more disadvantageous choices than affective controls (patients without history of suicide attempt) [7]. In another study, these same authors compared suicide attempters and affective controls using an adapted version of the IGT during functional neuroimaging and showed that patients with history of suicide attempts (a) performed worse on a decision-making task, (b) had decreased activation in the OFC (and occipital cortex) for the contrast between disadvantageous and advantageous choices, and (c) had no difference for the contrast between wins and losses, which implies impairment of guiding of safe behavior [8]. Taken together, these findings suggest that suicide attempters were not able to evaluate risky choices appropriately. Interestingly, a component of impulsivity called “lack of premeditation” was associated to disadvantageous decision-making, according to some authors [9].

In addition, a link of evidence between metabolic dysfunction, prefrontal cortex, and suicidal behavior was the observation of serotonergic dysfunction from *in vivo* brain imaging study of nonfatal suicidal behavior using positron emission tomography (PET) scanning with 11-C-methyl-tryptophan (an analogue of tryptophan) that showed less uptake in medial orbital PFC in association with lethality of suicidal behavior [10] and another study found lower prefrontal cortical 5-HT_{2A} binding in suicide attempters [11]. Besides, evidence from neuroimaging research has suggested the OFC as a crucial region involved in impulsive behavior; for instance, some authors have demonstrated a negative correlation between cortical thickness of the OFC and Barrat Impulsiveness Scale-II (BIS-II) total and motor domain in healthy adults [12].

While a number of structural neuroimaging research techniques have been utilized to investigate a host of psychiatry disorders [13], Proton Magnetic Resonance Spectroscopy (H⁺MRS) is a noninvasive method that allows biochemical constituents to be directly assayed *in vivo*. Numerous studies utilizing this neuroimaging technique in BD have identified a number of metabolite changes in specific brain regions, such as the medial OFC, but most of these studies show mixed results, probably due to methodological issues, such as inclusion of patients with types I and II, in several phases of the illness, and medicated or drug-naïve patients [14–20]. Besides, information in terms of metabolic changes measured by H⁺MRS in BD suicide attempters is even scarcer.

To our knowledge, there are no previous studies comparing metabolic levels in frontal cortical gray matter measured by H⁺MRS in suicide and nonsuicide attempters in a homogeneous sample of type I euthymic BD outpatients.

So, the purpose of this study was to investigate metabolic levels of N-acetyl-aspartate (NAA), myo-inositol (mI), choline (Cho), and creatine (Cr) in the medial orbital prefrontal cortex in euthymic bipolar type I outpatients, with and without history of suicide attempts. We hypothesized that the neuronal metabolic function may be impaired in the cortical gray matter by H⁺MRS, even in the euthymic phase of the

bipolar illness. In addition, we speculated that the patients with suicide attempts have more metabolic dysfunction in this area compared with the patients without history of suicidal behavior.

2. Methods

2.1. Participants. This study is part of a larger project of evaluation and treatment of patients with bipolar disorder treated at the research Center in Salvador-Bahia-Brazil (Mood and Anxiety Program of the Federal University of Bahia-CETHA), in which data is continuously collected. Patients were recruited from this center and were interviewed using the Structured Clinical Interview with the DSM-IV axis I (SCID-I) [21], the Hamilton Depression Rating Scale (HDRS) [22], the Young Mania Rating Scale (YMRS) [23], and the Barratt Impulsiveness Scale (BIS-11). The BIS is a self-report questionnaire composed of 30 items with Likert-type questions, rated from 1 (rarely/never) to 4 (almost always/always). Scoring yields a total score and 3 subscale scores derived by factor analysis: attention, motor, and nonplanning. Score varies from 30 to 120 and there is no established cut-off point [24]. The BIS differs from performance-based or cognitive measures of impulsivity as scores reflect self-rated behaviors rather than discrete cognitive processes and thus may be closer to psychiatric symptomatology. The euthymia criteria were scores for both the YMRS and HDRS below 7 points for at least two months. Demographic and clinical data were gathered through a questionnaire, and all assessment instruments were administered by two trained experts in psychiatry. Patients were classified as having positive suicidal history if they reported one or more self-injurious acts committed with intent to die.

Healthy controls were recruited among the patients' social network and were interviewed using the same evaluation instruments. The choice of these controls as a group was to try to prevent bias associated with differences in sociodemographic data between groups. None of these subjects had a current or past Axis I DSM-IV psychiatric disorder or a first-degree relative with an Axis I psychiatric disorder.

Exclusion criteria of all subjects were age less than 18 and more than 60 years, current serious medical conditions, history of head trauma and neurological disorders or substance abuse at any time, and current medical problems in the preceding six months.

2.2. Structural Magnetic Resonance Imaging HMRS Procedure. All MRI scans and H⁺MRS were acquired at the Image Memorial Clinic, Medicina Diagnóstica-Bahia-Brazil, using a 1.5-T Symphony Master/Class Siemens scanner (Ellagen, Germany) and conducted and interpreted in a blind manner by one research assistant (GLR), trained in H⁺MRS acquisition and spectral analysis and a second research assistant (MVR) confirmed in a blind manner the position of the voxel. The placement of the voxel was visually inspected to minimize white matter and CSF contents. To evaluate intrarater reproducibility, the same rater repeated the metabolites

measurements twice, at least one week apart, for ten randomly selected subjects. Intrarater reliability was found to be high for all measurement ($Kappa = 1,0$; $P = 0,0041$).

The interpupillary line in comparison with transverse landmark light on the scanner was used to denote the position of each subject's head and T2-weighted images were obtained to localize the H⁺MRS voxel. To minimize head movements, the forehead was affixed with adhesive tape to the MRI stretcher and neck support was provided when necessary.

A single 8 cm³ voxel water-suppressed H⁺MRS was prescribed on the medial orbital prefrontal cortex, and spectra were acquired using point resolved spectroscopy sequence (TR = 1500 ms, TE = 30 ms, flip angle = 90°, NEX = 128). The most inferior of the gray matter voxel was positioned at a minimum level of 1.5 mm above the orbits and a maximum level of 3 mm.

2.3. Statistical Analysis. The data were entered into the Software Statistical Package for Social Sciences (SPSS) version 17.0, and the STATA statistical software package, version 11.0, was used in the statistical analysis. Associations were accepted when a P value was less than 5% (P value ≤ 0.05). The one-tailed test was used to ANCOVA and ANOVA, and for chi-squared test we used two-tailed test.

In the metabolites analyses the assumptions of normal distribution (One-Sample Kolmogorov-Smirnov Test) and equal variance around the required mean (Levene's Test of Equality of Error Variances) that tests the null hypothesis that the error variance of the dependent variable is equal across groups were satisfied by all the metabolites evaluated. To verify the possible differences in clinical and demographic characteristics between attempters, nonattempters, and healthy controls, chi-squared, Student's t -tests, Fisher's exact test, or univariate analysis of variance (ANOVA), with post hoc analysis (Bonferroni correction), were used, where appropriate. Only the length of illness did not have a normal distribution.

Lastly, the multivariate analysis (ANCOVA) model was used to compare the results of the metabolites between groups and adjusted for clinical and demographic variables. The statistical analysis of metabolites NAA, Cho, Cr, mI, NAA/Cr, Cho/Cr, and mI/Cr was done in two steps: step 1 compared non-suicide attempters, suicide attempters, and healthy controls, and step 2 compared bipolar patients as a group and healthy controls.

The study was approved by the Local Medical Review Ethics Committee and was performed in accordance with the ethical standards of the Declaration of Helsinki. All subjects provided written informed consent prior to their inclusion in the study.

3. Results

We screened a total of 48 right-handed bipolar I outpatients and 8 were excluded, 5 because of a history of neurological illness or head trauma with loss of consciousness and 3 because they could not undergo an MRI exam. Twenty-five right-handed healthy controls were evaluated and 3

were excluded, 1 because of previous head trauma and 2 because they could not complete the MRI exam. Forty euthymic bipolar I patients, 19 with and 21 without a lifetime history of suicide attempt, and 22 healthy controls underwent the sociodemographics and clinical evaluation. During the subjects' assessments with MRI scan with H⁺MRS, 2 bipolar patients with suicide attempts, 2 bipolar patients without suicide attempts, and 6 healthy controls were excluded from the H⁺MRS analysis, because of the failure to meet spectral quality standards arising from susceptibility or movement artifacts.

Among attempters, 11 attempted once, 4 attempted twice, and 4 attempted three times; suicide attempt methods included overdose/poisoning, cutting, hanging, and jumping from heights. Finally, 5 patients adopted two different methods of suicide.

There were no significant differences between suicidal and nonsuicidal bipolar patients and healthy controls with respect to age, gender, and years of education ($P > 0.5$). There were also no significant differences between group of bipolar patients for age of onset, type of first episode, length of illness, history of psychiatric hospitalizations, number of psychiatric hospitalizations, lifetime psychoses, and family history of suicide or attempted suicide. However, the suicidal group had significantly more history of psychiatric comorbidities than nonsuicidal group ($P = 0.03$). All BD patients were on medication (mostly mood stabilizers).

Considering all selected patients and controls, suicide attempters showed higher mean scores than nonattempters and healthy controls in BIS total (67.3 ± 14.8 ; 58.3 ± 8.6 ; 58.5 ± 9.0 , resp.), BIS attentional (20.5 ± 3.9 ; 16.6 ± 2.5 ; 17.5 ± 3.4 , resp.), BIS nonplanning (26.4 ± 6.2 ; 22.1 ± 4.9 ; 23.1 ± 4.4 , resp.), and motor (20.4 ± 6.0 ; 19.5 ± 4.9 ; 17.9 ± 4.0 , resp.) and the ANOVA with post hoc analysis (Bonferroni correction) revealed that the differences were significant in BIS total ($F = 4.58$, $P = 0.01$), BIS attentional ($F = 7.75$, $P = 0.001$), and BIS nonplanning ($F = 4.44$, $P = 0.02$). The BIS motor was not significant ($F = 1.31$; $P = 0.28$). All these clinical and demographic data have been described in a previous article published by our group [25].

Conversely, when only patients submitted to H⁺MRS scan are included in the BIS analysis, suicide attempters showed higher mean scores than nonattempters and healthy controls in BIS total (65.3 ± 14.7 ; 59 ± 8.8 ; 58.5 ± 9.6 , resp.), BIS attentional (20.2 ± 4.0 ; 17.1 ± 2.5 ; 17.5 ± 3.7 , resp.), and BIS nonplanning (25.8 ± 6.3 ; 22.1 ± 4.3 ; 22.5 ± 4.3 , resp.), but the ANOVA with post hoc analysis (Bonferroni correction) revealed that only in attentional impulsivity the difference was significant ($F = 4.09$, $P = 0.031$). The BIS motor showed no significant lower mean score in suicide attempters compared to nonattempters and healthy controls (19.3 ± 5.8 ; 19.8 ± 4.9 ; 18.6 ± 4.4 , resp.). As a group, BD patients also showed higher mean scores compared to healthy controls in BIS total (62 ± 12.18 versus 58 ± 9.64), BIS attentional (18.5 ± 3.62 versus 17.5 ± 3.68), BIS motor (19.5 ± 5.26 versus 18.5 ± 4.42), and BIS nonplanning (23.8 ± 5.6 versus 22.5 ± 4.33), but no significant difference was demonstrated (Table 1).

4. Spectral Analysis

There were no differences on H⁺MRS between BD suicide, nonsuicide attempters, and healthy controls (NAA: ($F = 1.41$; $P = 0.252$), mI: ($F = 0.18$; $P = 0.829$), Cho: ($F = 0.26$; $P = 0.769$), Cr: ($F = 0.61$; $P = 0.543$), NAA/Cr: ($F = 0.19$; $P = 0.822$), Cho/Cr: ($F = 1.16$; $P = 0.320$), and mI/Cr: ($F = 0.07$; $P = 0.933$)) (Table 2). In addition, we did not find any evidence of metabolic abnormality in BD patients as a group, compared to healthy subjects (NAA: ($F = 2.13$; $P = 0.15$), mI: ($F = 0$, $P = 0.994$), Cho: ($F = 0.40$; $P = 0.527$), Cr: ($F = 0$; $P = 0.991$), NAA/Cr: ($F = 0.38$; $P = 0.536$), Cho/Cr: ($F = 1.27$; $P = 0.264$), mI/Cr: ($F = 0.13$; $P = 0.719$)).

5. Discussion

The findings from the present study are in agreement with some papers that did not find differences in brain metabolite levels in the prefrontal cortex of bipolar patients compared to healthy controls [15, 26]. Also, these results could be explained by the use of the same methodology, such as only evaluating BD I patients under strict criteria of euthymia; assessed patients had been receiving lithium alone for a long time or associated with another psychiatric drug, including other mood stabilizers, antipsychotics, and antidepressants. Besides, gender and duration of illness of patients were also very similar to our sample. However, to our knowledge it is the first study that assessed the BD I group according to the history of attempted suicide and did not demonstrate differences on H⁺MRS between BD I suicide and nonsuicide attempters.

We speculated that these results may reflect mood-dependent alterations in brain metabolic activity, since studies that included both euthymic and noneuthymic patients have shown changes on metabolite spectra [14, 16, 17]. Another possibility is that the chronic use of psychotropic medical drugs may have a brain protective effect in this subgroup of patients. In fact, in our study, 30 patients (attempters and nonattempters, 75% of our sample) were receiving lithium, of which 21 were also receiving at least one psychiatric drug in addition to lithium. Ten patients were receiving an anticonvulsant associated or not with atypical antipsychotics. So, the combined chronic use of psychotropic drugs with neuroprotective or neurotrophic effects leading to a euthymic state for a longer period of time may improve neurometabolic function, at least where it is measured by H⁺MRS, even in a subgroup of patients with a severe characteristic, such as a history of suicide attempt.

In fact, according to our previous data, in our sample, 30.2% of the subjects were prescribed the first mood stabilizer in the year after the first affective episode ($FMS \leq 1$), 22% after the first year before the 5th year ($1 < FMS \leq 5$), and 47.8% 5 years after the first affective episode ($FMS > 5$). The lifetime prevalence of suicide attempts was 33.3% in the $FMS \leq 1$ group, 32.2% in the $1 < FMS \leq 5$ group, and 58.6% in the $FMS > 5$ group. After adjusting for potential sociodemographic and clinical confounders in the two groups of patients with less than a 5-year delay for FMS ($FMS \leq 1$ and $1 < FMS \leq 5$), the lower prevalence of suicide attempts remained significant

when compared with the $FMS > 5$. When the $FMS < 1$ reference group was compared with the $FMS > 5$ group, adjusting for the same sociodemographic and clinical confounders, the higher prevalence of suicide attempts remained significant in the latter group, with a statistically significant difference between these groups [26]. These results support the protective clinical effect of the use of mood stabilizers on the suicidal behavior.

However, it is also important to highlight that in our sample the patients have low rates of psychiatric comorbidities (35%) compared to other studies that may contribute with a more favorable evaluation of the illness [27]. Additionally, the patients in the present study did not have current serious medical conditions, history of head trauma and neurological disorders or substance abuse at any time or current medical problems in the preceding six months, which may constitute a relatively benign clinical subgroup of BD I patients.

In general, the most important factor in controlling the neuroprogression of BD is the use of efficacious drugs combined with psychoeducation that may prevent relapses and recurrences of the illness [28, 29]. Keeping this issue in mind, mood stabilizers, such as lithium and valproic acid, used for the majority of patients in this study, are the most prominent drugs approved by the United States Federal Drug Administration (FDA) for treatment of BD [30]. Lithium and valproic acid, respectively, through inhibition of glycogen synthase kinase-3 (GSK-3) and the histone deacetylases (HDACs), regulate the transcription and expression of neurotrophic, angiogenic, and neuroprotective proteins, such as brain-derived neurotrophic factor (BDNF), glial cell-line derived neurotrophic factor (GDNF), and angiogenic vascular endothelial growth factor (VEGF). Also, lithium in particular acts on factors that affect apoptotic signaling, such as Bcl-2, p53, Bax, caspase, and heat shock proteins (HSP). Finally, lithium contributes to induction of the ubiquitin-proteasome system and autophagy, two major intracellular quality control mechanisms for protein clearance that prevents abnormal protein accumulation. Overall, these findings highlight the properties of lithium and probably other mood stabilizers to suppress cell death, attenuate neuroinflammation, and promote angiogenesis and cellular plasticity in BD patients [31], which contribute to the reduction of neuronal loss and consequently prevent the cognitive deficits in bipolar patients.

The positive effects of psychopharmacologic treatment on neural plasticity in BD patients may also be demonstrated from neuroimaging studies. In fact, some studies have shown an increase in gray matter volume in whole brain of BD patients treated with lithium [32], and untreated patients showed decreased left anterior cingulate volumes compared with either healthy controls or lithium-treated patients [33]. The NAA was also reported to be increased in the prefrontal cortex after lithium treatment [34]. These results may suggest evidence *in vivo* that the use of mood stabilizers, especially lithium, influences neurotrophic mechanisms in BD patients. Additionally, other authors who measured neuropsychological performance of BD patients in total remission under treatment with lithium as monotherapy demonstrated better

TABLE 1: Demographic and clinical data of the participants.

	BD I patients		Healthy controls (<i>n</i> = 22)	Statistics
	Suicidal (<i>n</i> = 19)	Nonsuicidal (<i>n</i> = 21)		
Gender (<i>n</i>)				
Male	6	5	10	$\chi^2 = 2.31, P = 0.31$
Female	13	16	12	
Age, mean \pm SD (years)	39.8 \pm 11.4	42.0 \pm 8.6	37.7 \pm 13.5	$F = 0.75, P = 0.47$
Educational level, mean \pm SD (years)	12.0 \pm 3.0	11.2 \pm 3.7	11.2 \pm 2.7	$F = 0.37, P = 0.69$
Age of onset, mean \pm SD (years)	24.3 \pm 9.0	25.3 \pm 9.4	NA	$t = 0.34, P = 0.73$
Length of illness, mean \pm SD (years)	15.6 \pm 7.2	16.5 \pm 10.7	NA	$t = 0.31, P = 0.75$
Hospitalizations (<i>n</i>)				
Yes	13	18	NA	$\chi^2 = 1.71, P = 0.26$
No	6	3	NA	
Number of hospitalizations, mean \pm SD	6.0 \pm 5.8	3.1 \pm 2.8	NA	$t = -1.80, P = 0.08$
Type of first episode				
Depression	11	10	NA	$\chi^2 = 1.84, P = 0.39$
Mania	7	11	NA	
Unknown	1	0	NA	
Lifetime psychoses (<i>n</i>)				
Yes	15	9	NA	$\chi^2 = 2.77, P = 0.96$
No	4	11	NA	
Family history of suicide (<i>n</i>)				
Yes	6	4	NA	$\chi^2 = 0.83, P = 0.47$
No	13	17	NA	
Family history of attempt suicide (<i>n</i>)				
Yes	2	4	NA	$\chi^2 = 0.56, P = 0.66$
No	17	17	NA	
Psychiatric comorbidities (<i>n</i>)				
Yes	10	4	NA	$\chi^2 = 4.51, P = 0.03^*$
No	9	16	NA	
[†] BIS total score, mean \pm SD	65.3 \pm 14.7	59 \pm 8.8	58.5 \pm 9.6	$F = 1.88, P = 0.312$
Attention score, mean \pm SD	20.2 \pm 4.0	17.1 \pm 2.5	17.5 \pm 3.7	$F = 4.09, P = 0.031^*$
Motor score, mean \pm SD	19.3 \pm 5.8	19.8 \pm 4.9	18.6 \pm 4.4	$F = 0.27, P = 0.28$
Nonplanning score, mean \pm SD	25.8 \pm 6.3	22.1 \pm 4.3	22.5 \pm 4.3	$F = 2.81, P = 1$

[†] Only from the patients submitted to H⁺MRS.

* Significant at the 0.05 level (2-tailed).

NA—no applicable.

performance on neuropsychological tests and higher plasma BDNF levels than BD patients with partial remission [35]. In summary, the benefits from optimal maintenance treatment regimens with lithium and probably other mood stabilizers go beyond the prevention of mood episodes and include neuroprotection, neural plasticity, and better functional and cognitive outcomes.

Other studies that have been conducted with BD patients compared to healthy subjects showed differences in the H⁺MRS scan, probably due to methodological issues, such as inclusion of euthymic and noneuthymic patients in different phases of the illness [14, 16, 17], assessment of more severe, hospitalized sample of patients and outpatients [14], inclusion of only medication-free patients at the time of the H⁺MRS scan [18], no stratification by age group (young versus old subjects) [36, 37], and inclusion of bipolar I and II patients [20], as previously described. Thus, these various

methodological differences preclude the interpretation of results between studies. We think it likely that there are different subgroups of bipolar patients with different patterns of metabolic spectra, depending on the disease phenotype, since BD is a very heterogeneous illness with various clinical presentations.

There is also a debate regarding impulsivity as being linked to higher suicidal behavior among BD patients. Our study included BIS-11 as a tool for investigating trait impulsivity and in a previous paper of our group, it was shown that suicide attempters rated higher mean scores than nonattempters and healthy controls in BIS total, BIS attentional, and BIS nonplanning [25]. But, when we analyzed BIS data only from the patients and controls who submitted to H⁺MRS scan, the only significant difference was in attentional impulsivity; as a group, BD patients also showed higher mean scores compared to healthy controls in BIS total,

TABLE 2: Medial orbital prefrontal cortex metabolic levels between bipolar patients with and without suicide attempt and healthy controls (mmol/l \pm SD).

Metabolic levels	BD I patients		HC	Statistics
	Suicidal <i>n</i> = 17 mean (SD)	Nonsuicidal <i>n</i> = 19 mean (SD)	<i>n</i> = 16 mean (SD)	
NAA	2.79 \pm 0.58	2.95 \pm 0.58	3.12 \pm 0.54	$F = 1.41, P = 0.252$
Myo-inositol	0.88 \pm 0.42	0.97 \pm 0.42	0.93 \pm 0.41	$F = 0.18, P = 0.829$
Choline	1.32 \pm 0.37	1.36 \pm 0.42	1.27 \pm 0.31	$F = 0.26, P = 0.769$
Creatine	1.53 \pm 0.39	1.70 \pm 0.53	1.63 \pm 0.43	$F = 0.61, P = 0.543$
NAA/creatine	1.86 \pm 0.36	1.85 \pm 0.39	1.93 \pm 0.51	$F = 0.19, P = 0.822$
Choline/creatine	0.86 \pm 0.17	0.81 \pm 0.14	0.78 \pm 0.11	$F = 1.16, P = 0.320$
Myo-inositol/creatine	0.61 \pm 0.31	0.63 \pm 0.35	0.59 \pm 0.27	$F = 0.07, P = 0.933$
Choline	2.79 \pm 0.58	2.95 \pm 0.58	3.12 \pm 0.54	$F = 1.41, P = 0.252$
Creatine	0.88 \pm 0.42	0.97 \pm 0.42	0.93 \pm 0.41	$F = 0.18, P = 0.829$

Significant at the 0.05 level (2-tailed).

Degrees of freedom = 1.

NAA: N-acetyl-aspartate.

HC: Healthy controls.

BIS attentional, BIS motor, and BIS nonplanning, but no significant difference was demonstrated. These results from BIS data may be explained in different ways. The reduced sample size may limit the power of analysis, and although the patients in our study fulfilled strict criteria for euthymia, the data support previous findings of increased impulsivity in bipolar I patients as a trait, compared with healthy controls [38, 39]. In this context, more impulsive subjects may be less tolerant and restless, resulting in movement artifacts on H⁺MRS scan, a procedure that requires more time for the acquisition on spectral curve than on MRI scan. As a consequence, nonsignificant differences can emerge from the analysis by exclusion of these patients that could not conclude the H⁺MRS scan.

Therefore, in the present study, the only result that persists is the higher levels of BIS attentional in bipolar I patients with history of suicide attempt, compared with nonsuicidal and healthy controls. BIS attentional is thought to reflect a person's tendency to rapidly shift attention and a lack of cognitive persistence with the inability to tolerate cognitive complexity [40], and attention problems might predispose an individual to make rapid and inappropriate attributions and not to reappraise a potential conflict situation, increasing the likelihood of aggressive behavior, especially impulsive aggression [41]. In fact, some authors have demonstrated that impulsive aggression increases the risk of suicide behavior in patients with BD [42]. BIS nonplanning refers to a lack of consideration of future consequences, and its relationship with an inability to delay reward-related responses was demonstrated [43], which may reflect a sense of hopelessness about the future; in an event-related potential study, some authors have demonstrated a relationship between reduced P300 amplitude and suicide behavior and hopelessness but not with depressed mood [44]; a population-based, case-control study of nearly lethal suicide attempts found impulsive attempters with higher scores in Beck Hopelessness Scale

than planned attempters and hopelessness, but not depression, distinguished impulsive attempters from the controls [45]. Ultimately, BIS motor is meant to measure the tendency of hasty or reckless action and it has been associated with manic symptoms [46]. Curiously, some authors have found a negative correlation between lethality of the attempts and motor impulsivity (i.e., higher lethality was associated with decreased motor impulsivity) [47]. In fact, it is important to highlight that in our second analysis of BIS, the motor impulsivity showed lower scores in the suicidal than in the nonsuicidal group. All these findings raise the complexity of research about impulsivity in BD patients.

Further complicating the relationship between impulsivity and suicidal behavior, a systematic review showed mixed evidence for an association between impulsivity and suicidal behavior from the studies; only 3 out of 11 studies found significant differences in overall impulsivity (BIS total scores) between attempters and nonattempters and no general consensus regarding association between BIS subscales and suicidal behavior was well established [48] and a recent meta-analysis was conducted for assessing the link between impulsivity and suicidal behavior; the authors have suggested impulsivity as a distal risk factor for suicide [49], reflecting the necessity for further investigation in this topic.

Although one of the strengths of our study is that it consisted of well-characterized euthymic BD I outpatients, without neurologic problems or other severe current medical comorbidities, with low rates of psychiatric disorders, which may result in a homogenous sample of BD patients, the results of the current study must be considered within the context of several limitations; first of all, the sample size of BD patients was reasonable for a study using a neuroimaging technique, but the number of BD was rather small, which leads to limitations in statistical power. Second, the single voxel technique requires a large voxel and limits the acquisition of small volumes of interest. Third, this study was conducted in

an urban tertiary hospital serving a low- or middle-income population and these results may not be generalizable to other care services settings. Lastly, as described before, our sample was small and heterogeneous in terms of lethality and number of suicide attempts; so it was not possible to categorize according to frequency or lethality risk of suicidal behavior. Also, it was not possible to determine the lag time between suicide attempt and neuroimaging procedure as well.

6. Conclusion

Our results suggest that the brain metabolites measured by H^+ MRS are normal in the medial orbital frontal lobe in a subgroup of medicated BD I euthymic outpatients, suicide attempters or not, which may represent a phase-dependent metabolic profile or positive neurotrophic effects of the pharmacological treatment in this area. However, additional studies are needed on larger patient samples in order to clarify these issues.

Conflict of Interests

None of the authors have an affiliation with or financial interest in any organization that might pose a conflict of interests.

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References

- [1] M. Pompili, X. Gonda, G. Serafini et al., "Epidemiology of suicide in bipolar disorders: a systematic review of the literature," *Bipolar Disorders*, vol. 15, no. 5, pp. 457–490, 2013.
- [2] A. C. Swann, D. M. Dougherty, P. J. Pazzaglia, M. Pham, J. L. Steinberg, and F. G. Moeller, "Increased impulsivity associated with severity of suicide attempt history in patients with bipolar disorder," *The American Journal of Psychiatry*, vol. 162, no. 9, pp. 1680–1687, 2005.
- [3] B. Carpiniello, L. Lai, S. Pirarba, C. Sardu, and F. Pinna, "Impulsivity and aggressiveness in bipolar disorder with comorbid borderline personality disorder," *Psychiatry Research*, vol. 188, no. 1, pp. 40–44, 2011.
- [4] M. C. Brower and B. H. Price, "Neuropsychiatry of frontal lobe dysfunction in violent and criminal behaviour: a critical review," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 71, no. 6, pp. 720–726, 2001.
- [5] C. A. Winstanley, D. E. H. Theobald, R. N. Cardinal, and T. W. Robbins, "Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice," *The Journal of Neuroscience*, vol. 24, no. 20, pp. 4718–4722, 2004.
- [6] M. L. Phillips, W. C. Drevets, S. L. Rauch, and R. Lane, "Neurobiology of emotion perception I: the neural basis of normal emotion perception," *Biological Psychiatry*, vol. 54, no. 5, pp. 504–514, 2003.
- [7] F. Jollant, F. Bellivier, M. Leboyer et al., "Impaired decision making in suicide attempters," *The American Journal of Psychiatry*, vol. 162, no. 2, pp. 304–310, 2005.
- [8] F. Jollant, N. S. Lawrence, E. Olie et al., "Decreased activation of lateral orbitofrontal cortex during risky choices under uncertainty is associated with disadvantageous decision-making and suicidal behavior," *NeuroImage*, vol. 51, no. 3, pp. 1275–1281, 2010.
- [9] A. Zermatten, M. van der Linden, M. D'Acromont, F. Jermann, and A. Bechara, "Impulsivity and decision making," *Journal of Nervous and Mental Disease*, vol. 193, no. 10, pp. 647–650, 2005.
- [10] M. Leyton, V. Paquette, P. Gravel et al., " α - $[^{11}C]$ methyl-L-tryptophan trapping in the orbital and ventral medial prefrontal cortex of suicide attempters," *European Neuropsychopharmacology*, vol. 16, no. 3, pp. 220–223, 2006.
- [11] K. Audenaert, K. van Laere, F. Dumont et al., "Decreased frontal serotonin 5-HT_{2A} receptor binding index in deliberate self-harm patients," *European Journal of Nuclear Medicine*, vol. 28, no. 2, pp. 175–182, 2001.
- [12] C. Schilling, S. Kühn, A. Romanowski, F. Schubert, N. Kathmann, and J. Gallinat, "Cortical thickness correlates with impulsiveness in healthy adults," *NeuroImage*, vol. 59, no. 1, pp. 824–830, 2012.
- [13] I. K. Lyoo, J. Hwang, M. Sim, B. J. Dunn, and P. F. Renshaw, "Advances in magnetic resonance imaging methods for the evaluation of bipolar disorder," *CNS Spectrums*, vol. 11, no. 4, pp. 269–280, 2006.
- [14] K. M. Cecil, M. P. DelBello, R. Morey, and S. M. Strakowski, "Frontal lobe differences in bipolar disorder as determined by proton MR spectroscopy," *Bipolar Disorders*, vol. 4, no. 6, pp. 357–365, 2002.
- [15] J. A. D. M. S. Amaral, R. S. Tamada, C. K. Issler et al., "A 1 H MRS study of the anterior cingulate gyrus in euthymic bipolar patients," *Human Psychopharmacology*, vol. 21, no. 4, pp. 215–220, 2006.
- [16] G. S. Malhi, B. Ivanovski, W. Wen, J. Lagopoulos, K. Moss, and P. Sachdev, "Measuring mania metabolites: a longitudinal proton spectroscopy study of hypomania," *Acta Psychiatrica Scandinavica. Supplementum*, no. 434, pp. 57–66, 2007.
- [17] M. A. Frye, J. Watzl, S. Banakar et al., "Increased anterior cingulate/medial prefrontal cortical glutamate and creatine in bipolar depression," *Neuropsychopharmacology*, vol. 32, no. 12, pp. 2490–2499, 2007.
- [18] N. C. Patel, K. M. Cecil, S. M. Strakowski, C. M. Adler, and M. P. DelBello, "Neurochemical alterations in adolescent bipolar depression: a proton magnetic resonance spectroscopy pilot study of the prefrontal cortex," *Journal of Child and Adolescent Psychopharmacology*, vol. 18, no. 6, pp. 623–627, 2008.
- [19] B. P. Forester, C. T. Finn, Y. A. Berlow, M. Wardrop, P. F. Renshaw, and C. M. Moore, "Brain lithium, N-acetyl aspartate and myo-inositol levels in older adults with bipolar disorder treated with lithium: a lithium-7 and proton magnetic resonance spectroscopy study," *Bipolar Disorders*, vol. 10, no. 6, pp. 691–700, 2008.

- [20] R. Senaratne, A. M. Milne, G. M. MacQueen, and G. B. C. Hall, "Increased choline-containing compounds in the orbitofrontal cortex and hippocampus in euthymic patients with bipolar disorder: a proton magnetic resonance spectroscopy study," *Psychiatry Research: Neuroimaging*, vol. 172, no. 3, pp. 205–209, 2009.
- [21] M. B. First, R. L. Spitzer, M. Gibbon, and J. B. Williams, *Structured Clinical Interview for DSM-IV Axis I Disorders—Clinical Version (SCID-CV)*, American Psychiatry Press, Washington, DC, USA, 1997.
- [22] M. Hamilton, "A rating scale for depression," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 23, pp. 56–62, 1960.
- [23] J. A. A. Vilela, J. A. S. Crippa, C. M. Del-Ben, and S. R. Loureiro, "Reliability and validity of a Portuguese version of the Young Mania Rating Scale," *Brazilian Journal of Medical and Biological Research*, vol. 38, no. 9, pp. 1429–1439, 2005.
- [24] L. F. Malloy-Diniz, P. Mattos, W. B. Leite et al., "Translation and cultural adaptation of Barratt Impulsiveness Scale (BIS-11) for administration in Brazilian adults," *Jornal Brasileiro de Psiquiatria*, vol. 59, no. 2, pp. 99–105, 2010.
- [25] F. Nery-Fernandes, M. V. Rocha, A. Jackowski et al., "Reduced posterior corpus callosum area in suicidal and non-suicidal patients with bipolar disorder," *Journal of Affective Disorders*, vol. 142, no. 1–3, pp. 150–155, 2012.
- [26] H. Scherk, M. Backens, T. Schneider-Axmann et al., "Cortical neurochemistry in euthymic patients with bipolar I disorder," *World Journal of Biological Psychiatry*, vol. 10, no. 4, pp. 285–294, 2009.
- [27] F. Nery-Fernandes, L. C. Quarantini, A. Galvão-De-Almeida, M. V. Rocha, F. Kapczinski, and N. Miranda-Scippa, "Lower rates of comorbidities in euthymic bipolar patients," *World Journal of Biological Psychiatry*, vol. 10, part 2, no. 4, pp. 474–479, 2009.
- [28] F. Nery-Fernandes, L. C. Quarantini, J. L. Guimarães et al., "Is there an association between suicide attempt and delay of initiation of mood stabilizers in bipolar I disorder?" *Journal of Affective Disorders*, vol. 136, no. 3, pp. 1082–1087, 2012.
- [29] C. S. Gama, M. Kunz, P. V. S. Magalhães, and F. Kapczinski, "Staging and neuroprogression in bipolar disorder: a systematic review of the literature," *Revista Brasileira de Psiquiatria*, vol. 35, no. 1, pp. 70–74, 2013.
- [30] L. N. Yatham, S. H. Kennedy, S. V. Parikh et al., "Canadian network for mood and anxiety treatments (CANMAT) and international society for bipolar disorder (ISBD) collaborative update of CANMAT guidelines for the management of patients with bipolar disorder: update 2013," *Bipolar Disorders*, vol. 15, no. 1, pp. 1–44, 2013.
- [31] C.-T. Chiu, Z. Wang, J. G. Hunsberger, and D.-M. Chuang, "Therapeutic potential of mood stabilizers lithium and valproic acid: beyond bipolar disorder," *Pharmacological Reviews*, vol. 65, no. 1, pp. 105–142, 2013.
- [32] R. B. Sassi, M. Nicoletti, P. Brambilla et al., "Increased gray matter volume in lithium-treated bipolar disorder patients," *Neuroscience Letters*, vol. 329, no. 2, pp. 243–245, 2002.
- [33] R. B. Sassi, P. Brambilla, J. P. Hatch et al., "Reduced left anterior cingulate volumes in untreated bipolar patients," *Biological Psychiatry*, vol. 56, no. 7, pp. 467–475, 2004.
- [34] T. Hajek, M. Bauer, A. Pfennig et al., "Large positive effect of lithium on prefrontal cortex N-acetylaspartate in patients with bipolar disorder: 2-centre study," *Journal of Psychiatry and Neuroscience*, vol. 37, no. 3, pp. 185–192, 2012.
- [35] J. K. Rybakowski and A. Suwalska, "Excellent lithium responders have normal cognitive functions and plasma BDNF levels," *International Journal of Neuropsychopharmacology*, vol. 13, no. 5, pp. 617–622, 2010.
- [36] P. Davanzo, M. A. Thomas, K. Yue et al., "Decreased anterior cingulate myo-inositol/creatine spectroscopy resonance with lithium treatment in children with bipolar disorder," *Neuropsychopharmacology*, vol. 24, no. 4, pp. 359–369, 2001.
- [37] S. C. Caetano, R. L. Olvera, J. P. Hatch et al., "Lower N-acetyl-aspartate levels in prefrontal cortices in pediatric bipolar disorder: a ¹H magnetic resonance spectroscopy study," *Journal of the American Academy of Child and Adolescent Psychiatry*, vol. 50, no. 1, pp. 85–94, 2011.
- [38] A. C. Swann, M. Lijffijt, S. D. Lane, J. L. Steinberg, and F. G. Moeller, "Interactions between bipolar disorder and antisocial personality disorder in trait impulsivity and severity of illness," *Acta Psychiatrica Scandinavica*, vol. 121, no. 6, pp. 453–461, 2010.
- [39] S. M. Strakowski, D. E. Fleck, M. P. DelBello et al., "Impulsivity across the course of bipolar disorder," *Bipolar Disorders*, vol. 12, no. 3, pp. 285–297, 2010.
- [40] R. L. Powers, M. Russo, K. Mahon et al., "Impulsivity in bipolar disorder: relationships with neurocognitive dysfunction and substance use history," *Bipolar Disorders*, vol. 15, no. 8, pp. 876–884, 2013.
- [41] E. L. Swing and C. A. Anderson, "The role of attention problems and impulsiveness in media violence effects on aggression," *Aggressive Behavior*, vol. 40, no. 3, pp. 197–203, 2014.
- [42] M. A. Oquendo, H. Galfalvy, S. Russo et al., "Prospective study of clinical predictors of suicidal acts after a major depressive episode in patients with major depressive disorder or bipolar disorder," *The American Journal of Psychiatry*, vol. 161, no. 8, pp. 1433–1441, 2004.
- [43] A. C. Swann, J. M. Bjork, F. G. Moeller, and D. M. Dougherty, "Two models of impulsivity: relationship to personality traits and psychopathology," *Biological Psychiatry*, vol. 51, no. 12, pp. 988–994, 2002.
- [44] M. Hansenne, W. Pitchot, A. Gonzalez Moreno, I. Urcelay Zaldua, and M. Ansseau, "Suicidal behavior in depressive disorder: an event-related potential study," *Biological Psychiatry*, vol. 40, no. 2, pp. 116–122, 1996.
- [45] T. R. Simon, A. C. Swann, K. E. Powell, L. B. Potter, M.-J. Kresnow, and P. W. O'Carroll, "Characteristics of impulsive suicide attempts and attempters," *Suicide and Life-Threatening Behavior*, vol. 32, supplement 1, pp. 49–59, 2002.
- [46] A. C. Swann, J. L. Steinberg, M. Lijffijt, and F. G. Moeller, "Impulsivity: differential relationship to depression and mania in bipolar disorder," *Journal of Affective Disorders*, vol. 106, no. 3, pp. 241–248, 2008.
- [47] A. M. Gilbert, J. L. Garno, R. J. Braga et al., "Clinical and cognitive correlates of suicide attempts in bipolar disorder: is suicide predictable?" *Journal of Clinical Psychiatry*, vol. 72, no. 8, pp. 1027–1033, 2011.
- [48] H. B. Watkins and T. D. Meyer, "Is there an empirical link between impulsivity and suicidality in bipolar disorders? A review of the current literature and the potential psychological implications of the relationship," *Bipolar Disorders*, vol. 15, no. 5, pp. 542–558, 2013.
- [49] M. D. Anestis, K. A. Soberay, P. M. Gutierrez, T. D. Hernández, and T. E. Joiner, "Reconsidering the link between impulsivity and suicidal behavior," *Personality and Social Psychology Review*, vol. 18, no. 4, pp. 366–386, 2014.