Benefits of animal models to understand the pathophysiology of depressive disorders

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1. Introduction

Major depressive disorder (MDD) is a potentially life-threatening mental disorder imposing severe social and economic burden worldwide. Despite the existence of effective antidepressant treatment strategies the exact pathophysiology of the disease is still unknown. Large number of animal models of MDD have been developed over the years, but all of them suffer from significant shortcomings. Despite their limitations these models have been extensively used in academic research and drug development. The aim of this review is to highlight the benefits of animal models of MDD. We focus here on recent experimental data where animal models were used to examine current theories of this complex disease. We argue, that despite their evident imperfections, these models provide invaluable help to understand cellular and molecular mechanisms contributing to the development of MDD. Furthermore, animal models are utilized in research to find clinically useful biomarkers. We discuss recent neuroimaging and microRNA studies since these investigations yielded promising candidates for biomarkers. Finally, we briefly summarize recent progresses in drug development, i.e. the FDA approval of two novel antidepressant drugs: S-ketamine and brexanolone (allopregnanolone). Deeper understanding of the exact molecular and cellular mechanisms of action responsible for the antidepressant efficacy of these rapid acting drugs could aid us to design further compounds with similar effectiveness, but less side effects. Animal studies are likely to provide valuable help in this endeavor.

Abbreviations: AD, axial diffusivity; AMPA, amino-hydroxy-methyl-isoxazolepropionic acid; BDNF, brain-derived neurotropic factor; CMS, chronic mild stress; CNS, central nervous system; CRH, corticotropin-releasing hormone; CSF, cerebrospinal fluid; DTI, diffusion tensor imaging; FA, fractional anisotropy; fMRI, functional magnetic resonance imaging; FSL, flinders sensitive line; GABA, gamma-aminobutyric acid; GAD, glutamic acid decarboxylase; Glu, glutamate; GSK3, glycogen synthase kinase-3; 1H MRS, proton magnetic resonance spectroscopy; MD, mean diffusivity; MDD, major depressive disorder; mPFC, medial prefrontal cortex; MRI, magnetic resonance imaging; NMDA, N-methyl-D-aspartate; PET, positron emission tomography; PFC, prefrontal cortex; PPD, postpartum depression; PV, parvalbumin; RD, radial diffusivity; SERT, serotonin transporter; shRNA, short hairpin RNA; SSRI, selective serotonin reuptake inhibitor; SST, somatostatin

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either on genetic manipulations, drug treatment or the application of various social, or environmental stressors (Czéh et al., 2016; Gururajan et al., 2019; Pryce and Fuchs, 2017; Wang et al., 2017b; Willner, 2017). Despite their limitations, these models have been extensively used in drug development and in academic research to understand disease pathophysiology. They continue to provide valuable information even in an era when such new and powerful methods are available as the human-induced pluripotent stem cell (iPSC) technologies which are excellent model systems to study the genetic and cellular mechanisms underlying MDD (Soliman et al., 2017). Furthermore, as recent findings indicate chronic stress models can replicate not only the behavioral and cellular aspects of depression, but they also share common transcriptional signatures with MDD (Scarpa et al., 2020).

Numerous excellent reviews have been published recently which provide detailed descriptions on the currently available animal models and discuss the pros and cons of using them (Antoniuk et al., 2019; Czéh et al., 2016; Gururajan et al., 2019; Harro, 2019; Planchez et al., 2019; Pryce and Fuchs, 2017; Wang et al., 2017b; Willner, 2017). Since this is not a rapidly developing field, truly novel models emerge only occasionally and it may take years to thoroughly validate a new model thus, it is difficult to add fresh information to the available reviews. Therefore, we decided to take another approach. The aim of this review is to present examples how nowadays animal models aid us to explore current concepts of the pathophysiology of the disease. We will also discuss animal studies where potential biomarkers are investigated, since objective diagnostic markers would be exceedingly instrumental both in the clinical practice as well as in drug development.

The traditional hypothesis for the pathophysiology of MDD was the “monoamine theory of depression” which postulated that disrupted serotonergic neurotransmission is the main neurobiological factor underlying the clinical symptoms (Ressler and Nemeroff, 2000; Schildkraut, 1965; Schildkraut and Kety, 1967). For many years this was an outstanding working hypothesis, but since then, it has been appreciated that this theory has several limitations (Massart et al., 2012; Wong and Licinio, 2004) and therefore new theories have been put forward which focused on other neurotransmitter systems, endocrine or inflammatory disturbances, gene–environment interactions, changes of neuroplasticity, or on the role of gut microbiota (Otte et al., 2016). Essentially, these theories are built on clinical observations and the animal studies are used to refine and validate the new concepts.

2. The neuroplasticity theory of depression

The neuroplasticity theory of depression has been formulated about 20 years ago (Duman et al., 2000; Manji et al., 2003, 2001). This theory postulates that neuroplasticity is a core feature of the healthy brain and it is essential for the adaptation to environmental challenges. Impaired neuroplasticity is the cellular basis of depressed mood and contribute to the cognitive bias and impairments which are often present in depressed patients (Pittenger and Duman, 2008; Price and Duman, 2020). According to this concept the different classes of antidepressant drugs share a common mechanism of action, i.e. they normalize the impairment of neuroplasticity (Castrén, 2005). These ideas were largely based on the clinical neuroimaging data demonstrating reduced volume of different limbic structures in depressed patients (Drevets, 2000a, 2000b; Drevets et al., 2008) and on the observation that antidepressant treatment can prevent or normalize this volume decrease (Czéh et al., 2001; Sheline et al., 2003). Although the exact cellular changes contributing to these volumetric alterations are still not completely understood (Czéh and Lucassen, 2007; MacQueen and Frodl, 2011; Malychkin and Coupland, 2015; Schoenfeld et al., 2017), this concept is continuously refined and functions as a good working hypothesis (Price and Duman, 2020).

Animal experiments made significant contribution to the development of this theory (Table 1) mainly because comparable morphological changes have been found in chronic stress models as in depressed patients (Lucassen et al., 2014; Pittenger and Duman, 2008). A vast number of experiments demonstrated molecular and cellular changes in response to stress and the reversal by antidepressant treatment. Most of these studies focused on key limbic brain areas such as the hippocampus, prefrontal cortex (PFC) and amygdala. Animal studies investigating neurogenesis in the adult dentate gyrus made a significant contribution to the neuroplasticity theory of depression as this form of cellular plasticity has been identified as a key player in the neurobiology of depression (Dranovsky and Hen, 2006; Hill et al., 2015; Kempermann and Kronenberg, 2003; Lino de Oliveira et al., 2020; Lucassen et al., 2010a; Malberg et al., 2006; Santarelli et al., 2003; Snyder et al., 2011). Impaired neurogenesis has been later confirmed in the hippocampi of depressed patients as well (Lucassen et al., 2010b), whereas antidepressant medication had a normalizing effect (Boldrini et al., 2012, 2009). Although the importance of adult hippocampal neurogenesis in the pathophysiology of depression has been questioned repeatedly (Eliava et al., 2017; Duque and Spector, 2019), this concept continues to inspire the scientific community. Another form of cellular plasticity which played a crucial role in the creation of the neuroplasticity theory is the dendritic reorganization of pyramidal neurons in response to stress or antidepressant treatment which has been reported in the hippocampus (Magaríños et al., 1996; Magaríños et al., 1997), amygdala (Pillai et al., 2012; Vyas et al., 2002) and also in the prefrontal cortex (Bessa et al., 2009; Liston et al., 2006; Radley et al., 2004). Numerous molecular pathways have been studied in the animal experiments and neurotrophic factors, most prominently BDNF (brain-derived neurotropic factor), have been pointed out as chief regulators of neuroplasticity (Brunoni et al., 2008; Castrén and Rantamäki, 2010; Duman and Monteggia, 2006; Groves, 2007; Martinovitch et al., 2007; Price and Duman, 2020).

3. Disturbed synaptic communication and the role of glial cells

Synaptic connections are key functional and structural elements of the central nervous system (CNS) and accurate synaptic transmission is essential for neuronal communication in the healthy brain. The “synaptogenetic hypothesis of depression” postulates that dysfunctional synaptic transmission is a fundamental element of the pathophysiology of MDD (Duman et al., 2019, 2016; Duman and Agahajanian, 2012; Marsden, 2013). Since in the neocortex the majority of the synapses are glutamatergic and to a lesser extent GABAergic thus, the synaptogenetic theory is largely focusing on these two neurotransmitter systems. Clinical studies document disturbed functioning and reduced number of synapses in the PFC and hippocampus of depressed patients (Holmes et al., 2019; Kang et al., 2012). Animal experiments yield similar data and report on stress-induced disturbances in synaptic communication (Joëls and Baram, 2009; Joëls and De Kloet, 1989; Kim and Diamond, 2002; Popoli et al., 2012) and reduced number of synapses in the hippocampus (Magaríños et al., 1999; Sousa et al., 2000) and PFC (Csabai et al., 2018) of rodents.

It is now well established that glial cells play an active role in synaptic transmission and they are regarded as the third element of the tripartite synapse (Halassa et al., 2007; Perea et al., 2009; Volterra and Meldolesi, 2005). Postmortem histopathological studies document reduced number of glia in the PFC, hippocampus and amygdala of depressed patients (Bowley et al., 2002; Cobb et al., 2016; Czéh and Nagy, 2018; Rajkowska, 2000; Sild et al., 2017; Stockmeier et al., 2004). Disturbed functioning of glial cells contributes to the alterations of cortical glutamatergic and GABAergic signal transmission in depression (Choudary et al., 2005; Verkrhatsky et al., 2016). Until now all major glial cell types have been implicated in the pathophysiology of depression thus, astrocytes (Wang et al., 2017a), microglia (Holmes et al., 2018; Yirmiya et al., 2015) and oligodendrocytes (Boda, 2019; Edgar and Sibille, 2012) all seem to contribute to the pathogenesis of MDD.

In harmony with the clinical findings, animal studies provide experimental evidence on stress-induced astrocytic deficits in the
### Table 1
Key animal studies contributing to the development of the neuroplasticity theory of depression.

| Authors            | Year | Animal model / treatment                                                                 | Species | Investigated brain area | Main findings                                                                 |
|--------------------|------|-----------------------------------------------------------------------------------------|---------|-------------------------|-------------------------------------------------------------------------------|
| Magariños et al.   | 1996 | Chronic psychosocial stress and treatment with the antiepileptic drug phenytoin (which interferes with excitatory amino acid action). | tree shrews | hippocampal CA3         | Chronic stress induced dendritic atrophy of CA3 pyramidal neurons which was prevented by the phenytoin treatment. |
| Gould et al.       | 1997 | A single episode of psychosocial stress.                                                 | tree shrews | hippocampal dentate gyrus | Acute stress reduced cell proliferation in the dentate gyrus.                |
| Malberg et al.     | 2000 | Short- and long-term drug treatments with tranylcypromine, reboxetine, fluoxetine and haloperidol. | Sprague Dawley rats | hippocampal dentate gyrus | Chronic (but not short-term) treatment with different classes of antidepressants stimulated neurogenesis in the dentate gyrus of adult rats. |
| Sousa et al.       | 2000 | Chronic unpredictable stress.                                                            | Wistar rats | hippocampus              | Stress-induced rearrangement and loss of synapses in the hippocampus.        |
| Czéh et al.        | 2001 | Chronic psychosocial stress.                                                            | tree shrews | hippocampus              | The stress-induced inhibition of adult neurogenesis in the hippocampus was normalized by an antidepressant treatment with tianeptine. |
| Vyas et al.        | 2002 | Chronic immobilization stress.                                                          | Wistar rats | basolateral complex of the amygdala | Stellar neurons in the amygdala increased the complexity of their dendritic arborization in response to stress. |
| Santarelli et al.  | 2003 | Chronic unpredictable stress. X-ray treatment to block cell proliferation in the dentate gyrus. Drug treatments with fluoxetine, imipramine and desipramine. | I29/Sv/Ev mice (wild type and 5-HT1A receptor knock-out) | hippocampal dentate gyrus | Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants: i.e., disrupting antidepressant-induced neurogenesis blocks the behavioral responses to antidepressants. |
| Radley et al.      | 2004 | 21 days of daily restraint stress.                                                       | Sprague-Dawley rats | medial prefrontal cortex: layer II/III of the anterior cingulate cortex and prefrontal area | Stress-induced atrophy of the apical dendritic tree of pyramidal neurons in the medial prefrontal cortex. |
| Czéh et al.        | 2006 | Chronic psychosocial stress and antidepressant treatment with fluoxetine.                | tree shrews | hippocampus              | Chronic stress reduced the number of astrocytes. Fluoxetine treatment prevented the effect of stress. |
| Kreisel et al.     | 2014 | Chronic unpredictable stress.                                                            | transgenic mice expressing green fluorescent protein in microglial cells or Sprague-Dawley rats | hippocampus | Microglial disturbances underlie the stress-induced depressive-like behavior and suppressed adult neurogenesis. |
| Hill et al.        | 2015 | A bi-transgenic mouse model to genetically increase adult neurogenesis in combination with chronic corticosterone treatment (to mimic chronic stress). | iBax mice | hippocampal dentate gyrus | Increasing adult hippocampal neurogenesis is sufficient to reduce anxiety and depression-related behaviors. |
| Miyata et al.      | 2016 | Chronic restraint stress in combination with water immersion.                            | C57/BL6 mice | corpus callosum and other white matter tracts | Chronic stress disrupts the organization of the Ranvier nodes and induces various molecular changes in oligodendrocytes. |
| Csabai et al.      | 2018 | Chronic mild stress.                                                                    | Wistar rats | infralimbic cortex       | Chronic stress reduced the number of synapses and myelinated axons in the medial prefrontal cortex of rats. |
Table 2

| Authors            | Year | Animal model / treatment | Species                        | Investigated brain area                                                                 | Main findings                                                                                                                                                                                                 |
|--------------------|------|--------------------------|--------------------------------|------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| B.Czéh and M. Simon |      |                          |                                |                                                                                          | **4. The glutamate and GABA systems as targets for antidepressant drug development**                                                                                                                |
|                    |      |                          |                                |                                                                                          | In the mature human brain, glutamate is the primary excitatory neurotransmitter, as more than 90% of the synaptic connections use glutamate (Glu), whereas gamma-aminobutyric acid (GABA) is the chief inhibitory neurotransmitter. These two systems came into focus for novel drug discovery for depression and other mood disorders. The scientific discoveries led to the FDA approval of two truly novel antidepressant drugs, ketamine and allopregnanolone which act on these two neurotransmitter systems.                                                                 |
|                    |      |                          |                                |                                                                                          | **4.1. Disturbed glutamate neurotransmission in MDD**                                                                                                                                                 |
|                    |      |                          |                                |                                                                                          | Clinical studies document altered glutamate levels in the serum, cerebrospinal fluid and also directly in the brains of depressed patients, while postmortem analysis reveal functional or expression changes of NMDA receptor subunits (Feyissa et al., 2009; Moriguchi et al., 2019; Murrrough et al., 2017; Sanacora et al., 2008). Gliat cells (primarily astrocytes) play a significant role in these alterations as they actively regulate glutamate signaling, by removing glutamate from the synaptic cleft and releasing gliotransmitters acting on NMDA receptors as well as by expressing metabotropic glutamate receptors. Animal studies document that stress exposure induce glutamate release in the hippocampus and PFC (Mohaddam et al., 1993; Mohaddam et al., 1994; Murrough et al., 2018) and a consequent downregulation of NMDA receptors (Harvey et al., 2004). At the same time, NMDA receptor activation contributes to the stress-induced inhibition of adult hippocampal neurogenesis (Gould et al., 1997) and to the stress-induced dendritic reorganization of pyramidal cells (Christian et al., 2011; Martin and Wellman, 2011). These effects can be reversed by NMDA receptor antagonists. A study using mouse models reported that ketamine and other NMDA receptor antagonists had a rapid (within 30 min) antidepressant-like effect in the forced swim test and this effect was mediated by BDNF (Auyer et al., 2011). In another animal experiment, a single administration of ketamine could restore (within 24 h) the chronic stress-induced abnormalities of behavior and the molecular and cellular changes in stress vulnerable rats (Tornese et al., 2019). Furthermore, in a chronic stress model it has been shown that stress can reduce the AMPA receptor-mediated excitation of the temporoammonic synapses at the dendritic domes of CA1 pyramidal cells and this alteration was restored by chronic antidepressant treatment with fluoxetine (Kallarackal et al., 2013). |
4.2. Disturbed GABAergic neurotransmission in MDD

Dysfunctional cortical GABAergic networks have been proposed to be causally related to the pathophysiology of MDD (Croarkin et al., 2011; Levinson et al., 2016; Luscher et al., 2011; Luscher and Fuchs, 2012; Schmitz et al., 2017). This theory emerged based on the in vivo studies which found altered GABA concentrations in the prefrontal cortex of depressed patients using MR spectroscopy (Abdallah et al., 2015; Draganov et al., 2020; Hasler et al., 2007; Romeo et al., 2018) and on the postmortem findings documenting the loss of cortical GABAergic neurons (Maciag et al., 2010; Rajkowska et al., 2007) and indications of deficits in GABA synthesis (Karolewicz et al., 2010; Thompson et al., 2009).

Animal studies provide growing number of evidence that stress exposure affects not only glutamatergic neurotransmission, but GABAergic network functions as well (Table 2). In the hippocampus, chronic stress disrupts the functioning of the parvalbumin-positive perisomatic inhibitory network (Holm et al., 2011; Hu et al., 2010). Reduced number of inhibitory neurons were reported in the hippocampi of chronically stressed rats, especially the parvalbumin, somatostatin, calretinin and neuropeptide Y expressing neurons were affected (Czeh et al., 2005; Czeh et al., 2015; Filipović et al., 2018; Ivana et al., 2019; Rossetti et al., 2018). Chronic stress exposure results in dendritic atrophy of GABAergic interneurons of the hippocampus (Gilabert-Juan et al., 2017) and amygdala (Gilabert-Juan et al., 2011), and reduced expression of GABA-synthesizing enzyme (glutamic acid decarboxylase 67, GAD67) have been found in limbic brain areas of the stressed animals (Banasr et al., 2017; Gilabert-Juan et al., 2011, 2017).

Relatively little is known on the effect of chronic stress on GABAergic neurons of the PFC. An early study found that 11 days of daily restraint stress led to a significant reduction in GABA_A receptor binding in the PFC (Gruen et al., 1995). More recently, a study which subjected mice to 21 days of restraint stress have found dendritic hypertrophy of somatostatin-positive (SST+) interneurons and reduced expression of GAD67 enzyme in the PFC (Gilabert-Juan et al., 2013). Complementing this finding, another study reported that SST+ positive cells display significantly greater transcriptome deregulations after chronic stress compared to the neighboring pyramidal neurons (Lin and Sible, 2015). A study investigating gender differences in stress-susceptibility found increased expression of parvalbumin (PV), but only in female mice and suggested that this increased vulnerability of the female prefrontal PV system may underlie the sex differences in the prevalence and symptomatology of stress-related mood disorders (Page et al., 2019; Shepard et al., 2016; Shepard and Coutellier, 2018). An electrophysiological study found increased inhibitory activity in the medial PFC of rats together with increased inhibitory synapses onto glutamatergic cells after 14 days of variable stress (McKlveen et al., 2016). In a comparable experimental design, we found decreased inhibitory activity in the medial PFC of rats subjected to 9 weeks of mild stress (Czeh et al., 2018) which suggests that the duration of the chronic stress exposure may eventually alter the impact of stress. More recently, a study using a 21 day of chronic unpredictable stress paradigm also reported decreased inhibitory activity in the medial PFC of rats (Ghosal et al., 2020). They also reported that stress reduced the expression of
several synaptic markers of GABAergic neurotransmission, i.e. stress reduced the expression levels of vesicular GABA transporter, GABA synthesizing enzyme (GAD67), and the postsynaptic protein gephyrin, a multi-functional protein that anchors inhibitory neurotransmitter receptors to the postsynaptic cytoskeleton (Ghosal et al., 2020). There is also evidence that chronic stress exposure can reduce the number of various interneuron subtypes in frontal cortical areas (Czéh et al., 2018; Varga et al., 2017), but it is not yet clear whether these changes indicate true cell loss, or only the expression level of cellular markers is reduced. Interestingly, recent data suggest that the GABAergic interneurons are the initial cellular targets of the rapid antidepressant actions of ketamine (Gerhard et al., 2020).

Overall, based on the current knowledge, we can conclude that the neocortical excitatory / inhibitory imbalance is a core feature of stress-related pathologies which is manifested in altered rhythmic network oscillations (Ito et al., 2020; McKlveen et al., 2019) which in turn leads to emotional and cognitive deficits that are typical symptoms of the stress-related psychiatric disorders (Fig. 1).

5. The Gut-Microbiota-Brain axis in depression

The gut microbiota (our “second brain”) is essential to maintain health and via the bidirectional communication between the gut and CNS it can contribute to the development of various neuropsychiatric disorders including MDD (Cryan et al., 2019; Foster and McVey Neufeld, 2013; Luna and Foster, 2015; Valles-Colomer et al., 2019; van de Wouw et al., 2019; Yang et al., 2020). A milestone study reported that depressed mood is associated with decreased gut microbiota richness and diversity and that fecal microbiota transplantation from depressed patients to microbiota-depleted rats can induce depressive-like behavior and metabolism in the recipient animals (Kelly et al., 2016).

Animal studies support the clinical data (Table 3) and a milestone study demonstrated that in germfree rats the absence of the gut microbiota enhanced anxiety-like behavior and the neuroendocrine response to stress (Crumeyrolle-Arias et al., 2014). More recently it has been shown that the gut microbiota contributes to the depression-like behavior and inflammatory processes in the ventral hippocampus of stress vulnerable rats (Pearson-Leary et al., 2020). A longitudinal study which monitored changes in fecal and plasma metabolomes during the development of depressive-like behaviors in rats exposed to chronic unpredictable mild stress revealed that changes in the abundance of fecal metabolites were associated with depressive-like behaviors and with altered levels of neurotransmitters in the hippocampus (Jianguo et al., 2019). The results of this study suggest that changes in amino acid metabolism by the gut microbiota contribute to changes in circulating amino acids and are associated with the behavior symptoms of depression (Jianguo et al., 2019). In turn, nurturing a beneficial gut microbiome with chronic prebiotic treatment can have both antidepressant and anxiolytic effects in mice (Burokas et al., 2017). A recent study demonstrated that psychobiotic therapy of mice with *Bifidobacterium breve CCFM1025* could reverse the chronic stress-induced depressive-like behavior, attenuated HPA-axis hyperfunction and inflammation, and modified BDNF and c-FOS expression in the brain (Tian et al., 2020).

In conclusion, we can state that therapeutic targeting of the gut microbiota seems to be a promising new approach and may eventually accomplish a completely new way of antidepressant treatment strategy with the application of nutritional neuropsychopharmacology (Adan et al., 2019). Obviously, there is still a long way to go until we can reach that point, since most of the results so far stem from small scale studies (Kazemi et al., 2019; Liu et al., 2019; Sanada et al., 2020). We need large scale, well-controlled randomized clinical trials to confirm the promising preliminary results (Jarbrink-Sehgal and Andreasson, 2020). Furthermore, the existing data provide very little information on the underlying mechanisms, i.e. the exact metabolic, intracellular and intercellular processes responsible for these effects. Animal studies could
aid us to unravel these issues.

6. In search of candidate biomarkers

A major limitation of understanding disease pathology and drug development is the fact that the diagnosis of MDD is still based on subjective criteria (First, 2013) and objective laboratory measures are not yet available. Unbiased biomarkers could aid the diagnosis and predict the onset or relapse of MDD as well as the response to medication or psychotherapy (Cristea et al., 2019; Kennis et al., 2020; Korgaonkar et al., 2019; Perlman et al., 2019). Objective biomarkers in preclinical studies would facilitate drug development. Intensive effort has been made to identify clinically useful biomarkers. Currently, neuroimaging is the most promising approach and amongst the serum markers, microRNA detection emerged as a conceivable target.

6.1. Neuroimaging

The continuously evolving functional neuroimaging approaches represent the forefront of MDD biomarker development (Fonseka et al., 2018; Korgaonkar et al., 2019; McGrath et al., 2013; Nazeri et al., 2020). For many years, the magnetic resonance imaging (MRI) studies focused on the volumetric changes of various limbic structures in MDD (Bora et al., 2012; Drevets et al., 2008; Hamilton et al., 2008; Kempton, 2011; Videbech and Ravndalke, 2004). Based on the findings of these studies hippocampal volume decrease emerged as a potential diagnostic marker (Campbell et al., 2004; Kempton, 2011; Koolschijn et al., 2009; Videbech and Ravndalke, 2004). However, hippocampal shrinkage is not specific for MDD and there are also numerous negative results in the literature. The preclinical data on hippocampal shrinkage in animal models is also ambiguous (Czéh et al., 2001; Kalisch et al., 2006; Tse et al., 2014). Nevertheless, imaging technologies are rapidly developing, new structural and functional imaging methods are invented continuously which enables us to analyze metabolic and microstructural changes or to quantify brain functions at a larger scale by evaluating dynamics within and between-systems (Bassett and Sporns, 2017).

Because of its non-invasive nature most of the neuroimaging studies are carried out directly on clinical samples. Neuroimaging studies in rodent models are still in a relative infancy. The main obstacles are that 1) small animal MR equipment is expensive and 2) because of the limited spatial resolution of MR imaging the rodent brain is too small to detect fine details. Despite all these hurdles, a growing number of imaging studies investigated structural and functional changes in rodent models (Table 4). Typically these studies employ various chronic stress models. The first MRI study examining the consequences of repeated immobilization stress used several imaging methods, i.e. high-resolution structural MRI, diffusion kurtosis imaging, and resting-state functional MRI (Henckens et al., 2015). This study found that stress increased connectivity in the somatosensory, visual, and default mode networks and increased the volume of the lateral ventricles, but it did not alter any grey matter volumes (Henckens et al., 2015). A more recent longitudinal neuroimaging study found that in stress susceptible animals, stress induced structural atrophy of several limbic and non-limbic brain areas, which was associated with increased functional connectivity in a network formed by these specific regions (Magalhães et al., 2018).

Proton magnetic resonance spectroscopy (1H MRS) is a MRI-based neuroimaging method which enables the direct measurement of specific metabolites in the living brain. 1H MRS studies of stressed animals reveal altered levels of brain metabolites and neurotransmitters similar to what has been reported in MDD patients (Banasr et al., 2010; Czéh et al., 2001; Delgado et al., 2011; Hemanth Kumar et al., 2012; Khan et al., 2018; Magalhães et al., 2019; Perrine et al., 2014; Sekar et al., 2019; Xi et al., 2011).

Table 4

| Key animal studies using neuroimaging to investigate stress-induced structural and functional changes in the brain. | Year | Animal model / treatment | Authors |
|---|---|---|---|
| 2001 | Chronic psychosocial stress for 4 weeks | Czéh et al. |
| 2011 | Chronic mild stress for 8 weeks | Delgado Y Palacios et al. |
| 2015 | Chronic immobilization stress for 2 weeks | Henckens et al. |
| 2015 | 1H MRS in vivo diffusion kurtosis imaging, structural MRI and DTI | Henckens et al. |
| 2016 | 1H MRS, diffusion kurtosis imaging, structural MRI | Anacker et al. |
| 2018 | 18F-FDG PET | van der Kooij et al. |
| 2018 | Chronic mild stress for 12 weeks | Czéh et al. |
| 2018 | 1H MRS, diffusion kurtosis imaging, structural MRI | Henckens et al. |
| 2019 | 1H MRS, diffusion kurtosis imaging, structural MRI | Magalhães et al. |
| Abbreviations: AD: axial diffusivity; DTI: diffusion tensor imaging; FA: fractional anisotropy; MRI: magnetic resonance imaging; 1H MRS: proton magnetic resonance spectroscopy; MDD: major depressive disorder; PET: positron emission tomography; RD: radial diffusivity. | | | |
technique, which enables the examination of the structural integrity of white matter structures by providing insights into the microstructure of pathways connecting brain structures. The typical readouts of DTI studies are mean diffusivity (MD) and fractional anisotropy (FA). MD represents the overall diffusion of water molecules regardless of directionality and FA corresponds to the degree of diffusion anisotropy. The FA value is thought to reflect fiber density, axonal diameter, and myelination in white matter tracts. Other DTI related parameters such as axial (AD) and radial diffusivity (RD) can reveal microstructural changes of myelinated axons. So far only a few studies employed diffusion MRI to investigate microstructural changes in the CNS of rats exposed to chronic stress and the generated results are somewhat ambiguous. The first study reported subtle substructural changes in the hippocampus of chronically stressed rats using in vivo diffusion kurtosis imaging (Delgado et al., 2011). Later, the same research group investigated diffusion properties of the PFC, caudate putamen and amygdala and found that mean kurtosis in the striatum was significantly different between the stress-susceptible and stress-resilient animals (Delgado y Palacios et al., 2014). A study using a repeated restrain stress protocol reported that using a high field (16.4 T) diffusion-weighted MRI was able to detect dendritic atrophy in the hippocampus (Vestergaard-Poulsen et al., 2011). Another study using the CMS model reported significant changes in MD, FA, AD and RD values in numerous brain areas indicating demyelination and axonal damage (Hemanth Kumar et al., 2014). Yet another study found no evidence for white matter microstructural changes in rats exposed to 10 days of repeated immobilization stress (Henckens et al., 2015). A more recent study which used tract-based spatial statistics analysis approach to explore the consequences of two weeks of repeated inescapable stress found that stress increased FA, but reduced MD and RD values in several white matter bundles of the brain (Magalhães et al., 2017). Others reported on increased FA in the hypothalamus and hippocampal CA3 in stress-susceptible mice after 10 days of social defeat stress (Anacker et al., 2016). Post-mortem diffusion MRI and diffusion kurtosis imaging studies documented specific microstructural changes in the hippocampus, amygdala and several cortical areas of rats exposed to chronic stress (Khan et al., 2016a, 2016b, 2018) and these findings have been confirmed by a recent in vivo DTI experiment (Liu et al., 2018b). More recently, in a longitudinal DTI experiment, we found reduced FA and increased MD, RD values in several major white matter tracts, and in addition to that, we could detect correlations between DTI metrics and the stress-induced changes in the cognitive performance of rats (Nagy et al., 2020).

There are a handful of studies which used small-animal positron emission tomography (PET) to study the stress response (Van Laeken et al., 2018; Wei et al., 2018), or stress-susceptibility (Van Der Kooij et al., 2018), dynamic changes of serotoninergic neurotransmission in different brain areas (Reisinger et al., 2019), or neuro-inflammation in the hippocampus (Wang et al., 2018) in rodents subjected to chronic stress. Another PET study used a different rat model of MDD, the Flinders Sensitive Line (FSL), and reported bilateral hypo-metabolism in the temporal lobes of the FSL rats (Thiele et al., 2016).

Overall, these studies are interesting and important. Interesting, since comparable changes have been found in clinical studies, for example PET studies report on prefrontal hypo-functionality in depressed individuals (Drevets et al., 2008, 1997) and recent meta-analytic studies evaluating the results of clinical DTI data report on similar white matter microstructural changes in depressive disorders, i.e. reduced FA and increased MD, RD values (Koshiyama et al., 2020; van Velzen et al., 2020). The animal studies are useful as they can examine the temporal dynamics of the stress response (Liu et al., 2018b; Magalhães et al., 2018; Nagy et al., 2020). Furthermore, the combination of neuroimaging with conventional light- or electron microscopic methods can advance the interpretation of neuroimaging data (Aswendt et al., 2017; Jelencu et al., 2016; Jespersen et al., 2010; Khan et al., 2019, 2016a). If the animal studies could reveal reproducible structural (or functional) changes specific to the animal models of MDD that could be a great utility in drug development. Clearly, more studies are needed to establish that.

6.2. Potential blood biomarkers: The microRNAs

An ideal biomarker should 1) reliably discriminate pathological from healthy cases, 2) should be relatively non-invasive and 3) inexpensive. Numerous candidates have been studied to identify peripheral biomarkers in depressive disorders. Most of the identified targets are markers of neuroendocrine, inflammatory and metabolic processes, or epigenetic agents (Gruzdov et al., 2019; Gurusurajan et al., 2016; Kennis et al., 2020; Nasca et al., 2018; Strawbridge et al., 2017; Young et al., 2016). Here, we briefly discuss a relatively new candidate the micro RNAs (Allen and Dwivedi, 2020; Ferrúa et al., 2019; Fries et al., 2019; Lopez et al., 2018).

MicroRNAs (miRNAs) regulate numerous cellular processes in the CNS, like cell formation, differentiation and death. MicroRNAs are relatively stable molecules compared to other mRNAs, since they are protected from degradation by forming complexes with proteins or by inclusion in exosomes. Furthermore, their expression levels appear to be cell or tissue type specific. Because of these characteristics, miRNAs are potent candidates to serve as biomarkers.

Animal studies focusing on miRNA changes are meaningful since most miRNA families are phylogenetically conserved from C. elegans to humans (Chiang et al., 2010; Wheeler et al., 2009). Numerous studies documented that stress exposure can influence the expression levels of various miRNAs in the hippocampus, amygdala and PPC of the stressed animals (Table 5) (Buran et al., 2017; Dwivedi, 2011; Meerson et al., 2010; Rinaldi et al., 2010; Xu et al., 2017; Zhou et al., 2018; Zurawek et al., 2017) and some of these expression changes are in harmony with the miRNA changes found in the blood of depressed patients (Bocchio-Chiavetto et al., 2013). Bioinformatic analysis revealed that many of these miRNAs potentially target several cellular and molecular pathways that are implicated in depression including the ERK/BDNF signaling pathway and the serotonin transporter (Zhao et al., 2017; Zhou et al., 2018; Zurawek et al., 2017). One study reported that bilateral intra-hippocampal infusions of miR124a-expressing lentiviral vectors exacerbated depression-like behavior in the chronic social defeat stress paradigm, whereas they observed an anti-depressant like effect, when miR124a-silencers were injected into the hippocampus (Bahi et al., 2014). These findings were partially replicated in a later study which reported on a dysregulated hippocampal miR-124 expression in the CMS model which could be blocked by an antidepressant drug treatment (Higuchi et al., 2016).

Determination of microRNA alterations in the serum has greater translational relevance and there are animal studies which investigated miRNA changes not only in the CNS, but also in the blood of the stressed animals. For example, a study which examined 376 mature miRNAs to find potential peripheral biomarkers related to stress could identify only one, miR-16 which was associated with the stress-resilient phenotype (Zurawek et al., 2016). Serum levels of miR-16 was increased in the stress-resilient rats on specific weeks of the chronic stress exposure, but the stress-susceptible rats did not show any change in their serum miR-16 levels (Zurawek et al., 2016). One well-known target of miR-16 is the serotonin transporter protein, and it has been demonstrated that treatment with the SSRI drug fluoxetine (Prozac) can increase miR-16 levels in the raphe nuclei, which in turn reduces serotonin transporter protein expression (Baudry et al., 2010). Furthermore, a recent study found reduced expression level of miR-16 in the cerebrospinal fluid (CSF) of CMS treated rats and a positive correlation between the expression levels miR-16 in the CSF and in the raphe nuclei (Shao et al., 2018). Another study investigating stress-induced serum miRNA changes reported increased level of miR-34a-5p in the stress-resilient animals (Zurawek et al., 2017) and it has been shown that the miR-34 family is a critical regulator of the behavioral and...
Table 5
Key studies investigating microRNA changes in animal models of depression.

| Authors         | Year | Animal model / treatment                                                                 | Species          | Investigated brain area                                           | miRNA                  | Main findings                                                                                                                                                                                                 |
|-----------------|------|-------------------------------------------------------------------------------------------|------------------|-------------------------------------------------------------------|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Meerson et al.  | 2010 | Immobilization stress: acute stress (a single session) and chronic stress (14 days of daily immobilization). | rat              | hippocampal CA1 and the central nucleus of the amygdala           | miR-134, miR-183       | Acute stress increased miR-134 and miR-183 levels in the amygdala. Chronic stress decreased miR-134 levels in both the amygdala and CA1. This was the first study to demonstrate that the stress response in the mammalian brain involves miRNA-mediated control of other posttranscriptional regulators of gene expression. |
| Baudry et al.   | 2010 | Unpredictable chronic mild stress for 6 weeks and fluoxetine treatment.                   | Swiss-Kunming mice | raphe nuclei and locus coeruleus                                  | miR-16                 | SERT is a target of miR-16 and chronic treatment with the SSRI fluoxetine increased miR-16 levels in the raphe nuclei, which in turn reduced SERT expression. It was proposed that miR-16 contributes to the therapeutic action of SSRI antidepressants. |
| Rinakli et al.  | 2010 | Single or repeated exposures to restraint stress (1 or 5 days).                           | CD1 mice         | frontal cortex and hippocampus                                    | let-7a, miR-9, miR 26-a/b | Acute stress induced a transient increase of let-7a, miR-9 and miR 26-a/b expression selectively in the frontal cortex.                               |
| Bahi et al.     | 2014 | Lentiviral mediated overexpression of miR124a in combination with 21 days of social defeat stress. | Wistar rats      | hippocampus and cortex                                           | miR124a                | miR124a overexpression exacerbated depression-like behavior. However, an antidepressant like effect was observed when miR124a-silencers (siR124a) were injected into the hippocampus. |
| Zarawek et al.  | 2016 | Chronic mild stress for 2 and 7 weeks.                                                     | Wistar Han rats  | Various brain areas and serum.                                    | Various miRNAs         | They examined the serum levels of 376 mature miRNAs to find peripheral biomarkers associated with the stress-resilient phenotype. Stress-resilient rats had elevated serum levels of miR-16 after 7 weeks of chronic stress, suggesting that miR-16 may contribute to a “stress-resistant” behavioural phenotype. |
| Zarawek et al.  | 2017 | Chronic mild stress for 2 weeks.                                                          | Wistar Han rats  | ventral tegmental and prefrontal cortex                          | miR-18a-5p, miR-34a-5p, miR-135a-5p, miR-195-5p, miR-320-3p, miR-674-3p, miR-872-5p | Bioinformatic analysis revealed that all these miRNAs potentially target SERT. Chronic stress increased the expression of these miRNAs in ventral tegmental area, but decreased them in the prefrontal cortex. This effect was more pronounced in the stress-resilient animals. |
| Shao et al.     | 2018 | Chronic unpredictable mild stress for 21 days.                                             | Sprague Dawley rats | raphe nuclei and CSF                                      | miR-16                 | Stress reduced the expression levels of miR-16 in CSF and raphe and there was a positive correlation between miR-16 levels in the CSF and raphe. In contrast, negative correlation was found between CSF miR-16 and raphe SERT protein. |

Abbreviations: CSF: cerebrospinal fluid; SERT: serotonin transporter; SSRI: selective serotonin reuptake inhibitor.
neurochemical stress-response (Andolina et al., 2018, 2016).

In sum, we may conclude that circulatory microRNAs are attractive candidates to serve as clinical biomarkers for the diagnosing of MDD, but definitively more research is needed, since the currently available data is rather inconclusive. A large number (approx. 180) of miRNAs have been identified to be significantly related to depression, but so far very few candidates show reproducible data. In the clinical settings, the most replicated candidate was miRNA-132 while miRNA-16 showed the most reproducible data in the animal studies (Yuan et al., 2018).

7. The utility of animal models in antidepressant drug development

Mental disorders represent an enormous unmet medical need. The history of antidepressant drug discovery is a mixture of time periods with serendipity, great success and severe disappointments. The accidental discovery of the first drugs with antidepressant efficacy in the 1950s, the tricyclic antidepressants (TCAs) and the monoamine oxidase inhibitors (MAOIs), was followed by an enthusiastic period, when a series of rationally designed class of psychotropic medications, the selective serotonin re-uptake inhibitors (SSRIs) and serotonin and noradrenaline reuptake inhibitors (SNRIs), were developed and marketed in the late ’80 and ’90s (Pereira and Hiroaki-Sato, 2018). The dramatic increase of prescription rates of antidepressant drugs helped millions of sufferers and gained large profits to the pharmaceutical industry (Pirraglia et al., 2003). But then, an era came without any significant development and the pharmaceutical industry complained about the lack of new molecular targets, the limitations of animal models, and the lack of biomarkers for clinical trials (Berton et al., 2012; Hyman, 2014).

Mental disorders have been labelled as ‘too difficult’ to tackle (Hyman, 2014). The lack of new molecular targets, the limitations of animal models, and the lack of biomarkers for clinical trials (Berton et al., 2012; Hyman, 2014). Mental disorders have been labelled as ‘too difficult’ to tackle (Hyman, 2014). But then, in March 2019, the US Food and Drug Administration (FDA) approved two new antidepressant drugs: the intranasal S-ketamine therapy (Kryst et al., 2020; Turner, 2019). Furthermore, we have been articulated regarding the safety aspects of prolonged S-ketamine effects of prolonged treatment are still preliminary and serious concerns have been articulated regarding the safety aspects of prolonged ketamine therapy (Kryst et al., 2020; Turner, 2019). Furthermore, we know very little about the exact mechanism of action how S-ketamine exerts its antidepressant effect.

Animal studies help us to unravel the molecular and cellular actions of ketamine (Table 6). For example, a very recent study demonstrated that a specific NMDA receptor subunit (GluN2B) on GABAergic interneurons are the initial cellular trigger for the rapid antidepressant actions of ketamine (Gerhard et al., 2020). Ketamine activates not only the NMDA receptors but also the amino-hydroxy-methyl-isoxazolepropionic acid (AMPA) receptors (Koike et al., 2011; Zhou et al., 2014), which in turn upregulates mammalian target of rapamycin (mTOR) and BDNF in the hippocampus and prefrontal cortex of rats (Zhou et al., 2014). Other candidate mechanisms are the modulation of the intracellular signaling pathways Calcium/Calmodulin-Dependent Protein Kinase II and the Eukaryotic Elongation Factor 2 Kinase pathway (Adaiikkan et al., 2018; Auyt et al., 2011). Yet another study found that ketamine treatment inhibits brain glycogen synthase kinase-3 (GSK3) which was necessary for the rapid antidepressant-like effect of ketamine in the mouse model of learned helplessness (Beurel et al., 2011). More recently, it has been reported that subanesthetic dose of ketamine treatment can acutely suppress the activity of somatostatin-positive interneurons in the medial PFC and by that it modulates synaptically evoked calcium transients in the apical dendritic spines of pyramidal neurons (Ali et al., 2020). This cellular effect of ketamine could influence frontal cortex-dependent behaviors and cortico-cortical connectivity (Ali et al., 2020). Overall, the current working hypothesis is that rapid-acting antidepressants like ketamine stimulate synaptic plasticity, via increasing BDNF production, and by that they reactivate cortical plasticity, which leads to the readjustment of neuronal networks to better adapt to the environmental difficulties (Garcia et al., 2008; Castrén and Rantamäki, 2010; Price and Duman, 2020).

7.2. Postpartum depression and allopregnanolone

Postpartum depression (PPD) is a distinct major depressive disorder which may affect up to 10–20% of women after delivery, and thus, it is one of the most common neurobiological complications of childbirth. PPD is a serious mood disorder which hinders women’s ability to function, and it is a leading cause of maternal suicide. PPD has been associated with impaired mother-infant bonding and with adverse effects on the cognitive, behavioral, and emotional development of the child. In March 2019, brexanolone has been approved by the FDA for IV treatment of PPD and this is the first drug to be approved by the FDA for this indication (Lüscher and Möhler, 2019; Meltzer-Brody and Kanes, 2020; Walton and Maguire, 2019). Allopregnanolone (brexanolone) is a neuroactive steroid and positive allosteric modulator of both synaptic and extra-synaptic GABA<sub>α</sub> receptors (Scott, 2019). Similarly to ketamine, the exact antidepressant mechanism of action is not fully understood. The working hypothesis is that by allosterically enhancing GABA<sub>α</sub> receptor function, allopregnanolone enhance GABAergic inhibition (Lüscher and Möhler, 2019; Meltzer-Brody and Kanes, 2020; Walton and Maguire, 2019). Double-blind, randomized, placebo-controlled clinical trials demonstrate that brexanolone treatment is significantly more effective compared with placebo and results in a significant and clinically meaningful decrease in the total score of the Hamilton Rating Scale for Depression (HAM-D) (Kanes et al., 2017; Meltzer-Brody et al., 2018). Notably, a single infusion of brexanolone appears to have an ultra-rapid antidepressant effect lasting for up to one week.

Despite the frequent occurrence and severe consequences of PPD only a few animal models have been created to mimic this condition (Fernandez et al., 2014; Perani and Slattery, 2014). The most commonly used model involves a hormone-simulated pregnancy and then a subsequent ‘postpartum’ withdrawal of estradiol which can precipitate depressive-like symptoms in female rats (Baka et al., 2017; Green et al., 2009; Stoffel and Craft, 2004; Suda et al., 2008). Another rat model is based on the administration of high levels of corticosterone to the dams during the postpartum period which results in behavioral changes and reduced hippocampal cell proliferation in the offspring (Brummette et al., 2012). In addition to that, there is a preclinical PPD model which uses transgenic mice, a mouse line which is deficient for the 8 subunit of the GABA<sub>α</sub> receptor (Maguire and Mody, 2008). These animals exhibit depression-like behavior during the postpartum period together with abnormal maternal behavior, which in turn results in decreased pup survival (Maguire and Mody, 2008). Interestingly, these mice display ordinary behavior as long as the animals become pregnant and deliver their offspring.

The neurosteroids are steroid hormone metabolites synthesized by neurons and astrocytes and they differentially modulate phasic and
Table 6
Key animal studies investigating the molecular, cellular and behavioral effects of ketamine or allopregnanolone treatment.

| Authors          | Year | Animal model                                                                 | Treatment                                                                 | Species                                                                 | Investigated brain area | Main findings                                                                                                                                 |
|------------------|------|------------------------------------------------------------------------------|----------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| Garcia et al.    | 2008 | Control animals were treated with ketamine or imipramine and afterwards BDNF protein levels were determined in the hippocampus. | Single application of ketamine or imipramine at various doses.             | Wistar rats                                                              | hippocampus             | High dose of ketamine increased BDNF protein levels in the hippocampus suggesting this effect might be necessary to produce a rapid onset of antidepressant action. |
| Autry et al.     | 2011 | Control and transgenic mice as well as mice subjected to the learned helplessness paradigm were treated with ketamine and other NMDA and AMPA receptor antagonists. | Ketamine, MK-801, CPP, NMda and NBQX. C57BL/6 mice, inducible Bdnf knockouts and conditional Ntrk2-knockout | C57BL/6 mice, inducible Bdnf knockouts and conditional Ntrk2-knockout | hippocampus             | Ketamine and other NMDA receptor antagonists produce fast-acting behavioral antidepressant-like effects in mouse models, and these effects depend on the rapid synthesis of brain-derived neurotrophic factor. |
| Koike et al.     | 2011 | Learned helplessness paradigm.                                                | Ketamine treatment in combination with an AMPA receptor antagonist.        | ICR mice and Sprague-Dawley rats                                         | none                    | AMPA receptors mediate both the rapid and sustained antidepressant-like effects of ketamine. A synthetic, neuroactive steroid was effective at decreasing depressive-like behaviors and improving maternal care in preclinical models of postpartum depression |
| Melón et al.     | 2018 | Transgenic mouse lines to mimic postpartum depression.                       | Acute and chronic treatment with a novel, synthetic, neuroactive steroid developed by SAGE Therapeutics (SAGE-516). | Female Wild Type mice, mice with a global knockout of the gene encoding the GABA_4 receptor δ subunit (Gabra4 δ−/− mice) and mice lacking KCC2 specifically in CRH neurons (KCC2/Crh mice). | none                    | A synthetic, neuroactive steroid was effective at decreasing depressive-like behaviors and improving maternal care in preclinical models of postpartum depression |
| Ali et al.       | 2020 | Two-photon calcium imaging to characterize SST+ interneurons, SST+ axons, pyramidal neurons, and pyramidal dendritic spines in awake, head-fixed mice. | Ketamine                                                                   | C57BL/6 J mice and SST-ires-Cre transgenic mice                             | medial prefrontal cortex | Ketamine inhibits the activity of SST+ interneurons in the medial prefrontal cortex. This in turn, leads to greater synaptically evoked calcium transients in the apical dendritic spines of pyramidal neurons. |
| Gerhard et al.   | 2020 | GluN2B-NMDA receptor knockdown on GABAergic interneurons and glutamatergic principle neurons in the medial prefrontal cortex. | Ketamine treatment in combination with viral shRNA and conditional mutation to produce cell-specific knockdown or deletion of the NMDA receptor subunit, GluN2B. | wild-type C57BL/6 mice and various transgenic mouse lines                | medial prefrontal cortex | They demonstrate that GluN2B-NMDA receptors on GABAergic interneurons are the initial cellular targets for the rapid antidepressant actions of ketamine. |

Abbreviations: BDNF: brain-derived-neurotrophic factor; CPP: a selective NMDA receptor antagonist; CRH: corticotropin-releasing hormone; KCC2: a $K^+$/Cl$^-$ co-transporter, which is required for effective GABAergic inhibition; MK-801: a highly potent, selective, and non-competitive NMDA receptor antagonist; NBQX: a highly selective competitive antagonist of AMPA and kainate ionotropic glutamate receptors; Ntrk2: neurotrophic tyrosine kinase, receptor, type 2; shRNA: short hairpin RNA; SST: somatostatin.
tonic GABAergic inhibition in the CNS (Lüscher and Möhler, 2019). Neurosteroid biosynthesis is downregulated in depressed patients and SSRI treatment can normalize the cerebrospinal fluid content of neurosteroids (Uzunova et al., 1998). Similar findings have been reported in animal studies, e.g. long-term social isolation stress reduced the concentration of neuroactive steroids in the brain and GABA_A receptor function was disturbed as well (Serra et al., 2002). In contrast, administration of allopregnanolone to animals subjected to chronic social isolation stress could normalize the symptoms of depressive-like behavior and the stress-induced impairment of adult hippocampal neurogenesis (Evans et al., 2012). Furthermore, a synthetic neuroactive steroid could alleviate the abnormal postpartum behavior in the transgenic mouse model of PPD (Melón et al., 2018).

Similarly to ketamine, the exact antidepressant mechanism of action of allopregnanolone is largely unknown. The rapid effect of allopregnanolone could be explained by the positive allosteric modulation of GABA_A receptors, and it may also act on other receptors such as pregnane xenoobiotic receptors or membrane progesterone receptors (Frye et al., 2014; Guennoun et al., 2015). The long-lasting effect of allopregnanolone is even more difficult to explain. Several mechanisms have been implicated such as the normalization of the disturbed HPA-axis, or the modulation of dentate granule cell activity which in turn is linked to resilience (Lüscher and Möhler, 2019; Meltzer-Brody and Kanes, 2020; Walton and Maguire, 2019). Deeper understanding of the exact molecular and cellular mechanisms of action responsible for the antidepressant effects of these rapid acting antidepressant drugs could help us to design further compounds with similar efficacy and less side effects. Animal models are indispensable tools for such endeavor.

8. Conclusions

Based on our current understanding it appears that all major cell types of the CNS contribute to the pathophysiology of MDD. Both excitatory and inhibitory neurons, as well as the glial cells are involved, but we know very little about the sequence of events. The key questions are: Which cell type is the weakest link? Is there a specific cellular cascade mechanism leading to the pathophysiology, or all the cellular changes are taking place parallel to each other? It is still not clear whether all these cellular alterations are evidences of cellular “damage” or some of them represent compensatory mechanisms. Longitudinal studies focusing on several cell types simultaneously may yield answers to these questions.

Animal models for mental disorders received heavy criticisms and has been occasionally named as scapegoats for the lack of developments in psychiatric therapeutics. Despite all their significant shortcomings these models are extensively used in academic research and drug development. We should accept that none of the models can mimic all aspects of this complex disease, but they can provide opportunities to understand the specific genetic, molecular and cellular mechanisms contributing to the development of MDD.

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