**The ups and downs of cellular stress: the “MAM hypothesis” for Bipolar disorder pathophysiology**

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**Abstract**

Mental health problems constitute the largest single source of world economic burden, with an estimated global cost greater than cardiovascular disease, cancer or diabetes individually. In the European Union mental disorders affect millions of people and these numbers are expected to rise as result of Europe’s ageing population. Given the biological causes of many of these disorders, most studies focus on their molecular basis. Bipolar disorder (BD) is characterized by mood swings between depression and mania resulting in cognitive and functional impairments that require lifetime treatment. Recurrent mood episodes, residual symptoms, functional impairment, psychosocial disability with high rates of divorce, unemployment, drug abuse and suicide attempting, and significant medical comorbidities such as metabolic and cardiovascular diseases cannot be efficiently controlled even with proper use of current treatments. Moreover, delayed diagnosis/misdiagnosis is frequent because reliable biomarkers are absent. Therefore, a better understanding of BD pathophysiology is a prerequisite for the design of new drugs and their implementation in clinical practice as well as to develop biomarkers for a more accurate and earlier diagnosis and/or evaluation of therapeutic response. This review summarises the association between decreased cellular resilience towards stress and BD. Since the key stress-response mediator Mitochondria-Associated Membranes (MAMs) modulate several BD relevant processes such as mitochondrial dysfunction and oxidative stress, Ca²⁺ deregulation and cytoskeleton abnormalities, endoplasmic reticulum stress responses, loss of proteostasis and inflammasome activation, we propose the “MAM hypothesis” for BD pathophysiology. Targeting MAM-associated signaling pathways can be a promising investigation avenue to identify novel therapeutic strategies.

**Keywords:** Bipolar disorder, Endoplasmic reticulum, Mitochondria, Mitochondria-associated membranes, Cellular resilience, Cellular stress, Plasticity.
**Bipolar Disorder**

BD is a chronic psychiatric illness with a remitting course, affecting 2.4% of the general population [1], characterized by mood swings between manic and depressive states that result in cognitive and functional impairments, high health care costs and premature mortality [2,3].

BD is the sixth leading cause of disability worldwide responsible for loss of more disability-adjusted life-years than all forms of cancer and major neurological conditions [4]. BD is associated with greatly impaired quality of life and increased physical health burden [5]. Moreover, BD patients tend to have high rates of divorce, unemployment, drug abuse and crime as consequence of impaired social cognition, and its impact on patients can be devastating, with approximately 23% of patients with BD reported having suicide attempts [6]. Moreover, individuals with BD diagnosis have a high risk of medical comorbidities such as metabolic (diabetes, obesity and metabolic syndrome) and cardiovascular disorders [7].

Current knowledge of BD's neurobiology and pathophysiology is still modest [8] and with no clear biological markers available, early diagnosis is presently a great challenge to clinicians.

Pharmacological therapy is often the first-line treatment for BD, followed by psychological [9] and psychosocial interventions [10]. For maintenance pharmacological treatment the main goal is to prevent recurrences of mood episodes, using mood stabilizers (e.g. lithium, valproate, lamotrigine and carbamazepine) and atypical antipsychotics (e.g. olanzapine, aripiprazole, quetiapine and risperidone) or conventional antidepressants for depressive phases (e.g. selective serotonin reuptake inhibitors or buproprion) [11]. Psychosocial treatments include individual psychotherapies, supportive group therapies, education about the disease and focus on treatment adherence and self-care [12]. Furthermore, polypharmacy is still common in BD treatment, reflecting the gap between research and clinical practice. Currently available drugs are primarily targeted at relieving symptoms, are often only partially effective and have significant side effects. Thus, a better understanding of the underlying pathophysiological mechanisms to identify potential therapeutic targets is a prerequisite for the design of new drugs as well as to develop biomarkers that help in a more accurate and earlier diagnosis and/or to evaluate therapies response.

**Impaired cellular resilience**

A growing body of evidence suggests that mood disorders are associated with regional atrophic brain changes that may be closely associated with abnormalities in cellular plasticity, including the resilience of brain cells to resist or adapt to environmental stressors [13]. Structural neuroimaging and postmortem studies have highlighted anatomical and neuropathological alterations in BD patients such as ventricular enlargement, decreased levels of neuronal integrity markers and reduction of neuronal density in specific brain areas, suggesting abnormalities in the cellular resilience towards stressful conditions of neurons and glia cells [14]. These alterations can explain several clinical features such as the progressive shortening of inter-episode interval with each recurrence occurring in consort with reduced probability of treatment response as the illness progresses. This hypothesis is supported by recent genetic studies in postmortem prefrontal cortex samples, which identified the EGR3 regulatory unit (regulon) that translates environmental stimuli into long-term changes in the brain to be robustly repressed in BD patients [15], further suggesting that an impaired response to stress influences BD risk.

Cellular modeling in BD using lymphoblastoid cell lines, fibroblasts, olfactory neuronal epithelium and neurons reprogrammed from induced pluripotent stem cells (iPSCs) [16-18], has proven useful to understand its biological basis, and potential pathways have been identified, especially in cellular resilience-related mechanisms. The most replicated findings that show consistency with genome-wide association studies (GWAS), brain-imaging and post-mortem brain expression include abnormalities in endoplasmic reticulum (ER)-related stress responses, mitochondrial function and Ca²⁺ signaling, which are often reversed in vitro with lithium. Furthermore, patient-derived cellular models also support that alterations in microRNAs (miRNAs), glia and immune cell signaling, cytokine as well as oxidative stress, inflammasome activation, autophagy and apoptosis play a relevant role in BD pathophysiology [8,19-29].

**ER-mitochondria miscommunication**

The above BD-related events are associated with functions localized to a subdomain of the ER, known as mitochondria-associated membranes (MAMs), which are lipid raft-like domains closely opposed to mitochondria in such a way that the two organelles can physically and biochemically communicate with each other. ER-mitochondria juxtaposition is crucial for efficient inter-organelle Ca²⁺ transmission controlling mitochondrial bioenergetics and pro-survival/pro-death pathways and determining cell fate under stressful conditions [30, 31]. The last few years have been marked by increased research on the molecular structure and function of MAMs, which provided a greater knowledge of the structural proteins dynamically associated with the ER-mitochondria contacts under physiological and non-physiological conditions. MAMs functions elucidation allowed to understand that MAMs role is much more important than it would be anticipated. MAMs are responsible for regulating mitochondrial shape and motility, as well as bioenergetics and redox status. Besides that, MAMs have a central role in the modulation of several key processes for cell survival, such as ER stress, autophagy,
inflammasome signaling and apoptosis [32]. MAMs composition and abundance are highly dynamic being modulated by metabolic demands and cellular insults in order to adapt to different conditions. Under acute or chronic ER stress normal ER-mitochondria cross-talk is affected and this circumstance may cause several dysfunctions, such as metabolic impairment, changes in redox balance and cell death control, which, in turn, may be the source of various central nervous system (CNS) disorders where ER stress plays a central role [33,34] and as a consequence of that develop morphological and biochemical alterations in MAMs [35]. Therefore, changes of the ER-mitochondria axis could be responsible for the onset and progression of several diseases, including cancer, diabetes, obesity and neurodegenerative disorders [33]. Sigma-1 receptor (Sig-1R), an intracellular chaperone that resides specifically at the ER-mitochondria interface, modulates inter-organelle Ca\(^{2+}\) signaling [36]. Beyond these functions, MAM-resident Sig-1R is also important to trigger and to fit anti-stress responses. Under stress conditions, this receptor promotes passage of stress signals from the ER to the nucleus through its interaction with various receptors such as the N-methyl-D-aspartate receptors (NMDARs), ion channels, kinases and numerous regulatory key proteins residing on ER, MAM, nucleus or in the cytosol. Based on these complex intracellular actions the Sig-1R has been conceptualized as a pluripotent modulator in living systems with pleiotropic protective effects. Recent studies found that the Sig-1R regulates bioenergetics, free radical generation, oxidative stress, unfolded protein response (UPR) and cytokine signaling, as well as morphogenesis of neuronal cells, such as neurite outgrowth, synaptogenesis, and myelination, which can be disturbed by cellular stress [37]. Their activation may therefore control a variety of stress-related cellular systems, thus contributing to a cellular defense system that protects the nervous system against chronic stress. In the last two decades, a considerable amount of clinical data demonstrated the role of Sig-1R in various pathologies. In particular ample evidence including the presence of genetic variants within SIGMA1R and the interaction of numerous antidepressants with these receptors, suggested a role of Sig-1R in affective disorders [38,39]. Accordingly, a genetic polymorphism is a risk factor for schizophrenia and Sig-1R levels are significantly reduced in the brain of schizophrenic patients [40]. Currently, some drugs (e.g., fluvoxamine, fluoxetine, escitalopram, donepezil, ifenprodil), which have been used in humans, and some endogenous neurosteroids (e.g. dehydroepiandrosterone) have high to moderate affinity to Sig-1R and exert antidepressant-like and neuroprotective actions supporting their clinical implication in numerous neuropsychiatric diseases [38]. The significant advances in Sig-1R research can be translated into future pharmacological approaches able to control the cellular stress systems based on a better understanding of upstream and downstream intracellular signaling cascades [41].

The above evidences support the “MAM hypothesis” for BD pathophysiology (Figure 1), which considers that ER-mitochondria miscommunication at MAMs is an initial event leading to diminished cellular resilience to stressful conditions and that approaches targeting these specific inter-organelle structures can lead to new treatment strategies and early diagnosis biomarkers. This novel hypothesis is supported by studies demonstrating modifications of several MAM-modulated cellular processes in BD patients, namely Ca\(^{2+}\) dyshomeostasis and cytoskeleton abnormalities, changes in the ER stress responses, mitochondrial dysfunction and oxidative stress, proteostasis (autophagy) impairment and inflammasome activation.

### Ca\(^{2+}\) dyshomeostasis and cytoskeleton abnormalities

Over the last few years, several studies have identified common single polymorphisms (SNPs) in calcium voltage-gated channel subunit alpha 1C (CACNA1C) and ankyrin-3 (ANK3) in BD patients, implicating these as susceptibility genes for BD [42,43]. These findings support that Ca\(^{2+}\) deregulation is involved in BD pathophysiology, as both CACNA1C and ANK3 encode Ca\(^{2+}\) signaling-related proteins: Cav1.2 Ca\(^{2+}\) channel are important regulators of Ca\(^{2+}\) influx into cells stimulated by ER Ca\(^{2+}\) depletion and are critical for normal brain development and plasticity [44,45] and ankyrin directly interacts with the ER 1,4,5-inositol triphosphate receptor (IP3R), which is localized at MAMs and is responsible for ER Ca\(^{2+}\) release [46]. Carriers of the CACNA1C risk polymorphism rs1006737 exhibit greater age-related thickness of cortical brain areas widely associated with mood regulation in BD [47]. Furthermore, recent studies demonstrate that the genetic variation rs10761482 of the ANK3 gene affects age-related brain atrophy [48]. Bioinformatics analysis of differentially expressed proteins identified by proteomics in postsynaptic density from the anterior cingulate cortex of BD patients further implicated changes in Ca\(^{2+}\) signaling in BD pathophysiology [49]. Accordingly, L-type Ca\(^{2+}\) channel (LTCC) antagonists have been used in BD for over 30 years without becoming an established therapeutic approach, however, additional genetic, molecular and pharmacological data are required to improve the selectivity, efficacy and tolerability of LTCC antagonists [50].

Neurons derived from individuals with mutations and deficiencies in odd Oz/ten-m homolog 4, TENM4 (ODZ4) genes show abnormal characteristics [51]. ODZ4, a human homolog of the Drosophila pair-rule gene ten-m (odz), encodes teneurin, a transmembrane protein that organizes the cytoskeleton [52], a crucial event for ER-mitochondria contact and Ca\(^{2+}\) transfer [53]. Recently, by profiling the proteomics of BD-hiPSC-derived neurons, data obtained by Tobe and colleagues implicate aberrant posttranslation-modification of collapsin response mediator protein-2 (CRMP2), a cytoskeleton-binding protein that is pres-
Cellular stress response in bipolar disorder

**Figure 1.** The “Mitochondria-Associated ER Membrane (MAM) Hypothesis” for BD pathophysiology. Patient’s cells are chronically exposed to stressful conditions leading to MAMs disruption and subsequent perturbation of cells’ ability to cope with stress (cellular resilience), which arises from loss of proteostasis, changes in lipid and calcium homeostasis, dysregulation of intercellular communication, impairment of ER stress responses, mitochondrial dysfunction and oxidative stress, formation of inflammasome and induction of apoptosis, which all have been described in BD. Impairment of these MAM-regulated events decreasing cellular resilience can explain several BD outcomes namely its progressive nature, the functional outcome, the physical complications and also the therapeutic implications, supporting MAMs as promising therapeutic targets for BD.

- **Progression of BD**
  - Increased severity of episodes
  - Inter-episode subthreshold symptoms
  - Rapid cycling

- **Therapeutic implications**
  - Treatment resistance
  - Polypharmacy
  - Treatment noncompliance

- **Physical complications**
  - Obesity
  - Diabetes mellitus
  - Cardiovascular disease

- **Functional outcome**
  - Cognitive decline
  - Psychosocial impairment
  - Loss of autonomy

**Compromised ER stress response**

The ER consists of a large membranous network and is the cellular site for synthesis, folding, and maturation of most secreted and transmembrane proteins. It has many different functions including the translocation of proteins across the ER membrane, the integration of proteins into the membrane, the folding and modification of proteins in the ER lumen, the synthesis of phospholipids and steroids on the cytosolic side of the ER membrane, and the storage of Ca²⁺ ions in the ER lumen and their regulated release into the cytosol [19,33]. Lately, ER has been put forward as a key organelle in inducing chronic stress associated with many brain disorders including neuropsychiatric diseases such as BD [58].

ER stress is caused by the accumulation of unfolded and misfolded proteins in the ER lumen leading to disturbed...
ER homeostasis. ER stress activates a signaling cascade called the UPR, which triggers a set of transcriptional and translational events that restore ER homeostasis, promoting cell survival and adaptation [59,60]. If this adaptive response fails, a terminal UPR program commits such cells to apoptosis [61]. The outcomes of the cellular response are influenced by ER stress levels [62]. When ER stress is mild, the cell can recover and adapt. However, when ER stress is prolonged or too severe, these mechanisms fail to restore proteostasis leading to autophagy and apoptosis if the stress cannot be alleviated. Canonical mammalian pathways of the UPR pathway involve three specialized ER stress-sensing proteins: protein kinase R-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1α (IRE1α) and activation of transcription factor 6 (ATF6) [63]. In cells undergoing ER stress, the ER chaperone, glucose-regulated protein 78 (GRP78) dissociates from the ER transmembrane sensors [64] and promotes their activation, thus inducing phosphorylation and oligomerization of IRE1α and PERK, as well as the translocation of ATF6 to the Golgi where it is cleaved by Site 1 and Site 2 proteases (S1P and S2P) [63]. Active IRE1α processes mRNA encoding for X-box binding protein 1 (XBP1), a transcription factor that up-regulates genes that encode mediators of the ER-associated degradation (ERAD) pathway, organelle biogenesis and protein quality control [65]. PERK activation reduces protein load in the ER by decreasing general protein synthesis through phosphorylation of the α-subunit of eukaryotic translation-initiation factor 2α (eIF2α), which paradoxically increases selective translation of activating transcription factor 4 (ATF4) mRNA [66]. ATF4 is a member of the bZIP family of transcription factors that activates expression of several UPR target genes involved in antioxidant responses, such as the transcription factor Nrf2, apoptosis and autophagy [67,68]. In cells experiencing ER stress, ATF6 is cleaved at the Golgi apparatus allowing the newly released cytosolic domain to translocate to the nucleus where it regulates ER chaperones, ERAD-related genes, and proteins involved in organelle biogenesis [69]. Severe and/or chronic ER stress can have, however, negative effects leading to apoptosis through different pathways such as: the rise of reactive oxygen species (ROS) and Ca²⁺ levels, activation of the ER-resident caspase-12 (in rodents or caspase-4 in humans), CAAT/enhancer binding protein homologous protein (CHOP) or c-Jun NH2-terminal kinase (JNK) [70]. Several evidences highlight the role of ER-related stress response in BD. For instance, the fact that the expression of the BiP protein (GRP78 protein / glucose-regulated protein, 78 kDa), a chaperone that is highly expressed in the ER, can be up-regulated by different types of mood stabilizers, allowed to hypothesize that the ER, an organelle that has been described to be implicated in the cellular response to different forms of cellular stress, may be involved in the pathophysiology of BD. Lately, evidence emerged revealing that lithium and valproic acid (VPA), drugs widely used in the treatment of BD, increase the expression of ER chaperones and activate the unfolded protein response element (UPRE), suggesting that their mode of action includes UPR activation [71]. Furthermore, genetic association studies have given an important contribution in order to strengthen this hypothesis by demonstrating significant association of GRP78 promoter polymorphisms with BD and linkage between other ER-stress associated genes like XBP1 (X-box binding protein 1) or GRP94 (glucose-regulated protein, 94 kDa, synonym for heat shock protein HSP90B1) and BD pathology [21]. XBP1, a pivotal gene in the ER stress response, has been considered a genetic risk factor for BD and a polymorphism in its promoter was found less effective to reduce ER stress in BD patients-derived cells [72]. Also, changes in the levels of several ER UPR-related proteins have been described in BD. In healthy controls but not in BD patients it was found an increase in the levels of GRP78, eIF2α-P, and CHOP after ER stress induction, which represents a cellular response to inhibit global protein synthesis and, therefore, functions as a defense mechanism triggered to cope with stress and restore ER homeostasis [19]. Tunicamycin-induced cell death was found significantly higher in patients compared to controls and, more importantly, early-stage patients did not differ from controls while the late-stage patients showed an impaired ER stress response [19]. These findings are in accordance with the “MAM hypothesis” for BD pathophysiology (Figure 1), which hypothesizes that ER-mitochondria miscommunication at MAMs is an initial event diminishing cellular resilience to stressful conditions in BD patients. Patients presenting Darier’s disease with a mutation in the ER Ca²⁺ pump SERCA have high rates of comorbid BD and/or the presence of manic-like symptoms [73] further supporting deregulated ER stress responses in BD. Moreover, a Bcl-2 polymorphism was shown to contribute to Ca²⁺ dyshomeostasis in BD through direct regulation of ER IP3R and Bcl-2 knockout mice have increased anxiety-like behaviours [74]. Accordingly, deregulation of Ca²⁺ signaling is one of the most reproducible observations in BD and is supported by the alteration of transcripts involved in Ca²⁺ signaling in iPSCs from BD patients [22]. Finally, Disrupted-In-Schizophrenia I (DISC1), a genetic candidate for BD, also modulates transcriptional responses to ER stress [75].

Mitochondrial dysfunction

Mitochondria are small organelles that play a central role in cell metabolism because they are the main energy providers by converting metabolites to adenosine-5'-triphosphate (ATP). Based on the chemiosmotic theory, most of the ATP produced by ATP synthase arises from the electrochemical gradient generated by the electron transport chain across the inner membranes of mitochondria [76]. Furthermore, mitochondria are also key organelles in diverse pathophysiological contexts, such as the regulation
of free radicals production, Ca²⁺ homeostasis and redox signaling, and also take part in the intrinsic pathway of apoptosis [76,77]. Besides that, mitochondria are involved in modulation of neuronal activity, neuroplasticity and morphogenesis because their distribution and activity are key factors for the normal neuronal development and synaptic plasticity. For instance, they control the neurotransmitter’s exocytosis and ion homeostasis in presynaptic nerve terminals [76,78].

There is increasing evidence showing that mitochondrial dysfunction is implicated in different diseases, such as cardiovascular diseases, neuromuscular neuropathies and neurodegenerative and neuropsychiatric disorders [76]. Impaired mitochondria function can arise from mutations or polymorphisms of mitochondrial DNA (mtDNA), deregulation of free radicals production leading to oxidative stress, impaired phospholipid metabolism and glycolytic shift, ATP synthesis decrement and changes in Ca²⁺ homeostasis, which were already implicated in mood disorders [76,77]. Kato and Kato were the first to propose the mitochondrial dysfunction hypothesis for BD based on the presence of mtDNA abnormalities and amino acid substitutions in specific candidate genes in BD patients, such as the mtDNA polymorphisms 5178C and 10398A; they found that these abnormalities were associated with decreased brain intracellular pH and alteration of intracellular Ca²⁺ signaling, respectively [79]. Numerous evidences that emerged recently support that impaired mitochondrial function is associated with mood disorders and in particular with BD [76,77,80,81].

Multiple lines of evidence suggest that mood abnormalities arising from alterations of mitochondria and energy production are a key feature in the pathophysiology of BD [82]. Evidence of mitochondrial dysfunction in BD includes decreased levels and activity of nuclear-encoded subunits of mitochondrial respiratory complexes, decreased pH and altered oxidative phosphorylation in the brains of BD patients [83]. Elevated lactate levels in BD patient’s brain suggest a shift from aerobic to anaerobic metabolism, further supporting mitochondrial dysfunction in BD [82]. Changes in the expression of mitochondria-related genes also corroborate the mitochondrial impairment in BD. Maeda and colleagues [84] showed a decrease in DISC1 expression in lymphoblasts with the DISC1 risk haplotype for BD, when compared to lymphoblasts from control subjects. Scola and colleagues demonstrated that patients with BD have a differential gene expression that increases the sensitive towards dysfunction of the electron transfer process [85]. The NDUFV2 gene, which is required for the first step in the electron transfer process, NDUFS8, which controls the electron relay, and NDUFS7, which is responsible for the reduction of ubiquinone to ubiquinol, are down-regulated in BD patients. Later on, an up-regulation of NDUFV2 was detected in depressed state when compared with euthymic state [86]. Yoshimi and colleagues obtained additional evidences corroborating the theory of mitochondrial dysfunction in BD. Through metabolomic assays of cerebrospinal fluid (CSF) obtained from BD patients, they observed that deregulation of isocitrate metabolism in the mitochondrial citric acid cycle is relevant in the pathogenesis of BD [87]. Moreover, mitochondrial dysfunction has been identified as the cause of the neuroprogression and cognitive impairment observed in BD. As mentioned above, brain mitochondria are essential for neurotransmission and neuronal plasticity, including long-term potentiation induction, which is required for cellular resilience under stress conditions and behavioral adaptation. Therefore, mitochondrial dysfunction will consequently lead to cellular resilience alterations, which play a major role in BD [77]. Mood stabilizers are used to treat BD because they have modest antidepressant properties and avoid the recurrence of new mood episodes [88]. Recently, several studies clarified the mechanisms of action of these stabilizers, revealing that they affect mitochondrial function [77].

Taken together, the above evidence allowed to face mitochondrial dysfunction as the most robust alteration found in the postmortem BD brain [77].

According to the MAM hypothesis, communication between ER and mitochondria at MAMs level is required to regulate cell homeostasis and allow maintenance of cellular resilience under stress conditions. Thus, if both mitochondrial integrity and function are impaired this communication will be affected, and the downstream events will be compromised.

**Oxidative stress**

A cell is in an oxidative stress state when an imbalance between the production of ROS and antioxidant activities occurs [89]. Mitochondria, in addition to be the major energy source of the cells, are also considered the main origin of ROS since superoxide radical is generated as a consequence of the electron transport chain, which may give rise to oxidative stress and subsequent cell damage. As result of accumulation of oxidative lesions, several physiological functions can be affected, increasing disease’s incidence concomitantly with a reduction in life span [90]. Despite the physiologic relevance of low and intermediary ROS levels that preserve cell survival [91] oxidative stress has been faced as a potential unifying mechanism contributing to several human pathologies since high ROS concentrations, above the clearance capacity of the cell cause oxidative stress, mitochondrial dysfunction, cellular damage, and, in numerous cases, cell death [92]. Accordingly, increasing evidence suggests the involvement of oxidative stress in the pathology and progression of BD and several studies report the increase in the levels of oxidative stress markers in BD patients [26,77]. Lipid peroxidation and nitric oxide levels were shown significantly increased in red blood cells or serum from BD patients compared to healthy controls [93,94] and increased protein oxidation and ni-
Proteostasis was detected in synaptosomes and mitochondria isolated from patients’ postmortem prefrontal cortex [95]. 4-hydroxy-2-nonenal (HNE), which is considered a strong marker of oxidative stress that leads to the formation of HNE-protein adducts able to alter cellular homeostasis and cause the development of a pathological state, was found in the cortex of bipolar patients [96]. Oxidative damage of nucleic acids was also observed and was found to be increased in peripheral and post-mortem patient brain samples [93,94,97,98]. However, contradictory data on the activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase was reported in BD patients [94]. Recent evidences support that the cellular effects of oxidative stress worsen with time and number of manic episodes. Despite unchanged levels of the main cerebral antioxidant glutathione were found in the anterior cingulate cortex of BD patients in comparison with healthy controls [99], Rosa and collaborators, using blood samples from patients with different ages of disease onset, showed that glutathione levels are lower in BD patients and observed a negative correlation with the age at onset [100]. In addition, higher protein carbonyl and lipid hydroperoxide content were determined in adults compared to adolescents with BD [101] and the increased levels of an early component of the peroxidation chain in euthymic older patients with BD support the hypothesis of a persistent effect of ROS in patients with BD into late life [102]. It was observed in a recent exploratory study that the plasma levels of oxidative stress markers were lower in adolescents with fully syndromal BD than controls, while levels in the at-risk groups were between healthy controls and fully syndromal BD supporting a role of oxidative stress in BD risk progression [103]. Furthermore, Andreazza and colleagues observed that superoxide dismutase activity was higher in manic and depressed patients compared to euthymic patients and controls [104]. Of relevance, the antioxidant properties of numerous mood stabilizers were described [105-110].

**Proteostasis (autophagy) impairment**

Protein misfolding and aggregation have been described as relevant events in many neurodegenerative and neuropsychiatric diseases [111]. The majority of neuropsychiatric diseases, such as schizophrenia, BD, depression and autism are associated with variations in the DISC1 gene that is expressed in neuronal dendritic spines controlling spine and synapse development. These variations seem to promote DISC aggregation since dimers, octamers, higher oligomers and insoluble aggregates of DISC1 have been identified in some of these chronic illnesses. The formation of DISC1 aggregates was recently associated with a decrease in free soluble protein and a gain of toxic function leading to the impairment of mitochondrial axonal transport [111]. Thus, the occurrence of certain mutations can trigger the accumulation of misfolded proteins, which might lead to the formation of protein aggregates [34,111] that can affect several mechanisms, such as mitochondrial function, slowing axonal transport and promoting oxidative stress [111].

Proteostasis, which means regulated protein homeostasis, is a complex process that requires the dynamic coordination between efficient folding of newly synthesized proteins, quality control and degradation mechanisms to reduce the load of unfolded and/or misfolded proteins to prevent abnormal protein aggregation [34]. Chaperones are central players in proteostasis maintenance since they are responsible for protein folding in cells, acting as an effective first line defense against protein misfolding through the recognition of non-native conformations of polypeptides that are sent for refolding. When this protective mechanism, which allows recovering the native conformation of proteins, fails degradation pathways—ubiquitin proteasome pathway (UPS) and autophagy—are activated. UPS and lysosomal autophagic degradation pathways are complementary in their mode of action, acting as a second barrier to restore proteostasis. When the proteasome is defective or saturated, or if there is an excess of ROS, the accumulation of misfolded proteins occurs leading to the formation of both toxic soluble oligomers and larger aggregates that can then be eliminated by autophagy [34,111]. However, in several neurodegenerative disorders such as Parkinson’s disease and amyotrophic lateral sclerosis, autophagic vacuoles (autophagosomes or autophagolysosomes) accumulate suggesting lysosomal dysfunction. Due to the impairment of the autophagic pathway, misfolded protein and aggregates are not cleared from the cells resulting in cell death [111].

Lithium, a classic mood stabilizer, was also proposed for treatment of Huntington’s disease (HD) and ALS because it was shown to be able to induce autophagy. In combination with two other mood-stabilizing and anti-convulsant drugs, VPA and carbamazepine (CBZ), lithium was suggested to induce autophagy through inhibition of inositol monophosphatase (IMPase), an enzyme that catalyzes the hydrolysis of inositol monophosphate into free inositol [111,112]. The fact that lithium is a drug widely used in the treatment of BD, together with the evidence showing that patients with neurodegenerative disorders where it has already been demonstrated the impairment in autophagy—exhibit several neuropsychiatric comorbidities like depression and apathy, anticipate autophagy deregulation in BD. According to this evidence, an increment in autophagy could be beneficial in BD treatment. However, it is important to take into account that these are indirect evidences, indicating that further studies are required.

**Inflammasome activation**

The majority of diseases, namely brain diseases, have been associated with an important inflammatory component [113]. Inflammation is a protective immune response triggered by the immune system in response to harmful stimu-
li, such as pathogens, dead cells or irritants. Immune activation within the CNS occurs to aid repair and regeneration of tissues, hence preventing cell death. However, it can often contribute enhancing neuronal damage. Thus, the inflammatory response in the CNS may have both neuroprotective and deleterious effects, depending on the circumstances [113,114]. The innate immune system is the first to be activated to trigger an immunologic response and it acts through sensing of pathogen-associated molecular patterns (PAMPs) derived from invading pathogens, and danger-associated molecular patterns (DAMPs) [115]. In the CNS, pattern-recognition receptors are primarily expressed by microglia, macrophages, and astrocytes. Microglia cells are often termed the "sentinel of brain parenchyma" because they are constantly in a state of alert to detect pathogen's invasion through the pattern-recognition receptors (PRR) that they express [116]. They can display two different types of receptors: Toll-like receptors (TLRs) that are membrane spanning receptors, and Nod-like receptors (NLRs), which are cytoplasmic sensors able to oligomerize and form a platform for the inflammasome [115,117].

Inflammasomes are cytosolic multimolecular complexes that need to be activated to initiate and sustain the inflammatory response and they are formed by microglia and macrophages within the $\text{CNS}$ [114,115]. They are usually constituted by three main components: a cytosolic pattern-recognition receptor, the enzyme caspase 1 and an adaptor protein, apoptosis-associated speck-like protein containing a CARD (ASC), that facilitates the interaction between the former components. According to the variable amino-terminal domain displayed, there are several subfamilies within the NLR family. Whereas members of the NLRP subfamily carry an N-terminal pyrin domain (PYD) that interacts with the pyrin domain of ASC to bridge the complex to pro-caspase-1, the NLRs containing a caspase activation and recruitment domain (CARD) bind directly to pro-caspase 1. The inflammasome assembly leads to caspase-1 activation that converts the cytokine precursors pro-IL-1β and pro-IL-18 into mature and bioactive form IL-1β and IL-18, respectively, which are two pro-inflammatory cytokines involved in neuroimmuno-modulation, neuroinflammation and neurodegeneration. These cytokines trigger signaling cascades that culminate in a type of inflammatory neuronal death termed pyroptosis [114,115,117,118]. There are two well-established pathways for NLRP3 inflammasome activation and an alternative NLRP3 inflammasome activation pathway was recently described that occurs exclusively in human monocytes [115,118,119].

Strong evidence has emerged implying the involvement of inflammasomes in the initiation or progression of diseases with a high social and health impact, such as metabolic disorders and neurological diseases, including meningitis, stroke and Alzheimer's disease [114,115]. Furthermore, activation of the immune system has been consistently reported in BD [120,121] and in the last few years, evidence has been emerged implying activation of the NLRP3 inflammasome in BD pathophysiology [17]. Altered levels of inflammatory cytokines were shown in the brain and periphery of patients with BD, suggesting that activation of the inflammatory system may play a role in the pathophysiology of BD [120]. Recent studies suggest that mitochondrial production of ROS may be linked to inflammatory activation. In fact, increased ROS production in result of inhibition of complex I of the mitochondrial respiratory chain led to increased levels of inflammatory factors such as IL-1, caspase 1, and NF-B and was recently linked to activation of an inflammatory redox sensor, the NLRP3. Post-mortem analysis of frontal cortex from BD patients showed lower levels of complex I and NDUFS7, a subunit of complex I, concomitantly with higher levels of mitochondrial NLRP3 and ASC and increased levels of caspase 1, IL-1β, IL-6, TNFα and IL-10 [17], suggesting that brain immune-activation in patients with BD is associated with mitochondrial dysfunction and NLRP3-inflammasome activation. Because inflammasome activation is recognized as a regulatory function associated with the specific membranous ER-mitochondria microdomains [122] a link between interorganelle miscommunication and NLRP3 activation in BD can be anticipated. Recently, it was found that genetic deficiency in vitro of caspase-1 decreased depressive- and anxiety-like behaviors and prevented the exacerbation of depressive-like behaviors following chronic stress [123]. This linkage is also supported by the fact that the induction of an inflammatory response in other neuropsychiatric diseases requires inflammasome activation [115]. Finally, Haneklaus and colleagues demonstrated that both NLRP3 inflammasome formation and IL-1β production are regulated by microRNAs that have been implicated in BD pathophysiology [124].

**Intercellular communication mediated by microRNAs**

MicroRNAs (miRNAs) are a class of endogenous, small, non-coding RNAs that post-transcriptionally regulate gene expression and are highly expressed in the brain, which have emerged as essential regulators of neuronal development, differentiation, and neuroplasticity [125]. miRNAs are present in tissues and in circulating fluids, particularly in the blood and several reports highlight differences of composition in disease states [126]. A peripheral blood compartment that may be particularly relevant are the exosomes, cellular miRNAs-containing secretory vesicles able to attach recipient cells and release miRNAs potentially modulating their function [127]. Recent results clearly indicate that miRNAs are important mediators of stress responses and their deregulation has been implicated in a variety of stress-related neuropathological conditions [128,129]. Accordingly, UPR regulates the expression of many miRNAs that play an important role in the regulation of life and death decisions during ER stress [130]. There is
accumulated evidence linking deregulation of numerous miRNAs with neuropsychiatric disorders, including BD. The problem of multiple susceptibility genes of small effect in BD has led to an increased interest in miRNAs. The mood stabilizer VPA used for treatment of BD has been shown to induce proteasomal degradation of Dicer, which causes a general down-regulation of miRNA expression, suggesting that VPA have some influence on BD psychopathology through the modification of miRNA biogenesis [131]. Evidence from postmortem cortical brain tissue from affected individuals, as well as from BD patient-derived neuronal cultures generated by reprogramming of human fibroblasts into iPSCs subsequently differentiated into neurons, demonstrate the deregulation of specific miRNAs in BD compared to control subjects [20]. Some of these miRNA, such as miR-34a, directly target the BD risk genes ANK3 and voltage-dependent L-type calcium channel subunit beta-3 (CACNB3) and modulate neuronal differentiation, expression of synaptic proteins and neuronal morphology [28]. Genome-wide analysis followed by target gene and pathway analysis of brain-expressed miRNAs support that miR-499, miR-708 and miR-1908 genes and their targets may be implicated in the development of BD [29]. MiR-499 regulates mitochondrial dynamics and apoptotic pathways involving the Ca²⁺-dependent protein phosphatase calcineurin [132] and the risk gene for psychiatric disorders CACNB2.51 is a miR-499 target gene [29]. Recent pathway analysis also indicates a potential role of miR-499 in the regulation of the actin cytoskeleton [29], that is crucial for neuronal cell migration and maturation, neurite outgrowth and maintenance of synaptic density and plasticity. The miRNA miR-708 is located in the first intron of ODZ4, a human homolog of the Drosophila pair-rule gene ten-m (odz), which has been reported as a genome-wide significant susceptibility gene for BD. It was recently found that miR-708 regulates the expression of neuronatin, which is a membrane protein in the ER, and neuronatin-mediated regulation of intracellular Ca²⁺ levels has been implicated in cell migration and neural induction within embryonic stem cells [133]. MiR-1908 was showed to belong to a miRNA cluster that downregulates the MARK1 signaling pathway, thus altering cell proliferation and differentiation [134]. Kim and colleagues [135] identified and validated DLGAP4, GRIN1, STX1A, CLSTN1 and GRM4, which all function in neuronal glutamatergic synapses, as target genes of the recently identified BD-associated miRNA, miR-1908-5p, supporting the hypothesis that neuronal synapses could be a key converging pathway associated miRNA, miR-1908-5p, supporting the hypothesis that disturbance of MAMs, which are crucial for determining cell fate under stress conditions, plays a major role in BD pathophysiology and that MAMs are relevant targets to develop new treatments and biomarkers for early diagnosis.

Concluding remarks

The former Director of the National Institute of Mental Health (USA), Tom Insel, said recently: “Without biology, there is no treatment for mental disorders”. BD is a chronic mental illness that follows a relapsing/remitting course requiring lifetime treatment. However, few treatments are available and have limited efficacy. Lack of biological markers is another difficulty in clinical practice. A growing body of evidence suggests that BD pathogenesis is closely related with cellular plasticity defects, including in brain cells ability to resist or adapt to environmental stressors (cellular resilience). Results obtained in the last years using postmortem brain tissue from BD patients and also patient-derived cellular models support the hypothesis that disturbance of MAMs, which are crucial for determining cell fate under stress conditions, plays a major role in BD pathophysiology and that MAMs are relevant targets to develop new treatments and biomarkers for early diagnosis.

Abbreviations

ANK3: ankyrin-3; ASC: apoptosis-associated speck-like protein containing CARD; ATF4: activating transcription factor 4; ATFs: activation of transcription factor 6; ATP: adenosine-5'-triphosphate; BD: Bipolar disorder; CACNA1C: calcium voltage-gated channel subunit alpha 1C; CACNB3: Calcium channel subunit beta-3; CARD: caspase activation and recruitment domain; CBZ: carbamazepine; CHOP: CAAT/enhancer binding protein homologous protein; CNS: central nervous system; CRMP2: collapsin response mediator protein-2; DAMPs: danger-associated molecular patterns; DISC1: Disrupted-In-Schizophrenia 1; eIF2α: α-subunit of eukaryotic translation-initiation factor 2α; ER: endoplasmic reticulum; ERAD: ER-associated degradation; GRP78: glucose-regulated protein 78; GWAS: genome-wide association studies; HNE: 4-hydroxy-2-nonenal; iPSCs: induced pluripotent stem cells; IRE1α: inositol-requiring enzyme 1α; iRNAs: microRNAs; JNK: c-Jun NH2-terminal kinase; LTCC: L-type Ca²⁺ channel; MAMs: Mitochondria-Associated Membranes; miRNAs: MicroRNAs; mtDNA: mitochondrial DNA; nArgBP2: neural abelson-related gene-binding protein 2; NLRs: Nod-like receptors; NMDARs: N-methyl-D-aspartate receptors; PAMPs: pathogen-associated molecular patterns; PERK: protein kinase R-like endoplasmic reticulum kinase; PRR: pattern-recognition receptors; PYD: pyrin domain; ROS: reactive oxygen species; SAPAP3: SAP90/PS95-associated protein 3; Shank3: SH3 and multiple ankyrin repeat domains; SiP and SiP: Site 1 and Site 2 proteases; Sig-1R: Sigma-1 receptor; SNPs: single polymorphisms; TLRs: Toll-like receptors; UPRE: unfolded protein response element; UPS: ubiquitin proteasome pathway; VPA: valproic acid; XBP1: X-box binding protein 1

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Author's contribution

All authors contributed to the content, organization and writing of the manuscript.

Competing interests

The authors declare no conflict of interest.
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Cellular stress response in bipolar disorder


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Cellular stress response in bipolar disorder


