Morphometric post-mortem studies in bipolar disorder: possible association with oxidative stress and apoptosis

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Abstract
Despite extensive research in the last decades, the pathophysiology of bipolar disorder (BD) remains unclear. Access to post-mortem brain tissue of subjects who had BD offers an opportunity to investigate neurobiology and this approach has led to some progress, particularly, due to the availability of more sophisticated molecular and cellular biological methodologies and well characterized brain collections over the past decade. Here we review the findings of morphometric post-mortem studies in BD and interpret them in the context of a potential physiopathological mechanism involving oxidative stress and apoptosis. A review of the literature was conducted to identify post-mortem studies that investigated cellular changes such as number, density and size of neurons and glia, in brains of subjects with BD. We found decreased density of neurons and glia and decreased size of neurons in frontal and subcortical areas of the brain. Based on recent studies that found evidence of increased apoptosis and oxidative stress in BD, we hypothesize that the cell abnormalities described are due to an increase in the apoptotic process that can be triggered, through its intrinsic pathway, by the existence of an exacerbated production of reactive oxygen species and oxidative damage in the disease.

Introduction
Bipolar disorder (BD) is a chronic mental illness characterized by recurrent episodes of mania and depression. Despite extensive research over the last 50 yr, the precise mechanisms underlying expression of symptoms in BD remain unknown, but some progress has been achieved in understanding of the disease. For instance, family and twin studies have confirmed that genetic factors influence susceptibility to BD, although specific genes have not been unequivocally identified (O’Donovan et al. 2009). Further, there is evidence that non-heritable factors play an important role in the aetiology of the disease and environmental influences can lead to durable changes in gene expression (Rutten & Mill, 2009).

To investigate the complex neurobiology of this disease, different approaches have been used. In addition to genetics studies, human studies in vivo have mainly utilized neuroimaging and biochemical strategies. The first has repeatedly shown abnormalities in the brains of BD patients and implicated frontal cortex-limbic circuitries in the pathophysiology of BD (Keener & Phillips, 2007). The second has focused for decades on an imbalance of neurotransmitters in this disorder. This approach has led to some progress (Brambilla et al. 2003; Daban et al. 2005; Kugaya & Sanacora, 2005; Sanacora, 2008; Yatham, 2005), and application of sophisticated molecular and cellular biological methodologies recently have further amplified the understanding of the pathophysiology of BD.
and clarified extracellular and intracellular biochemical pathways, epigenetic mechanisms, cell damage and dysfunction of intracellular organelles (Harrison, 2002; Kato, 2006, 2008; Rutten & Mill, 2009; Schloesser et al. 2008; Young, 2001; Young et al. 2002).

In this paper we review the findings from post-mortem morphometric studies which support the existence of cell damage in frontal cortex-limbic circuitry in BD. Although different mechanisms have been postulated to explain cell damage in BD (Kato, 2006, 2008; Konradi et al. 2004; Rao et al. 2010; Schloesser et al. 2008; Wang, 2007), we discuss here the role of oxidative stress (Andreaazzi et al. 2008; Ranjekar et al. 2003; Wang et al. 2009) and how it may trigger the process of apoptosis. Finally, we provide a hypothesis of how an increase in the process of apoptosis, due to oxidative stress, may explain the cellular loss in BD.

Search description

We report here a review of post-mortem studies that investigated cellular changes in brains of subjects with BD. Our inclusion criteria were: (1) studies that investigated grey matter of frontal-limbic areas; (2) studies that investigated number, density and size of neurons and glia; and (3) presence of three or more studies of the area. We searched PubMed using the key words ‘bipolar disorder’, ‘cell count’, ‘cell size’ and ‘brain’. Sixty-five studies were retrieved and 23 were selected based on the above criteria. References in each identified publication were manually searched and three more studies were found. Finally, 26 papers were included in this review and covered the following brain areas: dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC), hippocampus, amygdala and thalamus.

Frontal areas

The frontal areas have received great attention in the investigation of the pathophysiology of mood disorders, especially BD. In this context, the three next sections will focus on describing the post-mortem morphometric findings in these areas in BD.

DLPFC

This brain area corresponds to Brodmann area (BA) 9 and BA 46, and is mainly related to executive functions (Burruss et al. 2000). We identified seven quantitative post-mortem studies in this cortical region in which glial and neuron alterations were found (Table 1). In a study which included 10 subjects, Rajkowska et al. (2001) found a decrease in density of neurons in layer III (16–22%). More detailed analyses revealed that this global decrease was due to pyramidal neurons which, when separately analysed, were reduced in layers III (a, b, c) and Va (17–30%), while the number of non-pyramidal neurons was unchanged. A trend towards a decrease in neuron size was also detected in this study. Cotter et al. (2002b), using BD brains supplied by The Stanley Foundation (SF), observed a decrease in neuron size in layers V and VI, although no alteration in density was noted. The failure to find decreased neuron density might be due to the different brain collection used compared to Rajkowska et al. (2001). Law & Harrison (2003) also used samples from SF and had similar findings. They found a decrease in size in pyramidal neurons at layer V, but there was no difference in neuron density between BD and controls. Vostrikov et al. (2007) also found a decrease in neuron size, although restricted to the sub-layer IIIc. Sakai et al. (2008), found a decrease in neuron density in layer IV (28%) in the brains of BD subjects from Tokyo Institute of Psychiatry, but no differences were evident in neuron size compared to controls. The density of GABAergic neurons using immunohistochemistry techniques was investigated and the neurons were classified by size. The authors found an increase in the density of large calretinin marked neurons in layer II, a specific sub-population of GABAergic neurons. On the other hand, Beasley et al. (2002b) did not find differences between BD and controls in respect to non-pyramidal neurons, although there was a trend towards decrease in another sub-population of non-pyramidal neurons (calbindin reactive neurons) in layers II and III. In an investigation of the interstitial neuron density of DLPFC white matter, no difference between BD and controls was noted (Beasley et al. 2002a).

Concerning glia, Rajkowska et al. (2001), studied 10 brains of BD subjects and 11 normal controls, and found a 19% reduction in glial density in layer IIIc of DLPFC, and a similar tendency to decrease in layer Vb (12%). When the authors considered only medium-sized glial cells (unspecified type), the density reduction was even more evident (35–45%) occurring in all layers. On the other hand, the density of glial cells increased by 32–100% when only the larger-sized cells were considered. These findings possibly indicate that a change in size of glial cells (a smaller number of medium cells and a larger number of larger cells) takes place in BD, rather than a global decrease in the density of glial cells (Rajkowska, 2002). However, it is still unclear whether reduced glial density is specific to a
<table>
<thead>
<tr>
<th>DLPFC</th>
<th>n</th>
<th>Brain collection</th>
<th>Method</th>
<th>Glia density</th>
<th>Neuron size</th>
<th>Neuron density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rajkowska et al. (2001)</td>
<td>10BD/11C</td>
<td>HBTRC and CWRU</td>
<td>3D</td>
<td>Decreased (19%) sublayer IIIc</td>
<td>NI</td>
<td>PN decrease (17 to 30%) in layers III (a, b, c) and Va</td>
</tr>
<tr>
<td>Beasley et al. (2002a, b)</td>
<td>15BD/15C</td>
<td>SFNC</td>
<td>2D</td>
<td>NI</td>
<td>NI</td>
<td>Trend to decrease in sub-population of GABAergic neurons (calbindin) in layers II and III</td>
</tr>
<tr>
<td>Cotter et al. (2002a, b)</td>
<td>15BD/15C</td>
<td>SFNC</td>
<td>2D</td>
<td>No alterations</td>
<td>Decrease layers V (14%) and VI (18%)</td>
<td>No alterations</td>
</tr>
<tr>
<td>Law &amp; Harrison (2003)</td>
<td>15BD/15C</td>
<td>SFNC</td>
<td>2D</td>
<td>NI</td>
<td>PN decrease in layer V</td>
<td>No alterations</td>
</tr>
<tr>
<td>Uranova et al. (2004)</td>
<td>15BD/15C</td>
<td>SFNC</td>
<td>3D</td>
<td>Oligodendrocytes decrease (29%) layer VI</td>
<td>NI</td>
<td>No alterations</td>
</tr>
<tr>
<td>Vostrikov et al. (2007)</td>
<td>15BD/15C</td>
<td>SFNC</td>
<td>2D</td>
<td>Perineuronal oligodendrocytes decrease layer III (a, b, c)</td>
<td>Decrease layer IIIc</td>
<td>NI</td>
</tr>
<tr>
<td>Sakai et al. (2008)</td>
<td>5BD/5C</td>
<td>TIPMH</td>
<td>2D</td>
<td>No alterations</td>
<td>No alterations</td>
<td>Global decrease (28%) layer IV / increase of large GABAergic neurons (calretinin) layer II (68%)</td>
</tr>
</tbody>
</table>

BD, Bipolar disorder; C, controls; PN, pyramidal neurons; NI, not investigated; DLPFC, dorsolateral prefrontal cortex; HBTRC, Harvard Brain Tissue Resource Center; CWRU, Case Western Reserve University; SFNC, Stanley Foundation Neuropathology Consortium; TIPMH, Tokyo Institute of Psychiatry and Matsuzawa Hospital.
sub-population of glial cells (i.e. astrocyte, oligodendrocyte, microglia), or not. Subsequent studies showed that oligodendrocyte density is reduced in layers III and VI (Uranova et al. 2004; Vostrikov et al. 2007). An earlier electron microscopic study (Uranova et al. 2001) revealed prominent signs of apoptosis and necrosis of the oligodendrocytes in the prefrontal cortex of BD. Neuropathological studies focusing specifically on other glial subpopulations (astrocytes and microglia) in BD are still not completed. On the other hand, two studies failed to identify any glial alterations in the DLPFC of BD subjects (Cotter et al. 2002b; Sakai et al. 2008).

In summary, these neuropathology data suggest that cellular alterations do exist in DLPFC of BD brains, although the results are sometimes conflicting and not always replicated. Differences in brain samples might be one of the reasons for inconsistency in findings. One possible explanation for the difference may concern the age of death in the SF collection, which is primarily composed of younger subjects (42.3 yr) than the Harvard Brain Bank (64 yr). Older populations have been found to be more susceptible to decreased cell densities in post-mortem studies (Rajkowska et al. 2005). Briefly, a decrease in neuronal density and somal size and a decrease in glial density were observed. Some of those studies suggest that specific cell types, like pyramidal neurons and oligodendrocytes are more vulnerable in BD.

**ACC**

This region corresponds to BA 24 and BA 25 and is centrally involved in the regulation of emotional responses (Fountoulakis et al. 2008). Seven quantitative studies investigated this area (Table 2). Regarding neurons, Benes et al. (2001), observed a decrease in density of non-pyramidal neurons in layer II (27%) of the pregenual area. This finding was further investigated by the same group (Woo et al. 2004). They replicated the finding of decreased density of non-pyramidal neurons on layer II of BD (35%) and also found that this was more prominent (60%) in non-pyramidal neurons that received inputs from glutamatergic neurons from other areas of the brain. Moreover, in a study of 21 BD subjects, Bouras et al. (2001), investigated BA 24 (dorsal and subgenual subareas), and found a decrease in neuronal density, although in different layers (III, V and VI). Chana et al. (2003) investigated the supragenual area (BA 24c), where they found neurons of reduced size (16%) in layer V and an increase in neuron density in layer VI. Cotter et al. (2002a), in their investigations on number...
and density of GABAergic neurons in ACC of BD, noted a decrease in density of calbindin-positive neurons, while the densities of parvalbumin- and calretinin-positive neurons remained unaltered. Ongür et al. (1998) and Cotter et al. (2001) did not find alterations in neurons. Both used SF brain collection. Differences in time of fixation and post-mortem interval (PMI) between patients and controls might have contributed to the negative findings.

Regarding glia, Ongür et al. (1998) investigated 18 brains of BD subjects and identified a decrease in the density and number (reduction of 41%) of the glial cells in the subgenual area of the ACC. When these authors divided the brains into two subgroups based on the presence (n = 4) or absence (n = 10) of a family history of BD, the decrease in density and number of neurons was evident only in the brains of subjects with familial BD. These findings were noteworthy as they corresponded to neuroimaging findings from the same group of researchers, which identified a decrease in grey-matter volumes in the same area in a group of patients with familial BD (Drevets et al. 1997).

Interestingly, three subsequent studies failed to find glial alterations in BD and two of these studies (Cotter et al. 2001; Chana et al. 2003) used the same brain samples from SF. However, it is important to note that these studies investigated different subareas of the ACC, such as the pre-genual (Benes et al. 2001) and the supragenual (Cotter et al. 2001; Chana et al. 2003). Furthermore, in contrast to the studies by Cotter et al. (2001) and Chana et al. (2003) which assigned brain samples to diagnostic groups provided by SF, Ongür et al. (1998) conducted a re-evaluation of case-history summary and based on this, the diagnosis of one patient was changed from schizophrenia to BD. Hence, the diagnostic grouping for one brain sample was different between these studies and this may also have contributed to the discrepancy in findings between these studies.

In summary, most post-mortem studies in the ACC reveal a decrease in neuronal density and somal size. Only one study found an increase in neuron size. A decrease in glial density was found in a subgroup of subjects with familial BD, but this finding was not replicated in subsequent studies.

**Subcortical areas**

There are fewer neuropathological studies of limbic or subcortical areas than frontal area studies (Table 3). In the hippocampus, a reduction in the number of non-pyramidal neurons was described in the stratum pyramidale of CA2 subfield (Benes et al. 1998) and in neuronal size in CA1 subfield (Liu et al. 2007). In contrast, one study did not find any alterations in cell counting in the hippocampus of BD brains (Müller et al. 2001).

In the amygdala, a decrease in the total number and density of neurons, a reduction in the total volume of the lateral nucleus, as well as decrease in neuronal density in the accessory basal nuclei were noted by Berretta et al. (2007). Similarly, a decrease in size of the cellular bodies of the neurons (29.7% in the lateral nucleus, and 28.3% in the accessory basal parvocellular nucleus) was also described (Bezchlibnyk et al. 2007). However, two other studies (Bowley et al. 2002; Hamidi et al. 2004) did not find changes in neurons and glia between BD and controls, and a post-mortem study comparing volumes of subcortical structures found no difference in amygdala between BD and controls (Bielau et al. 2005), showing that results regarding changes in amygdala are contradictory.

In the thalamus, a reduction in the number of oligodendrocytes was found in the anterior principal thalamic nucleus and centromedian nucleus in a subset of BD subjects who had experienced psychotic symptoms (Byne et al. 2008). Moreover, thalamus volume was found to be significantly smaller in BD subjects than in controls (Bielau et al. 2005). Other studies did not find changes in neuron and glia number, density or size between BD subjects and controls in the thalamus (Chana et al. 2008; Young et al. 2004, 2007) (Table 3). More studies are needed to clarify cellular changes in the thalamus of BD subjects.

The neuropathological findings reviewed here are in accord with several neuroimaging studies that also noted alterations in the areas included above. ^3^H magnetic resonance spectroscopy studies have consistently reported decreased levels of N-acetyl aspartate (NAA) in DLPFC (Molina et al. 2007; Olvera et al. 2007; Sassi et al. 2005; Winsberg et al. 2000) and hippocampus (Atmaca et al. 2006; Bertolino et al. 2003; Deicken et al. 2003; Scherk et al. 2008). Since NAA is a putative marker of neuronal integrity and mitochondrial dysfunction, reduction in NAA levels suggests a probable decrease in brain neuronal density or energy production (Moffett et al. 2007; Stork & Renshaw, 2005). Other studies on functional neuroimaging have shown decreased cortical activity in the DLPFC of BD subjects (Altshuler et al. 2008; Brooks et al. 2009). Furthermore, a decrease in volume has been consistently found by MRI studies in ACC (Arnone et al. 2009; Drevets et al. 1997; Kaur et al. 2005; Koo et al. 2008), hippocampus (Frazier et al. 2005) and amygdala (Pfeifer et al. 2008) in BD patients. Although this assumption is yet to be proven, cell alterations described...
### Table 3. Morphometric post-mortem studies in the hippocampus, amygdala and thalamus

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Study</th>
<th>Sample</th>
<th>Method</th>
<th>Glial size</th>
<th>Glia density/number</th>
<th>Neuron size</th>
<th>Neuron density/number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hippocampus</strong></td>
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<tr>
<td></td>
<td>Benes et al. (1998)</td>
<td>4BD/11C</td>
<td>HBTRC</td>
<td>2D</td>
<td>NI</td>
<td>NI</td>
<td>No alterations</td>
</tr>
<tr>
<td></td>
<td>Müller et al. (2001)</td>
<td>2BD/13MDD/16C</td>
<td>NBB</td>
<td>2D</td>
<td>NI</td>
<td>No alterations</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>Liu et al. (2007)</td>
<td>14BD/14C</td>
<td>SFNC</td>
<td>2D</td>
<td>NI</td>
<td>NI</td>
<td>PN decrease in CA1</td>
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<td>(12%)</td>
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<td><strong>Amygdala</strong></td>
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<tr>
<td></td>
<td>Bowley et al. (2002)</td>
<td>10BD/12C</td>
<td>HBTRC</td>
<td>3D</td>
<td>NI</td>
<td>No alterations</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>Hamidi et al. (2004)</td>
<td>9BD/10C</td>
<td>HBTRC</td>
<td>3D</td>
<td>NI</td>
<td>No alterations</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>Berretta et al. (2007)</td>
<td>10BD/12C</td>
<td>HBTRC</td>
<td>3D</td>
<td>NI</td>
<td>No alterations</td>
<td>No alterations</td>
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<td>decrease in LN and</td>
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<tr>
<td></td>
<td>Bezchlibnyk et al. (2007)</td>
<td>11BD/15C</td>
<td>SFNC</td>
<td>2D</td>
<td>No alteration</td>
<td>No alteration</td>
<td>Decrease (29% LN,</td>
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<td>28% ABPC)</td>
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<td><strong>Thalamus</strong></td>
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<td></td>
<td>Young et al. (2004)</td>
<td>13BD/11C</td>
<td>SFNC</td>
<td>3D</td>
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<td>NI</td>
<td>No alterations</td>
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<tr>
<td></td>
<td>Young et al. (2007)</td>
<td>11BD/15C</td>
<td>SFNC</td>
<td>3D</td>
<td>NI</td>
<td>No alterations</td>
<td>NI</td>
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<tr>
<td></td>
<td>Byrne et al. (2008)</td>
<td>15BD/15C</td>
<td>SFNC</td>
<td>3D</td>
<td>NI</td>
<td>Decreased number of oligodendrocytes in BD with psychosis</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>Chana et al. (2008)</td>
<td>15BD/15C</td>
<td>SFNC</td>
<td>3D</td>
<td>No alterations</td>
<td>No alteration</td>
<td>NI</td>
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</table>

BD, Bipolar disorder; C, controls; PN, pyramidal neurons; NPN, non-pyramidal neurons; NI, not investigated; OFC, orbitofrontal cortex; LN, lateral nucleus; ABPC, accessory basal parvocellular nucleus; ABN, accessory basal nucleus; HBTRC, Harvard Brain Tissue Resource Center; NBB, Netherlands Brain Bank; SFNC, Stanley Foundation Neuropathology Consortium.
above in neuropathological studies are detected as volume reduction in the *in-vivo* neuroimaging studies in the disease.

Although developmental mechanisms may also play a role, the neuropathological findings described above may be related to cell damage in the brain of BD subjects. However, post-mortem studies do not identify a gliosis reaction in those brains, a classic feature of neurodegenerative diseases, as seen in Huntington disease, for example. The findings of decreased density and size of neurons and glia could potentially be explained by different mechanisms leading to cell damage such as glutamate excitotoxicity and neuroinflammation (Rao et al. 2010), dysfunction of signalling pathways linked to apoptosis (Schloesser et al. 2008) and mitochondrial dysfunction (Kato, 2006; Konradi et al. 2004; Wang, 2007). Since those biological processes have been described elsewhere, we will focus here on a new mechanism, a malfunction in the process of apoptosis (Fig. 1) due to an overproduction of reactive oxygen species (ROS) (Fig. 2). Conceptual issues related to this hypothesis will be examined in the Discussion section.

**Limitations**

Alterations in cell size and density could theoretically be a consequence of differences in pH, tissue fixation time and PMI between BD subjects and controls. Tissue volume can shrink after prolonged fixation time, which will lead to decreased water percentage and consequently increased cellular density. Increasing pH can cause increase in density and higher PMI can lead to an increase in the size of neurons (Hayes et al. 1991). However, most of the studies reported have been careful about the influence of those variables and have controlled for them when performing analyses.

Most of the brains investigated in neuropathological studies were from BD subjects who had been medicated during life. This raises the question of potential bias caused by the influence of drugs on morphometric measures. Available evidence has implicated medications in cellular proliferation and volume increase. Chronic antipsychotic treatment has been associated with increased glial density in animals (Kodama et al. 2004; Selemon et al. 1999) and antidepressants may cause proliferation of oligodendrocytes and activate microglia in culture cells (Grundt & Nyland, 1994). Furthermore, neurotrophic effects of lithium have been shown in studies with animals and cultured rat cells (Nonaka et al. 1998; Omata et al. 2008; Rowe & Chuang, 2004; Yeste et al. 2007; Zhong et al. 2006). In humans, increased volume of hippocampus has been related to the use of lithium in neuroimaging studies (Yucel et al. 2007, 2008), as well as in prefrontal and subgenual areas (ACC).
(Moore et al. 2009). If the increase in volume is due to extracellular space, this could influence measures of cell density (Rajkowska, 2002). Furthermore, in a post-mortem study, Rajkowska et al. (2001) found a weak positive correlation between greater exposure to lithium and a wider layer IIIc in the DLPFC of BD brains. Regarding cell size, Berretta et al. (2007) reported a negative correlation in amygdala between neuronal somata size and lifetime lithium use and exposure to antipsychotics in the last 6 months of life. In the studies reviewed, researchers have used different approaches to control for these effects, such as within-group analyses between drug-free and medicated patients, correlations with lifetime use of medications and statistical methods, such as multiple regression models. However, comparisons between controls and subjects who have never been exposed to medications are necessary to exclude this potential effect in the final results.

Another confounding factor that needs to be clarified in future research is the influence of previous use of alcohol. Neurotoxic effects of alcohol are well known and some morphometric studies have shown alterations in glia and neurons in brains of alcoholics (Hercher et al. 2009; Miguel-Hidalgo et al. 2002; Miguel-Hidalgo & Rajkowska, 2003). Unfortunately, many brains collected for neuropathology studies have undergone alcohol abuse.

There are also technical limitations in neuropathological studies that need to be addressed. The field of quantitative morphology of the brain has gone through changes in the last two decades in respect to the method used to quantify cells in the tissue. A new group of techniques called design-based stereology (also known as 3D methods) has been introduced in place of so-called model-based techniques (2D methods) (Schmitz & Hof, 2005). This has lead to a debate in the literature about the validity of studies that do not use the new method. That is an important matter for this review, since some studies did not use the three-dimensional (3D) counting cell method, thus raising questions about the reliability of results with that method. However, some authors have argued that although the stereological method is intrinsically less biased, model-based studies can be equally valid when appropriate sampling and statistical corrections are done (Benes & Lange, 2001; Everall & Harrison, 2002; Geuna, 2000). Some authors criticize the validity of density measures because this can be affected by tissue shrinkage due to tissue preparation. They emphasize that the whole volume of the structure under study needs to be known, so the number of cells of the whole structure could be derived from density measures. Although this is a valid statement, it is not practical until now in face of the difficulties associated with availability of brain tissue (Everall & Harrison, 2002).
Apoptosis in BD

Cell death can occur as a result of the apoptotic process which is an endogenous cell programme that can be activated by many triggers. Apoptosis is morphologically characterized by rounding-up of the cell, reduction of cellular size (pyknosis), chromatin condensation, nuclear fragmentation (karyorrhexis), little or no ultrastructural modifications of cytoplasmic organelles, plasma membrane blebbing and engulfment by resident phagocytes (Kroemer et al. 2009). Before the final stage of death, the cell goes through different phases, including a reduction in its size. Interestingly, as seen in this review, reduction in cell size (neurons) is one of the most replicated findings in BD neuropathological studies. We hypothesize that the decrease in size is found in those cells which are in the first stage of apoptosis (Fig. 1).

Supporting this hypothesis, a qualitative post-mortem study using electron microscopy found signs of apoptosis in oligodendrocytes from the frontal lobe (BA 10) of BD brains, mainly cell shrinkage, decreased nuclear size and condensation of nuclei and nuclear chromatin (Uranova et al. 2001). Liu et al. (2007) also suggested that apoptosis could play a role in explaining their findings of reduction in size of pyramidal neurons in the hippocampus of BD brains, attributing that to diminished levels of an anti-apoptotic factor, Bcl-2. This molecule has an essential role in intrinsic apoptosis pathway and it was found to be up-regulated by lithium (Chen et al. 1999). In fact, different approaches have found evidence of increased apoptosis in BD more recently. A study in BA 9 of BD brains identified decreased levels of Bcl-2, together with decreased levels of BDNF, another anti-apoptotic factor. In addition, protein and mRNA levels of BAD, BAX, caspase-9 and caspase-3, molecules known as apoptotic factors, were found to be increased in this same region (Kim et al. 2010). Interestingly, BDNF seems to be related to the course of BD, because patients in the late stage of the disease show lower blood levels than patients with the first episode of mania (Kauer-Sant’Anna et al. 2009; Yatham et al. 2009), although decreased levels of BDNF seems to be present in BD since the beginning of the disease compared to controls (Palomino et al. 2006). Furthermore, a microarray study in the hippocampus of BD subjects identified an up-regulation of 19/44 genes related to the apoptosis pathway and a marked down-regulation in anti-apoptotic genes (Benes et al. 2006). Another way to identify apoptosis is to investigate the presence of DNA breaks in the cells, a hallmark feature of the process. Consistently, DNA fragmentation was found to be increased in non-GABAergic cells of layers V/VI of the ACC of BD subjects (Buttner et al. 2007).

In summary, post-mortem and in-vivo studies have found evidence of increased apoptosis in BD. The following sections will explore the evidence of increased oxidative stress in BD and provide a potential mechanism to explain how this redox dysfunction in the mitochondria can lead to apoptosis.

Oxidative stress in bipolar disorder

ROS are constantly produced in all cell types of the body. Under normal physiology, ROS production is detoxified by antioxidant defences. In situations where production of ROS exceeds the competence of the antioxidant system, oxidative damage to molecules can occur. Investigation of oxidative stress can be detected by evaluation of end-products of oxidative reactions and antioxidant enzyme activities (Fig. 2).

A considerable number of studies have shown alterations in the measures of antioxidant defences in BD. Most of the studies found increased activity of superoxide dismutase (SOD) in BD patients (Abdalla et al. 1986; Andreazza et al. 2007a; Kuloglu et al. 2002; Kunz et al. 2008; Machado-Vieira et al. 2007). There are only two studies reporting decreased levels of SOD in BD (Gergerlioglu et al. 2007; Selek et al. 2008). This increased activity of SOD may be a compensation mechanism and is indicative of a precedent increase in...
oxidative stress. Alterations in glutathione peroxidase (GPx) have also been reported in BD. Andreazza et al. (2007a) have found increased activity of GPx in euthymic BD patients, although this finding was not replicated (Abdalla et al. 1986; Andreazza et al. 2009; Kuloglu et al. 2002; Ranjekar et al. 2003). Catalase activity is decreased in euthymic patients (Andreazza et al. 2007a) and increased in unmedicated manic patients (Machado-Vieira et al. 2007). Kuloglu et al. (2002) also found decreased levels of catalase in BD patients. The differences in findings between the studies may be associated with confounding effects of medications. For instance, both GPx and SOD activities measured in neutrophils of BD patients were found to be decreased after treatment with lithium (Aliyaziciog˘lu et al. 2007). Other evidence of increased oxidative stress in BD comes from studies which have shown increased levels of nitric oxide (NO), another free radical, in BD (Gergerlioglu et al. 2007; Selek et al. 2008). Furthermore, a down-regulation in components of the mitochondrial electron transport chain in the post-mortem frontal cortex was found in BD subjects. This can lead to malfunctioning of the ETC and higher ROS production (Sun et al. 2006).

Oxidative damage to biomolecules also is described in BD. Machado-Vieira et al. (2007) found unmedicated manic patients to have increased levels of serum thio-barbituric acid reactive species (TBARS), a marker of lipid oxidative damage, and Andreazza et al. (2007a) identified that this alteration is present in mania, depression and euthymia. Moreover, a meta-analysis showed that TBARS was increased in BD with a large effect size (Andreazza et al. 2008). More recently, a post-mortem study by Wang et al. (2009) identified that 4-HNE levels, another marker of lipid oxidative damage, were significantly increased by 59% in the ACC of BD brains compared to controls. Lipid per-oxidation may affect oxidative phosphorylation, maintenance of mitochondrial membrane potential and mitochondrial Ca2+ buffering capacity leading to necrosis or apoptosis (Ott et al. 2007).

Damage to proteins has also been identified in brains of BD subjects. Recent data showed increased oxidation and nitration of mitochondrial proteins in post-mortem prefrontal cortex from BD subjects (Andreazza et al. 2010). In addition, a study using cultured rat cortical cells has also shown that valproate may prevent protein oxidation (Wang et al. 2003). Furthermore, nitration of proteins was found increased in the serum of BD patients compared to controls. In this study, there was a significant increase in 3-nitrotyrosine levels among patients in the early and late stages of BD (Andreazza et al. 2009).

DNA damage is potentially mutagenic and may contribute to cancer, premature ageing and neurodegenerative diseases (Ott et al. 2007). ROS oxidize guanine and generate 8-oxo-7,8-dihydroguanosine (8-OHG) in RNA and 8-oxo-7,8-dihydro-2-deoxyguanosine (8-OHdG) in DNA, which can be detected to identify and quantify RNA and DNA damage,
respectively. RNA oxidative damage was found significantly increased in CA1, CA3 and dentate gyrus regions of post-mortem hippocampus in BD subjects (Che et al. 2010). In summary, alterations in the antioxidant system, an increase in oxidant substances and the presence of damage to lipids, proteins and RNA have been found in BD. Therefore, oxidative stress seems to play an important role in the pathophysiology of this disease, besides being an open window to study new drugs with preventive action against cellular damage in BD.

Apoptosis and oxidative stress

An interesting viewpoint is the induction of apoptosis by oxidative stress. Recent studies about mechanisms of cytotoxicity and cell death have found that, in the apoptotic intrinsic pathway, this process can be triggered by the existence of an exacerbated production of ROS and by oxidative damage caused by those oxidative radicals (Galluzzi et al. 2009). One of the targets of ROS is mitochondrial DNA (mtDNA), whose proximity to the electron transport chain and lack of protective histones makes it strongly susceptible to damage. This can lead to cell damage due to impairment of mitochondrial respiration (Orrenius, 2007). Another critical event in the induction of apoptosis by ROS is cardiolipin peroxidation. Cardiolipin is bound to cytochrome c and its oxidation facilitates the detachment of cytochrome c, initiating the apoptotic process (Orrenius, 2007; Ott et al. 2007). The first stages of apoptosis are associated with cell suffering and more production of ROS, initiating a vicious circle and completing the process of cell death. Future studies are needed to clarify other details of the path leading to apoptosis due to increased production of ROS. In summary, the findings of cellular alterations in frontal and subcortical areas of the brain in BD might be connected with findings of oxidative stress and apoptosis in the disease. Alterations in cell size could represent a stage of the apoptosis process that culminates in cell death and consequent decrease in cell density. We hypothesize that those different findings could be linked in a unifying view to explain neurodegeneration in BD (Fig. 3).

Conclusion

This is an updated review of post-mortem cellular morphometric studies in BD. Consistent findings of morphometric and morphological cellular alterations are discussed in the context of recent evidences showing increased apoptosis and oxidative stress in BD. A pathological mechanism is described of how oxidative stress may lead to apoptosis and how those cellular alterations found could be stages in the process of apoptosis. Considering that lithium has neuroprotective effects and interacts with mechanisms related to apoptosis, a better understanding of this pathological mechanism in BD can certainly lead to the development of new drugs, which would represent a remarkable advance in the therapeutics of BD in the future.

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Statement of Interest

None.

References


