Animal models of major depression and their clinical implications

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**Abstract**

Major depressive disorder is a common, complex, and potentially life-threatening mental disorder that imposes a severe social and economic burden worldwide. Over the years, numerous animal models have been established to elucidate pathophysiology that underlies depression and to test novel antidepressant treatment strategies. Despite these substantial efforts, the animal models available currently are of limited utility for these purposes, probably because none of the models mimics this complex disorder fully. It is presumable that psychiatric illnesses, such as affective disorders, are related to the complexity of the human brain. Here, we summarize the animal models that are used most commonly for depression, and discuss their advantages and limitations. We discuss genetic models, including the recently developed optogenetic tools and the stress models, such as the social stress, chronic mild stress, learned helplessness, and early-life stress paradigms. Moreover, we summarize briefly the olfactory bulbectomy model, as well as models that are based on pharmacological manipulations and disruption of the circadian rhythm. Finally, we highlight common misinterpretations and often-neglected important issues in this field.

**Keywords:** Animal model, Chronic stress, CMS, Depression, Mood disorder

1. Introduction

1.1. Major depressive disorder

Major depressive disorder (MDD), the DSM-5 diagnosis of clinical depression (American Psychiatric Association, 2013), is a complex, multifactorial, heterogeneous, and (often) chronic mental disorder that affects ~120 million people worldwide (Kessler and Bromet, 2013). MDD imposes a severe social and economic burden globally (Ferrari et al., 2013). In 2010, MDD ranked second as the leading cause of global disability, as it accounted for 8.2% of the global years lived with disability. It is also a problem that is expanding, as recent WHO predictions indicate that, by 2030, depression will be the leading cause of disease burden globally (WHO report EB130/9, 2011). Although effective therapies exist, more-effective, faster-acting, and prophylactic remedies are desperately needed. To fulfill this goal, however, a paradigm switch must take place in the field of antidepressant drug research (Insel et al., 2013).

Despite extensive investigations, the exact neurobiological processes that lead to depression are not fully understood. The most widely accepted hypothesis regarding the underlying neuropathology of MDD is the monoamine imbalance hypothesis (Schildkraut, 1965), which emphasizes the role of disturbed monoamine neurotransmission in the synaptic cleft. Almost all of the antidepressants available currently are based on chance discoveries that occurred more than half a century ago. The first such compound reported was iproniazid, a drug that was originally registered for the treatment of tuberculosis. Iproniazid was found to elevate mood in tuberculosis patients, and subsequent studies of depressed patients without tuberculosis demonstrated its antidepressant effect. At about the same time, imipramine, which is an antihista-mine with a tricyclic structure, was found to have antidepressant effects. In subsequent studies, it was shown that imipramine and iproniazid increase the extracellular concentration of serotonin and noradrenaline by blocking their reuptake or by inhibiting monoamine oxidase.
(the main metabolizing enzyme of the two neurotransmitters), respectively. These discoveries revolutionized the treatment of mood disorders and led to the development of more-advanced antidepressants. Among them are the tricyclic antidepressants, which are believed to act by inhibiting the plasma membrane transporters of serotonin and/or noradrenaline. These molecules provided a template for developing modern classes of antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), noradrenaline reuptake inhibitors (NRIs), and dual serotonin/noradrenaline reuptake inhibitors (SNRIs). Since then, the significant limitations of the monoamine theory have been increasingly acknowledged, for example antidepressant drugs modulating monoamine transmission increase synaptic concentrations of monoamines within minutes, while their clinical effects take 2–4 weeks to become apparent. The monoamine theory does not explain the wide spectrum of macroscopic and microscopic structural changes that have been documented in depressed patients, the obvious alterations in numerous other neurotransmitter systems, the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, which is often present in these patients and the recently emphasized changes in gut microbiota (Mayer et al., 2014) and potential inflammatory mechanisms (Rosenblat et al., 2014) contributing to the pathophysiology. Not surprisingly, further theories have been proposed suggesting the involvement of several other neurotransmitter systems, such as glutamate (Sanacora et al., 2012), γ-aminobutyric acid (GABA) (Lüscher et al., 2011), and various neuropeptides, including the corticotropin-releasing hormone (CRH) (Lloyd and Nemeroff, 2011). Furthermore, new ideas have emphasized the role of dysfunctional glucocorticoid homeostasis and disturbed HPA-axis regulation (Holsboer, 2000; de Kloet et al., 2005; Frodl and O’Keane, 2013), or the possibility that inflammatory reactions underlie the disease (Rosenblat et al., 2014). Further theories stressed the significance of changes related to neuroplasticity and proposed a crucial role for adult hippocampal neurogenesis (Castren, 2005; Lucassen et al., 2010; Eisch and Petrik, 2012; Miller and Hen, 2014) and neurotrophic factors, such as the brain-derived neurotrophic factor (BDNF), in the pathophysiology of the disease (Numakawa et al., 2014). More recently, the contribution of epigenetic mechanisms, such as histone modifications and DNA methylation, that affect diverse neuronal pathways have been pointed out as important MDD contributing factors (Sun et al., 2013). Fig. 1 depicts a

Fig. 1. The pathophysiology of major depressive disorder. (A) Examples of three different cases with the onset and course of the illness, with the black bars representing the depressive episodes. Note that Case 1, which had an early onset in adolescence and had repeated depressive episodes, ending finally with a suicide is likely to have a very different pathophysiology compared to Case 3, which had a late onset and co-morbidity with a neurodegenerative disorder. (B) Factors known to influence the pathophysiology of the disorder and leading to a diseased brain (C), with significant alterations at numerous levels.
summary of the most well-known factors that have been proposed as contributing to the pathophysiology of the disorder and leading to a diseased brain. A further complicating issue is that distinct disorders with a very different pathophysiology may manifest the same clinical symptoms and, on that basis, be categorized as “major depressive disorder” (see, e.g., the different cases depicted in Fig. 1A, and Chapter 4.1).

Although depression tends to run in families, the human genome-wide association studies (GWAS) performed to date have failed to find reproducible genetic loci that contribute significantly to the disease (Bosker et al., 2011). Currently, MDD is thought to result from interactions between genetic predispositions and environmental factors, such as stress (Caspì et al., 2003; Risch et al., 2009). According to the current notion, while early life stress experiences, traumatic or negative life events are potent risk factors, they trigger MDD only in genetically susceptible individuals. We are just starting to uncover the genetic variants that moderate the effects of adversities and the molecular mechanisms mediating these gene-environment interactions (for reviews see e.g. Caspi and Moffitt, 2006; KlengeI and Binder, 2013; Mandelli and Serretti, 2013). Not surprisingly, most experimental models employ either genetic manipulations or some type of environmental stress, or the combination of both, to produce animals that exhibit phenotypes that are similar to the symptoms of depressed patients (e.g., reviewed by Nestler and Hyman, 2010; Berton et al., 2012). For commercial reasons, drug companies need valid models for the development of new therapies. However, in recent years, the pharmaceutical industry has become severely disappointed in their research of drugs to treat neuropsychiatric disorders, because of the lack of success in developing truly new compounds. They have condemned mental disorders as a challenge that is “too difficult” to attract major investment (Hyman, 2014). Clearly, the ideas that we can constantly be developed and the existing models are being re

Depression is a symptomatic heterogeneous disease, and its clinical-symptom profiles vary among patients. Some alterations in symptoms may even go in opposite directions, like psychomotor activity, sleep, and appetite changes. Consequently, individual models would be expected to simulate endophenotypes or a subset of symptoms, which are likely defined by the inducing conditions applied. For several reasons, it is essential to use realistic inducing conditions, etiological validity, and, thus, also to ensure the legitimacy of the underlying pathology. Furthermore, it should be noted that major depression is usually an episodic disease; consequently, genetic manipulations or inbreeding techniques that cause congenital behavioral abnormalities suffer from a lack of etiological validity. However, genetic models without congenital abnormalities, but with increased vulnerability to straining environmental factors, may be valid.

Unfortunately, none of the animal models available currently fulfills these three criteria completely. There are models that replicate some symptoms of MDD, and a few simple tests (such as the forced swimming test) have been useful for testing the efficacy of a specific group of antidepressant compounds (namely, SSRIs). Table 1 summarizes the major symptoms and potential biomarkers of MDD, the corresponding behavioral and physiological phenotypes in experimental animals, and the tests that can be used to assess them. Importantly, these simple tests or screening methods are not equal to the complex experimental paradigms that we regard as valid animal models of depression (for discussion, see Frazer and Morilak, 2005; Slattery and Cryan, 2014).

2. Animal models for understanding the pathophysiology of major depressive disorder

2.1. Genetic and optogenetic models

2.1.1. Traditional genetic mouse models of depression

Genetically engineered mice enable the investigation of the functional consequences of silencing or overexpressing candidate genes that are thought to contribute to the pathophysiology of the disease. A continuously increasing number of mutant mouse lines have been created, based on the improving knowledge of genes implicated in the pathogenesis of depression and its treatment. Researchers can choose between two main approaches: the use of either forward or reverse genetics. Forward genetics is an unbiased approach in which a large number of random mutations are generated in an organism (e.g., mice) using simple mutagenic techniques, followed by breeding and screening for individuals with the desired aberrant phenotype. After the generation of a mutant mouse line with the desired phenotype—in this case, depressive-like behavior—the responsible gene can be identified. The reverse genetic approach is used more commonly in scientific practice and involves genetic manipulations that result in either loss- or gain-of-function mutants. “Knockout mice” are the most well-known examples, in which a specific target gene is disrupted, resulting in a loss-of-function mutant. However, loss of function can be achieved using other tools, such as insertion of transgenes that produce an antisense mRNA of the target gene or of short hairpin RNAs directed against the gene of interest. Conversely, gain-of-function mutant mice carry additional copies of a specific gene in their genome or have been generated by knockin techniques. The generation of the early conventional knockout mice was an important milestone in neuroscience research aiming to uncover the underlying neurobiology of the disease. Since then, a rapid development of technologies has taken place in this field, such as the introduction of sophisticated conditional strategies (Branda and Dymecki, 2004). This new method enables an increasingly refined control of spatial and temporal gene expression. For example, mouse lines expressing Cre recombinase, which is a tyrosine recombinase enzyme derived from the P1 bacteriophage, selectively in neurons of a specific neurotransmitter type allow the genetic targeting of specific populations of neurons and neuronal pathways. However, targeted genes can potentially affect development; to circumvent this obstacle, mouse lines expressing the tamoxifen-inducible Cre recombinase variant
Table 1
Symptoms and potential biomarkers of MDD with the corresponding tests to measure behavioral and physiological changes in experimental animals.

<table>
<thead>
<tr>
<th>Depressive symptoms and potential biomarkers in patients</th>
<th>Related physiological or behavioral phenotype in experimental animals</th>
<th>Tests to measure behavior or method to assess physiological changes</th>
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</thead>
<tbody>
<tr>
<td>DSM-5 criteria</td>
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<tr>
<td>Markedly diminished interest or pleasure in activities</td>
<td>Anhedonic-like behavior</td>
<td>Sucrose preference</td>
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<tr>
<td>Markedly diminished interest or pleasure in activities</td>
<td>Anhedonic-like behavior</td>
<td>Intracranial self-stimulation</td>
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<td>Markedly diminished interest or pleasure in activities</td>
<td>Anhedonic-like behavior</td>
<td>Conditioned place-preference</td>
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<tr>
<td>Markedly diminished interest or pleasure in activities</td>
<td>Anhedonic-like behavior</td>
<td>Female urine snifing</td>
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<tr>
<td>Diminished ability to think or concentrate, or indecisiveness</td>
<td>Cognitive deficits</td>
<td>Body weight measurements</td>
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<tr>
<td>Recurrent thoughts of death, recurrent suicidal ideation</td>
<td>–</td>
<td>Measuring diurnal activity, sleep EEG</td>
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<tr>
<td>Recurrent thoughts of death, recurrent suicidal ideation</td>
<td>–</td>
<td>Open field test</td>
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<tr>
<td>Recurrent thoughts of death, recurrent suicidal ideation</td>
<td>–</td>
<td>Home cage activity</td>
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<td>Recurrent thoughts of death, recurrent suicidal ideation</td>
<td>–</td>
<td>Forced swim test</td>
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<td>Recurrent thoughts of death, recurrent suicidal ideation</td>
<td>–</td>
<td>Tail suspension test</td>
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<tr>
<td>Recurrent thoughts of death, recurrent suicidal ideation</td>
<td>–</td>
<td>Learned helplessness</td>
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<tr>
<td>Recurrent thoughts of death, recurrent suicidal ideation</td>
<td>–</td>
<td>Reduced home cage activity</td>
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<tr>
<td>Recurrent thoughts of death, recurrent suicidal ideation</td>
<td>–</td>
<td>Treadmill running</td>
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<tr>
<td>Further typical symptoms</td>
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<tr>
<td>Anxiety, agitation or restlessness — for example, excessive worrying, pacing, hand-wringing or an inability to sit still</td>
<td>Anxiety-related behavior</td>
<td>Open field test</td>
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<tr>
<td>Anxiety, agitation or restlessness — for example, excessive worrying, pacing, hand-wringing or an inability to sit still</td>
<td>Anxiety-related behavior</td>
<td>Elevated plus maze</td>
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<tr>
<td>Anxiety, agitation or restlessness — for example, excessive worrying, pacing, hand-wringing or an inability to sit still</td>
<td>Anxiety-related behavior</td>
<td>Dark-light box</td>
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<tr>
<td>Anxiety, agitation or restlessness — for example, excessive worrying, pacing, hand-wringing or an inability to sit still</td>
<td>Anxiety-related behavior</td>
<td>Novelty-induced hypophagia</td>
</tr>
<tr>
<td>Anxiety, agitation or restlessness — for example, excessive worrying, pacing, hand-wringing or an inability to sit still</td>
<td>Anxiety-related behavior</td>
<td>Marble burying</td>
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<tr>
<td>Anxiety, agitation or restlessness — for example, excessive worrying, pacing, hand-wringing or an inability to sit still</td>
<td>Anxiety-related behavior</td>
<td>Novel object exploration</td>
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<tr>
<td>Anxiety, agitation or restlessness — for example, excessive worrying, pacing, hand-wringing or an inability to sit still</td>
<td>Anxiety-related behavior</td>
<td>Modified hole board</td>
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<tr>
<td>Dysfunctional social behavior, frequent negative social interactions</td>
<td>Impulsivity</td>
<td>Time spent on social interaction/avoidance</td>
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<tr>
<td>Dysfunctional social behavior, frequent negative social interactions</td>
<td>Impulsivity</td>
<td>Continuous performance task, stop-signal task, go/no-go and delay-discounting paradigms</td>
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<tr>
<td>Unexplained physical problems, such as back pain or headaches</td>
<td>Increased pain sensitivity</td>
<td>Pain threshold measurements</td>
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<td>Potential “biomarkers”</td>
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<tr>
<td>Disturbed HPAaxis regulation</td>
<td>Disturbed HPA-axis regulation</td>
<td>Serum corticosterone levels</td>
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<td>Disturbed HPAaxis regulation</td>
<td>Disturbed HPA-axis regulation</td>
<td>Dexamethasone suppression test</td>
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<td>Disturbed HPAaxis regulation</td>
<td>Disturbed HPA-axis regulation</td>
<td>in vivo MRI</td>
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<td>Disturbed HPAaxis regulation</td>
<td>Disturbed HPA-axis regulation</td>
<td>post-mortem histology</td>
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<tr>
<td>Disturbed HPAaxis regulation</td>
<td>Disturbed HPA-axis regulation</td>
<td>Pro-inflammatory cytokines (IL-1, IL-6, TNF-alpha, IFN-gamma)</td>
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<tr>
<td>Hippocampal volume decrease</td>
<td>Hippocampal volume decrease</td>
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<td>Hippocampal volume decrease</td>
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<td>Inflammatory biomarkers</td>
<td>Serum markers for inflammation</td>
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<td>Inflammatory biomarkers</td>
<td>Serum markers for inflammation</td>
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</table>

*Note that assessing “Feelings of worthlessness or excessive or inappropriate guilt” is impossible in animals. From an anthropomorphic point of view one may suggest that when an animal “feels worthless” to groom itself properly then, subsequently its fur status will gradually deteriorate. Grooming and fur condition is often measured in these models, but without any clear idea what it represents.

CreERT2 have been developed, which allows temporal control of the expression of a gene of interest (Branda and Dymecki, 2004). Other conditional strategies, such as the RNA interference (RNAi) technology or virus-mediated genetic manipulations, are also gaining widespread use, as they are easier to use and permit fairly good control of spatial and temporal gene expression.

Earlier genetic models focused on the monoamine theory of depression and led to the generation of knockout mouse models such as the 5-HT1A receptor knockout mouse model (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998) or the noradrenaline transporter knockout mouse model (Xu et al., 2000). Based on the theories that suggest that HPA axis dysfunction contributes to the pathophysiology of the disease, transgenic animals have been generated in which the corticotropin-releasing hormone receptor-1 (CRH-R1 knockout, Timpl et al., 1998; Müller et al., 2003) or the type II glucocorticoid receptor (Pepin et al., 1992; Montkowski et al., 1995) was targeted, to mimic glucocorticoid homeostasis dysfunctions (Hesen et al., 1996; for review, see, e.g., Massart et al., 2012; Barkus, 2013). Other authors have tested heterozygous BDNF knockout mice, based on theories of a role for neurotrophic factors, such as BDNF, in the disease; surprisingly, these mice exhibited unaltered performance in the elevated plus maze and forced swim tests, as well as normal sucrose consumption (for review, see, e.g., Barkus, 2013).

These “traditional” genetic models, conventional the knock-out and knock-in mice, have several limitations. Among others, they mostly focus on only one protein (e.g. receptor or membrane transporter), and the genetic manipulations affect mostly the entire body and not only the pathophysiologically relevant brain areas. As discussed above, these models have often surprisingly limited or no face and predictive validity and have been used mainly because of their presumed etiological and construct validity. A further complicating issue is that most of the genetic models are based on mice and the animals are behaviorally characterized in tests that have been developed for rats (see 4.5.)

More recently, epigenetic events have been highlighted, and it has been suggested that transcriptional dysregulation underlies the behavioral manifestations of many psychiatric disorders, including major depression (Krishnan and Nestler, 2008; Sun et al., 2013). The discovery that monoamine oxidase inhibitors, which are a typical class of antidepressants, potently inhibit the nucleosomal demethylation of histone H3 lysine 4 provided strong support to this hypothesis (Lee et al., 2006). Whether these chromatin mechanisms account for the therapeutic efficacy of these drugs remains completely uncertain. Studies using animal models of depression, as well as post-mortem studies of human brains of depressed patients, have demonstrated altered patterns of histone acetylation and DNA methylation, as well as several additional...
chromatin modifications, in several limbic brain regions, which suggests the involvement of epigenetic events in this disease (reviewed by Sun et al., 2013).

2.1.2. Animal models involving optogenetic tools

Currently, optogenetic tools are the shining stars among the rapidly developing methods in the field of neuroscience. Technical progress in molecular biology has led to the development of a wide array of methods that enable the blockade or stimulation of neuronal activity with high anatomical, genetic, and temporal precision. For a brief overview of these techniques, see Fig. 2 (or www.openoptogenetics.org) or recent reviews (e.g., Kramer et al., 2013; Gautier et al., 2014; Packer et al., 2013). This approach has revolutionized the field of behavioral neuroscience and the means of studying disease-related brain circuits in animal models. Naturally, optogenetic tools have been applied to investigate the neurobiology of different psychiatric disorders (for review, see, e.g., Tye and Deisseroth, 2012; Deisseroth, 2014; Lammel et al., 2014; Steinberg et al., 2015). Briefly, this approach has been used to study the contribution of: dopaminergic neurons to the regulation of depressive-like behaviors (Chaudhury et al., 2013; Tye et al., 2013), prefrontal cortical circuits to the elicitation of antidepressant-like effects (Covington et al., 2010; Vialou et al., 2014), and neuronal circuits that control anxiety (Tye et al., 2011; Kim et al., 2013; Vialou et al., 2014). In these elegant studies, various behaviors were successfully manipulated, such as hedonic and anhedonic-like behaviors (Tsai et al., 2009), reward-seeking behaviors (Stuber et al., 2011), active- or passive-coping behavioral patterns (Warden et al., 2012; Tye et al., 2013), and the sleep–wake balance (Adamantidis et al., 2007). Notably, these behavioral patterns are altered in depressed patients (see Table 1).

There are high expectations in these optogenetic models, which are much more advanced compared to the “traditional” gene-knock-out or knock-in mice. But this type of research is still in an early phase and...
thus, it is difficult to assess its usefulness. So far these models seem to have good face and construct validities. Future studies should prove their predictive validity. It is difficult to assess the etiological validity of these models since they are often very “artificial” in a sense that they combine numerous rather invasive procedures to produce the desired effects. Notably, these models are technically challenging and expensive, but the use of the optogenetic toolkit is literally exploding, and this approach is expected to lead to the development of truly novel treatment strategies for mood disorders.

2.1.3. Animal models based on selective breeding

Rodent models exist in which animals are selected based on their specific features and are then bred over several generations, which yields inbred strains of individuals that show specific physiological or behavioral abnormalities. Some of these lines that are currently used as animal models of depression were originally generated for other purposes; in other lines, a specific depressive-like behavior was used as a selector for breeding. In inbred strains, the individuals are identical to each other genotypically, because of long inbreeding, which in this case is considered to be an advantage for further research. Such inbred lines can be used to investigate the underlying genetic background or molecular mechanisms that are responsible for the specific behavioral abnormalities observed.

One example is the Flinders Sensitive rat Line (FSL), which was originally selectively bred for increased response to an anticholinesterase agent (diisopropyl fluorophosphate, DFP; for a recent review, see, e.g., Overstreet and Wegener (2013)). Experimental work using this line over the years has revealed that FSL rats exhibit certain behavioral, neurochemical, and pharmacological features that have been reported in depressed individuals; moreover, it has been used to measure the efficacy of antidepressants (for comprehensive reviews see, e.g., Overstreet et al., 2005; Overstreet, 2012; Overstreet and Wegener, 2013). FSL rats have reduced appetite and psychomotor function but exhibit normal hedonic responses and cognitive function. Animals of this line have neurochemical changes in their serotonergic, cholinergic, dopaminergic, and NPY systems but have normal HPA axis and GABAergic regulation. FSL rats also exhibit an altered circadian rhythm, together with sleep and immune abnormalities that are similar to those observed in depressed individuals.

Another example is the Wistar–Kyoto (WKY) rat strain. Originally, this line was developed as the normotensive control strain for the spontaneously hypertensive rat (Okamoto and Aoki, 1963). Later, however, it turned out that WKY rats exhibited several hormonal, behavioral, and physiological abnormalities that were similar to those found in depressed patients. For example, WKY rats are hyperreactive to stress, show dysregulation of the HPA axis, and exhibit a depressive-like behavior in a wide range of behavioral tests (for summary see, e.g., Overstreet, 2012). Furthermore, two substrains of the WKY line have been developed based on behavioral performance in the forced swim test as a functional selector for breeding (Will et al., 2003).

Selective inbreeding of rats using the learned helplessness behavior as a selector (see 2.2.3.) yielded a strain with congenital learned helplessness behavior that displayed treatment-resistant helplessness even without previous exposure to uncontrollable shock (Henn and Vollmayr, 2005; Sartorius et al., 2007).

As anxiety is often present in depressed patients, it is worth noting here that Wistar rats have been selectively bred for either high (HAB) or low (LAB) anxiety-related behavior in the elevated plus-maze (Liebsch et al., 1998) and that later, a similar approach was used to generate inbred HAB/LAB mice (Krömer et al., 2005).

There are also selectively bred rat lines on the basis of their infantile trait related to anxiety. Infant rodents (pups) vocalize initially when separated from their mothers (dams) and littermates; this vocalization peaks at 45 kHz and are therefore called ultrasonic vocalizations or USV. Brunelli and co-workers selectively bred two lines of rats based on high and low rates of USV (Brunelli and Hofer, 2007). High-USV rats demonstrate “anxious”/“depressed” phenotypes in standard laboratory, whereas low-USV rats show an “aggressive” phenotype in adulthood.

Further selectively bred lines are the “high- and low-yawning rats” (HY and LY) which were selectively bred based on their spontaneous yawning frequency. Interestingly, HY rat dams spent less time in the nest, retrieved their pups faster, and show a longer latency to licking and mouthing the pups than the LY or outbred Sprague–Dawley animals (Ugarte et al., 2011). HY dams typically build lower quality nests, and produced offspring with lower body weight and often lost more pups during lactation than the LY dams (Ugarte et al., 2011). Thus, these lines show significant differences in maternal care, imposing different amount of stress on the pups (see also chapter 2.2.4).

In summary, the selective breeding lines provide an interesting approach. It is argued that, they have good face validity and their predictive validity has also been proven in some cases (see e.g. Overstreet et al., 2005; Overstreet and Wegener, 2013). However, their etiological and construct validities are questionable.

2.2. Models based on stress exposure

The stress hypothesis of mood disorders (e.g., Gold, 2015) has stimulated the development of several animal models that have been used as surrogates of depression (for recent reviews see, e.g., Hollis and Kabbaj, 2014; Slattery and Cryan, 2014). These models are based on very different stress types, which might model distinct subsets of patients/disorders. Overall, the different stress models seem to have good etiological, face and construct validities. However, as outlined below their predictive validity in some cases needs to be proven by further research.

2.2.1. Social stress

From a biological point of view and in line with the ideas of Barnett and Henry, it seems that the social environment is a considerable source of stress and that the two processes of fighting for control and losing control are of central importance to the psychosocial situation of individuals (Barnett, 1958, 1964; Henry and Stephens, 1977). In humans, loss of rank, social status, and/or control are examples of the more general class of loss events, which are increasingly recognized as the specific type of “life events” that are associated with a greater risk of depression (Brown, 1993). Based on these ideas, new animal models that were established using social perturbations as stressors have been validated. These models have heuristic value because they investigate the environmental challenges that an animal may meet in its everyday life. In social settings, this might mean loss of control by social defeat.

The resident–intruder paradigm, which is specific to rodents, is the most popular model of social defeat/stress and uses social conflict between members of the same species to generate emotional psychological stress. Classically, in this experimental setting, a male, the intruder, is transferred into the home cage of another male, the resident. If the animals are allowed to fight on a single occasion it is regarded as an acute stress exposure; if the intruder is exposed to the resident at several occasions, ranging from days to even weeks, it is regarded as a model of chronic stress. In some models, the intruder is transferred to the cage of a singly housed resident, whereas in other cases, the intruder replaces a cohabitating female in the resident’s cage. In all settings, the intruder is quickly attacked and subjugated. After the physical exposure, the intruder is often placed in a small protective cage before being returned to its home cage. In the protective cage, the animal is exposed to stressful psychogenic signals that are emitted by the resident, without experiencing physical harm. For a more comprehensive review of the different social defeat protocols in mice and rats, we refer the reader to the recent review by Hollis and Kabbaj (2014).

In various laboratory settings, this stress model works well when male individuals are being investigated. Until recently, it was thought that this approach does not work in female rodents because they do not fight with each other in a resident–intruder paradigm (Palanza,
stressor’s effects on social status, including the differentiation between stability of the dyad, which allows a clearer investigation of the main difference of the chronic psychosocial stress paradigm is the motivation for a few minutes. Compared with the sensory contact model, the model which allows continuous sensory contact but no physical interaction.

As pointed out by Koolhaas et al. (1997a), social defeat is a special kind of stressor and distinguishes itself from other stress paradigms with respect to the magnitude and the quality of the stress response. Moreover, it should be emphasized that social defeat induces changes in a variety of biophysiological parameters, each of which may have different temporal dynamics (Koolhaas et al., 1997b). In this context, it should be mentioned that the diurnal time point of exposure to a stressor is also critical. Mice subjected to chronic social defeat stress during the active phase developed more pathophysiological signs compared with those subjected to stress during the inactive phase (Bartlang et al., 2012).

2.2.1.1. Stress models in mice. Experiments with transgenic mice enable a unique approach. Namely, the combination of genetic predisposition with environmental stress which provides additional insights into disease mechanisms (e.g. Berton et al., 2006; Wang et al., 2011; Wagner et al., 2011). For this reason, stress models in mice have particular importance (Golden et al., 2011). Adult male mice aggressively attack unfamiliar male conspecifics in their own territory. Accordingly, the classic resident-intruder test has been designed according to basic investigations of mouse aggressive behavior that have been conducted on wild populations and laboratory strains (Benus et al., 1991; Parmigiani et al., 1998; Miczek et al., 2001). The resident-intruder or social defeat model is a useful tool for investigating the physiological and behavioral consequences of a single or repetitive social defeat (for details, please see Bartolomucci et al., 2009). Based on this model, Kudryavtseva and coworkers developed the “sensory contact model”. Because its behavioral and physiological effects are stable and reproducible, this protocol is among the more widely adopted procedures and has been validated by several laboratories. A detailed description of this procedurally complex protocol is given in a recent review (Kudryavtseva et al., 2014).

The model of chronic psychosocial stress for male mice proposed by Bartolomucci et al. (2004) was adapted from the “sensory contact model”, as was the psychosocial stress model in tree shrews (see below). Resident animals are individually housed for 1 week, to allow the establishment of an individual territory. Each resident receives an intruder mouse (coming from group housing), and the two animals are allowed to interact freely for 10 min. After the interaction, the two animals are separated by a perforated polystyrene-metal partition, which allows continuous sensory contact but no physical interaction. The partition is removed daily, e.g., for 21 days, allowing direct interaction for a few minutes. Compared with the sensory contact model, the main difference of the chronic psychosocial stress paradigm is the stability of the dyad, which allows a clearer investigation of the stressor’s effects on social status, including the differentiation between susceptible and unsusceptible individuals (Krishnan et al., 2007). Finally, it should be noted that repeated social defeat stress models have been successfully combined with optogenetic tools (e.g., Covington et al., 2010; Chaudhury et al., 2013; Vialou et al., 2014).

2.2.1.2. Social defeat stress models in rats. By analogy with the mice models, different social-stress paradigms are used in rats involving two or more animals in dyadic, group, or colony housing, respectively (for review, see, e.g., Blanchard et al., 2001; Miczek et al., 2008; Hollis and Kabbaj, 2014; Slattery and Cryan, 2014). These paradigms are believed to be more similar to human conditions than are nonsocial stress paradigms such as repeated restrain stress (Slattery and Cryan, 2014). In experimental animals subjected to social-stress-based paradigms, numerous central nervous, physiological, and behavioral effects can be observed that resemble those of human individuals who have been exposed to acute or chronic stressors.

Recently, we validated a new model of chronic social stress in rats based on the resident–intruder paradigm originally described by Miczek (1991) and Koolhaas et al. (1997a, 1997b). Anhedonic-like behavior, which is a core symptom of depressed patients, was induced in rats submitted to 5 weeks of social defeat (Rygula et al., 2005). Importantly, the stress-induced anhedonic-like behavior could be reversed in a time-dependent manner via the chronic administration of citalopram, thus revealing the predictive validity of the model (Rygula et al., 2006).

2.2.1.3. Psychosocial stress in tree shrews. Rodents are frequently used to model human mental illnesses. In recent years, however, evidence has accumulated that chronic psychosocial stress in a nonrodent species, the male tree shrew (Tupaia belangeri), represents a natural and valid paradigm for studying the behavioral, endocrine, and neurobiological changes that may underlie stress-related disorders, such as depression. Phylogenetically, tree shrews are placed together with primates and dromoptera within the clade Euarchonta (Kriegs et al., 2007). A recent genome analysis further supported the close affinity between tree shrews and primates (Fan et al., 2013). Tree shrews are diurnal day-active animals that are widely distributed in South-East Asia. In their natural habitat, males defend their territories vigorously against intruding conspecifs (Kawamichi and Kawamichi, 1979). Originally developed by Raab (1971) and later adopted by von Holst (1977), we used this pronounced territoriality to establish a naturally occurring challenging situation under experimental control in the laboratory. When living in visual and olfactory contact with a male conspecific that has defeated it, the subordinate tree shrew shows dramatic changes in behavior, physiology, endocrine function, and neuronal activity. Subordinates lose body weight and have reduced locomotor activity; their sleeping patterns are characterized by an increasing number of early-morning waking episodes, and their circadian rhythm is profoundly disturbed. Analysis of endocrine function in subordinates reveals consistently increased levels of the adrenocortical hormone cortisol, increased adrenal weight, increased concentration of noradrenaline (which indicates enhanced sympathetic activity), and reduced gonadal function (for review, see Fuchs and Flügge, 2002, and Table 2). As the distinct stress-induced behavioral, physiological, and central nervous system alterations observed in subordinate animals result exclusively from the cognitive interpretation of the continuous visual presence of the dominant conspecific (Raab and Storz, 1976; Raab and Oswald, 1980), this paradigm has been termed “psychosocial stress”.

As pointed out previously, some of the key symptoms of human affective disorders, such as depressed mood, loss of mental energy, or recurrent thoughts of death, cannot be modeled directly in animals. Importantly, the biobehavioral responses observed in subordinate tree shrews are similar to the signs and symptoms observed in depressed patients. Thus, the chronic psychosocial stress model in tree shrews has strong face validity for human depression (for review, see Fuchs, 2005).

To investigate whether the tree shrew model also possesses predictive validity, we treated subordinate shrews with conventional antidepressants, such as clomipramine, fluoxetine, tianeptine, and agomelatine, or with investigational drugs, such as neurokinin 1 receptor antagonists. It is important to note that (i) we determined serum concentrations and used the appropriate dose of the antidepressants that was necessary to reach therapeutically relevant levels, (ii) the daily oral treatment commenced only when the stress-induced behavioral and endocrine changes were reversing, (iii) the psychosocial stress
paradigm was continued during the treatment, (iv) the therapeutic action of the drug was assessed for the clinically appropriate period of time of 4 weeks, and (v) the action of the antidepressant had a time-dependent onset, requiring in some cases several weeks of chronic treatment to reach efficacy. Using this approach, we found a time-dependent restoration of endocrine, behavioral, and central nervous parameters in subordinate animals (and Fuchs et al., 1996; Magarinos et al., 1996; Czeh et al., 2001, 2005; van der Hart et al., 2002, 2005; Schmelting et al., 2014, and Fig. 3). In contrast, the anxiolytic drug diazepam was ineffective in this experimental setting (van Kampen et al., 2000). The results of our experiments with clomipramine were recently confirmed by Wang and coworkers, who showed that the core symptoms of depression could be reversed by chronic treatment of subordinate tree shrews with this conventional tricyclic antidepressant (Wang et al., 2013).

Based on these findings, the chronic psychosocial stress paradigm in tree shrews can be regarded as a “homologous model” of depression. It mimics several aspects of the human disease in the animal, the state of the animal is induced by stimuli that are similar to those that cause the condition in humans, and pharmacotherapy that is efficacious in human illness is effective in the model. The advantage of a homologous model is that it can probably contribute to the understanding of the brain biochemistry of depression and might lead to the development of effective drugs for the treatment of the illness.

The limitations of the current antidepressant medications, such as the delay in achieving a full therapeutic response, the substantial number of nonresponders, and unpleasant side effect profiles, merit the full exploration of all plausible novel agents with antidepressant action. Currently, antidepressant drugs are typically tested in rats (mostly from Wistar or Sprague–Dawley strains) assuming that their drug metabolism and receptor/transporter affinities are similar to humans. However this is often not the case. Pharmacokinetics should be evaluated more carefully, before testing antidepressant compounds and using other species (not only rats and mice) can be an advantage.

Molecular studies have revealed important structural differences between the rat/mouse and human receptors, which hampers the application of the results obtained in experimental animals to humans. Therefore, it is important to note that in tree shrews the DNA sequences of genes that encode (i) major stress-regulating receptor proteins, such as the glucocorticoid and the mineralocorticoid receptor (Meyer et al., 2000) and using other species (not only rats and mice) can be an advantage.

It is clear that the CMS paradigm was developed to mimic the human disease and that the CMS paradigm is a valid and realistic model of depression focusing on a core symptom of MDD; namely, anhedonia (in humans) or anhedonic-like behavior (in animals) (e.g., Willner, 2005). The CMS paradigm involves the exposure of animals to a series of mild stressors in an unpredictable manner (isolation or crowded housing, food or water deprivation, disruption of the dark–light cycle, tilting of home cages, dampened bedding, etc.) over a period of several weeks or even months (at least 2 weeks). The advantage of the CMS model is that the stress paradigm induces long-lasting changes in behavioral, neurochemical, neuroimmune, and neuroendocrinological parameters that resemble the dysfunctions observed in humans (Kramer et al., 1999; Kohlhause et al., 2011). Therefore, it is important to note that in tree shrews the DNA sequences of genes that encode (i) major stress-regulating receptor proteins, such as the glucocorticoid and the mineralocorticoid receptor (Meyer et al., 2000), (ii) the corticotropin-releasing factor receptors CRH1 and CRH2 (Palchaudhuri et al., 1998, 1999), and (iii) the alpha2A-adrenoceptor (Meyer et al., 2000) all have high homology to the equivalent human genes. These genes have a 90%–98% homology with the human sequences compared with an average of 80% for the corresponding sequences in rats. Additional information regarding the face and predictive validity, the tree shrew model obviously has a “molecular validity,” as demonstrated by its close similarity to humans regarding both the primary targets and the degradation routes of psychotropic compounds. This may indicate an advantage of this model over the widely used rodent models.

2.2.2. Chronic mild stress

The chronic mild stress (CMS) model is one of the most extensively validated and realistic models of depression focusing on a core symptom of MDD; namely, anhedonia (in humans) or anhedonic-like behavior (in animals) (e.g., Willner, 2005). The CMS paradigm involves the exposure of animals to a series of mild stressors in an unpredictable manner (isolation or crowded housing, food or water deprivation, disruption of the dark–light cycle, tilting of home cages, dampened bedding, etc.) over a period of several weeks or even months (at least 2 weeks). The advantage of the CMS model is that the stress paradigm induces long-lasting changes in behavioral, neurochemical, neuroimmune, and neuroendocrinological parameters that resemble the dysfunctions observed in humans (Kramer et al., 1999; Kohlhause et al., 2011).

Table 2

<table>
<thead>
<tr>
<th>Stress-induced changes in male tree shrews.</th>
<th>Effects of chronic psychosocial stress</th>
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<tbody>
<tr>
<td><strong>Physiological and neuroendocrine parameters</strong></td>
<td></td>
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<tr>
<td>Body weight</td>
<td>Decreased (Fuchs et al., 1993)</td>
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<tr>
<td>HPA axis</td>
<td>Non-adapting increase of urinary cortisol - no suppression by dexamethasone (Kramer et al., 1999; Kohlhause et al., 2011), structural changes in the pituitary (Heinzeller and Raab, 1982) and enlarged adrenal glands (von Holst, 1977)</td>
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<tr>
<td><strong>Sympathetic nervous system</strong></td>
<td></td>
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<tr>
<td>Gonadal system</td>
<td>Decreased testosterone (Kohlhaas et al., 2011) and testes weight (Fischer et al., 1985)</td>
</tr>
<tr>
<td>Sleep</td>
<td>Reduced slow wave sleep, more/longer awake phases (Fuchs and Flügge, 2002)</td>
</tr>
<tr>
<td>Circadian rhythm</td>
<td>Elevated core body temperature (Kohlhaas et al., 2011; Schmelting et al., 2014), heart rate (Stöhr, 1986) and oxygen consumption (Fuchs and Kleinrhein, 1986) during resting period</td>
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<tr>
<td><strong>Behavior</strong></td>
<td></td>
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<tr>
<td>General motor activity</td>
<td>Reduced (Kramer et al., 1999; Schmelting et al., 2014)</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>Reduced (Kramer et al., 1999)</td>
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<tr>
<td>Scent marking activity</td>
<td>Reduced (Kramer et al., 1999)</td>
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<tr>
<td>Food and water intake</td>
<td>Reduced (Kramer et al., 1999)</td>
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<tr>
<td><strong>Structural and functional changes in the brain</strong></td>
<td></td>
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<tr>
<td>Neurogenesis in the dentate gyrus</td>
<td>Inhibition of the proliferation of granule precursor cells (Gould et al., 1997; Czeh et al., 2001)</td>
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<tr>
<td>Retraction of dendrites</td>
<td>Retraction of apical dendrites of pyramidal neurons in the CA3 of the hippocampus (Magarinos et al., 1996)</td>
</tr>
<tr>
<td>Volume of the hippocampal formation</td>
<td>Volume reduced by approximately 10% (Ohl et al., 2000; Czeh et al., 2001)</td>
</tr>
<tr>
<td>Brain metabolites</td>
<td>Significantly decreased in vivo concentrations of N-acetyl-aspartate, creatine/phosphocreatine, and choline-containing compounds (Czeh et al., 2001)</td>
</tr>
<tr>
<td>Hippocampal gluco- and mineralocorticoid receptors</td>
<td>Downregulation of glucocorticoid receptors; regional up- and downregulation of mineralocorticoid receptors (Meyer et al., 2001)</td>
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<tr>
<td>CRH receptors</td>
<td>Downregulation of binding sites for 125I-ovine corticotropin releasing hormone (CRH) in anterior pituitary, dentate gyrus, CA1 and CA3 of the hippocampus, area 17, superior colliculus; upregulation of binding sites for 125I-ovine CRH in cortical regions, amygdala, chroid plexus (Fuchs and Flügge, 1995)</td>
</tr>
<tr>
<td>5-HT14 receptors</td>
<td>Gradual downregulation of heteroreceptors in hippocampus and cortical regions; fast renorinalization after stress or hormonal replacement (Flügge, 1995; Flügge et al., 1998)</td>
</tr>
<tr>
<td>Alpha2-adrenoceptors</td>
<td>Downregulation in brain regions involved in autonomic functions (Flügge, 1996; Flügge et al., 1992; Meyer et al., 2000)</td>
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<tr>
<td>Beta2-adrenoceptors</td>
<td>After 4 weeks downregulation in hippocampus and parietal cortex; transient effects in prefrontal cortex, olfactory area, and pulvinar nucleus (Flügge et al., 1997)</td>
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<tr>
<td>Beta2-adrenoceptors</td>
<td>After 4 weeks upregulation in pulvinar nucleus; transient effects in prefrontal cortex (Flügge et al., 1997)</td>
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depressed patients (see, e.g., Willner, 1997, 2005; Wiborg, 2013). Most importantly, the CMS paradigm induces anhedonic-like behavior, as demonstrated by various behavioral tests, and this deficit in reward sensitivity can be reversed by chronic, but not acute, antidepressant treatment.

Historically, the first chronic stress model of anhedonia was developed by Katz and Hersh (1981). At that time, those authors used harsh stressors, including electric shock, shaker stress, and cold swimming. The baseline corticosterone levels increased, and a reduced preference for sucrose consumption suggested a decrease in reward sensitivity, thus indicating an anhedonic-like condition (Katz and Hersh, 1981; Katz, 1982). Based on these observations, Willner modified the paradigm mainly by using milder stressors over a longer time period, to achieve more realistic inducing conditions. Thus, the Willner CMS model mimics human life stressors more closely than does the Katz model (Willner et al., 1987, 1992). The severity of the arsenal of microstressors applied is essential, as is the unpredictable sequential presentation of the stressors (Fig. 4). The primary impetus for optimizing the CMS paradigm was to simulate anhedonic-like behavior; consequently, hedonic measures became the primary readouts for the model. The preferred readout is sucrose consumption or preference. The effects induced by CMS are observed both in single-bottle consumption tests and in two-bottle, sucrose–water preference tests, as reviewed by Willner (1997, 2005). Moreover, we and others have shown that the observed decrease in sucrose consumption is specific and is not associated with a general decrease in fluid consumption; e.g., decreased thirst, as

![Fig. 3. The chronic psychosocial stress model of male tree shrews (Tupaia belangeri) as a potential animal model for depression. Graphical description of typical experimental design (A) and the experimental procedures (B). Notably, in all cases pilot studies were performed to determine the metabolic rate and the optimal concentration of the compounds under investigation (e.g., Czeh et al., 2005, 2006). When introducing a naive male into the cage of a socially experienced male this co-housing results in active competition for control over the territory, and after establishment of a clear dominant/subordinate relationship, the two animals were separated by a wire mesh barrier. For the subordinate male the daily interaction and continuous presence of the dominant conspecific is a severe stressor. Post mortem histopathological analysis revealed that exposing animals to such stressors over a period of 4–5 weeks inhibits neurogenesis in the adult hippocampus (C). Note that it was this model in which we demonstrated for the first time that the stress induced suppression of adult neurogenesis can be reversed by the treatment with an antidepressant drug (see Czeh et al., 2001).]
intake of plain water is unaffected by the CMS paradigm (Willner et al., 1992; Henningsen et al., 2012).

In addition, the caloric content of sucrose does not appear to have an effect, because similar effects were observed when sucrose was replaced with a calorie-free saccharine solution (Willner et al., 1987; Ayenu et al., 1995). Furthermore, a decrease in sucrose consumption was observed in both food-deprived and nondeprived animals (Willner et al., 1992; Willner, 1997). Thus, decreased sucrose consumption is not a physiological phenomenon; rather, it seems to be a mental phenomenon. The decrease in sucrose consumption suggests a decrease in reward sensitivity; this issue has been studied and proved extensively (for summary, see, e.g., Wiborg, 2013).

The protocol of CMS has been described extensively (e.g., Willner, 1997, 2005; Wiborg, 2013). Typically, outbred rats are preferred, but CMS protocols have also been developed for mice (Schweizer et al., 2009). The sucrose consumption test is used to quantify the hedonic state of the experimental animals. Rats are then divided, according to their sucrose intake, into two matched groups in such a manner that both the mean and standard deviation are similar in the two groups. One of the groups is left unchallenged in a separate room, and the other group is exposed sequentially to several microstressors (as shown in the scheme in Fig. 4). After repeating the stress paradigm weekly for several weeks, the majority of the rats will gradually and individually reduce their sucrose consumption, some more than others. Some rats are resilient and can cope with the applied stressors, to maintain homeostasis, whereas others are much more vulnerable or susceptible and enter an anhedonic-like state, including reduced reward sensitivity (Fig. 4). The genetic variability of an outbred strain underlies the heterogeneity in stress susceptibility observed within the rat population. To compare the extremes, we use operational cutoff values: rats with a conserved sucrose intake or a reduced sucrose consumption lower than 10% are defined as being stress resilient; whereas rats with a reduced sucrose intake greater than 30% are defined as being stress susceptible. An anhedonic-like state is gradually induced over 3–4 weeks of stress (Fig. 4). If the stress exposure is discontinued, rats will gradually recover spontaneously over 4–5 weeks, and another anhedonic-like episode may be induced by resuming stress exposure. The inducing conditions and the repetition of episodes mimic closely the naturalistic conditions in human life and MDD. Taken together, these features of the CMS model are unique in the preclinical modeling of depression and add much etiological validity to the CMS model. If the stress paradigm is sustained, the anhedonic-like state may be maintained for months, and rats are incapable of habituation because of the unpredictable nature of the stress regimen. Alternatively, if stress exposure is combined with chronic antidepressant treatment, rats may recover from the anhedonic-like state; however, a subdivision, typically around 50%, of the anhedonic-like rats will be refractory to treatment, which is very similar to therapeutic treatment refraction. Treatment refraction is another unique feature of our version of the CMS model (Christensen et al., 2011; Wiborg, 2013).

2.2.3. Learned helplessness

Helplessness and feelings of helplessness are core symptoms of MDD and are among the most thoroughly studied topics in the clinical and preclinical research of depression. In humans, learned helplessness (LH) describes a specific deficit in behavior to control aversive stimuli that is induced by prior exposure to uncontrollable aversive stimuli (for a recent review, see, e.g., Pryce et al., 2011). Not surprisingly, the learned helplessness paradigm was one of the earliest models that mimicked depression in animals and that was used to study the effects of uncontrollable stress (Seligman et al., 1968). Animals with learned helplessness are a complex animal model of depression that has good face, construct, and predictive validity; moreover, several pathophysiological concepts of depressive disorders have been validated using this model (for recent reviews, see Pryce et al., 2011; Vollmayr and Gass, 2013).
The classic experimental design consists of three groups, with two control groups. The first control group of animals is exposed to electric shocks that can be controlled, e.g., by shuttling (escaping) or lever pressing. The animals of the second group are coupled to the animals of the first group, which implies that each animal is connected to an animal of the first group receiving shocks of the same amount, duration, and pattern. These animals experience an uncontrollable stress, as they have no control whatsoever over the situation, because they receive electric shocks in an unpredictable, uncontrollable, and unavoidable manner. A third group of animals (second control) is not exposed to stress. Thus, animals exposed to uncontrollable shocks can be compared with animals exposed to controllable shocks (of exactly the same physical quality) and with unstressed controls. Studies employing this triadic design indicated that the deficits that follow exposure to stress are not caused by stress per se; rather, the uncontrollability of the stress was the critical determinant (Drughan et al., 1997).

2.2.4. Models based on early-life stress

Early-life stress models are based on the observation that unfavorable events and experiences that occur during in this critical developmental period of early life may cause a vulnerability for developing various types of diseases in later life. The models are based on initial studies performed in rodents (Weininger, 1953; Levine, 1957, 1967) and in nonhuman primates (Harlow and Zimmerman, 1959).

Over recent decades, evidence from epidemiological studies has indicated that prenatal (fetal) and/or postnatal (infant/child) environmental factors are associated substantially with the etiology of neuropsychiatric disorders. Negative experiences in early life, such as parental loss, abuse, and emotional and physical neglect, significantly increase the risk of developing an affective disorder later in life (for review, see, e.g., Heim et al., 2010; Lanius et al., 2010; Heim and Binder, 2012).

To date, many experimental approaches aimed at inducing early-life stress in rodents and nonhuman primates at critical developmental periods have been described. Many of these manipulations produce physiological and behavioral changes that persist well into adulthood and represent a risk factor for psychopathology (see, e.g., Newport et al., 2002; Pryce et al., 2005; Lucassen et al., 2013). Maternal separation is an experimental procedure that is used widely in this context. Many studies performed in rats have shown that a single or repeated separation of the pups from the mother leads to acute or long-term effects on physiology and behavior. Although maternal separation is the most common model of disruption of the mother–offspring relationship, the reports of its effects show contradictory findings for almost all parameters investigated (for review, see, e.g., Daly, 1973; Lehmann and Feldon, 2000). A possible explanation for this inconsistency may be that maternal separation has become a collective term for a variety of extremely different experimental manipulations (Lehmann and Feldon, 2000).

For example, the maternal deprivation stress, as a model for parental neglect, induces long lasting structural and functional consequences (e.g., Oomen et al., 2010, 2011). In contrast the repeated maternal separation model promotes and increases the extent of maternal care. Obviously it is less stressful for the pups (Entenhoven et al., 2008; Schmidt et al., 2011), and thus often results in opposite effects compared to the deprivation models. Interestingly, in a recent comprehensive review of this issue, Schmidt and colleagues question the validity of early-life stress paradigms such as maternal separation – at least in rodents – as robust models of depression (Schmidt et al., 2011). Based on the literature available, those authors conclude that future studies should investigate the extent to which the interplay between genetic predisposition and aversive or nonaversive stimuli in adulthood determines the outcome of early-stress experiences in later life challenges.

Recently, a chronic early-life stress model has been also developed which has both acute and long-lasting effects on the HPA system as well as on cognitive functions in adulthood. In this model the dam-pup interaction is disrupted by limiting the nesting and bedding material of the cages which results in abnormal, fragmented dam-pup interactions. Rearing pups in this stress-provoking environment has long lasting effects e.g. impaired hippocampus-dependent learning and memory functions as well as reduced survival of adult-born neurons (Rice et al., 2008; Naninck et al., 2015).

3. Other animal models

3.1. The olfactory bulbectomy model

Bilateral olfactory bulbectomy (OBX) is a surgical model of depression that results in changes in endocrine, immune, and neurotransmitter systems, as well as behavioral alterations that resemble the symptoms observed in patients with major depression (van Riezen et al., 1976; for reviews see Kelly et al., 1997; Song and Leonard, 2005; Hendriksen et al., 2014). Olfaction is extremely important in rodents, and their olfactory system forms part of the limbic region in which the amygdala and hippocampus contribute to the emotional and memory components of behavior. Importantly, the loss of olfaction alone resulting from bulbectomy is not the major factor that contributes to the behavioral abnormalities observed, as peripherally induced anosmia does not cause the same behavioral changes (Song and Leonard, 2005). The behavioral alterations observed after OBX are thought to result from dysfunctions and/or compensatory mechanisms of the cortical–hippocampal–amygdala circuits that involve changes in synaptic strength and/or loss of spine density in these limbic areas. The same neuroanatomical regions are dysfunctional in patients with major depression (Price and Drevets, 2012). The following behavioral changes have been observed after bilateral OBX: hyperactivity, changes in social behavior, enhanced nocturnal activity, deficits in learning and memory, and changes in taste-aversion behavior; however, no indication of anhedonic-like behavior has been reported (Kelly et al., 1997; Song and Leonard, 2005).

Alterations in the noradrenergic, serotonergic, cholinergic, GABAergic, and glutamatergic neurotransmitter systems are also associated with OBX. An enhanced nocturnal secretion of corticosterone is observed in OBX rats, which can be suppressed by the administration of dexamethasone. Furthermore, chronic, but not acute, administration of antidepressants largely corrects most of the behavioral, endocrine, immune, and neurotransmitter changes that occur after bulbectomy. Tricyclic antidepressants (amitriptyline and desipramine), atypical agents (mianserin), selective serotonin-reuptake inhibitors (paroxetine, sertraline, and fluvoxamine), reversible inhibitors of monoamine oxidase A ( moclobemide), and putative antidepressants such as 5–HT1A agonists (zolazopride and ipsapirone), noncompetitive N-methyl-D-aspartate antagonists (MK-801), and triazolobenzodiazepines (alprazolam and adinazolam)), have exhibited antidepressant-like activity in this model (for review, see Kelly et al., 1997; Song and Leonard, 2005).

In summary, this model seems to have both face and predictive validity, but etiological and construct validities are lacking.

3.2. Pharmacological models

Different approaches have been used to create animal models based on pharmacological manipulations aimed at disrupting primarily the monoamine balance. These models were based on the monoamine theory of depression (Schildkraut, 1965). One such model is the administration of reserpine (reviewed by Willner, 1984; O’Neil and Moore, 2003), which is an antihypertensive and antipsychotic drug that can deplete brain monoamines nonselectively, thereby inducing a syndrome of anhedonic-like state, locomotor hypomotility, and reduced body temperature in rodents (Leith and Barrett, 1980; Skalis et al., 2002). The psychostimulant withdrawal paradigm is another such pharmacological model (Barr and Markou, 2005). As an effect of withdrawal from drugs such as amphetamine or cocaine, rodents display behavioral changes that are highly analogous to some aspects of depression in humans, such as reward deficits (i.e., elevation in brain reward...
thresholds) and behaviors that are opposite to those observed after treatment with antidepressant drugs (e.g., decreased immobility in the forced swimming and tail suspension tests) (Barr and Markou, 2005).

Overall, these models seem to have some etiological and face validities. These models are unlikely to have any predictive validity and in addition their construct validity is also questionable.

3.3. Disruption of the circadian rhythm

Depressed patients often exhibit significant disruption of their circadian rhythms and sleep/wake cycle, and stabilizing the circadian rhythm can have a positive impact on depressive symptoms (for review, see, e.g., McClung, 2013). Similarly, disruption of circadian regulation in animals can induce depressive-like symptoms (e.g., Gonzalez and Aston-Jones, 2008; for review, see, e.g., Evans and Davidson, 2013), although this paradigm is rarely used currently. This model may have a unique etiological validity and also specific face validity. Further research is needed to evaluate its predictive and construct validities.

4. Common pitfalls, misinterpretations, and often-neglected issues in the field

4.1. Symptoms and comorbidity

MDD is a heterogeneous concept. Even though, the diagnostic manuals (ICD–10, DSM–5) give clear guidelines for the diagnosis, the clinical classification of mood disorders is a complex issue and differentiating MDD from other mood disorders (i.e., recurrent brief depression, minor depressive disorder, bipolar depression, and adjustment disorder with depressive symptoms) can be a challenging task. In this paper, we focus on MDD, since this is the most prevalent and clinically severe type of mood disorder. Furthermore, modeling MDD in animals has higher translational value than modeling for example bipolar depression. Similarly to other psychiatric disorders, MDD is a heterogenous diagnostic category. The clinical manifestation of MDD can be very variable (some symptoms may go into opposite directions), and currently, patients with different clinical features are classified into the same diagnostic category, even though they are likely to have a different underlying pathophysiology. For example, patients with a melancholic subtype of MDD experience anorexia and insomnia, whereas patients with an atypical subtype of MDD experience hyperphagia and hypersomnia. This issue is difficult to address and thus, often neglected when working with animal models. In light of the high complexity of MDD and of its high comorbidity with anxiety disorders, the chance of succeeding in developing comprehensive animal models that reflect accurately the relative influences of contributing factors in humans is probably quite poor. However, a specific symptom or subset of symptoms can be modeled.

4.2. Clinicians vs basic scientists

Clinicians and nonclinical neuroscientists need to become more involved in the collaborative development of both laboratory experiments and clinical research programs. Very few clinicians work on animal models of psychiatric disorder. Similarly, few basic scientists contribute to clinical research programs. Matthews et al. (2005) mention that a former leading researcher in animal models of depression confessed that despite having written about the topic for over 15 years, he had never actually met or spoken to someone with depression.

4.3. Models vs tests

Many laboratories are testing new compounds, to demonstrate their antidepressant efficacy or a depressive behavioral phenotype in animals as a result of genetic modifications or a specific pretreatment. The most important challenge for scientists working in this field is to establish how to assess a depressive-like status in laboratory animals. Table 1 summarizes the most commonly used laboratory tests and relates them to the clinical symptoms, as described in the DSM5. While some symptoms found in patients are easy to translate into behavioral or physiological changes in animals (e.g., anhedonia, altered diurnal rhythm, disturbed sleep pattern, and change in body weight), others are difficult or impossible to model. For example, it is impossible to generate an animal that will commit suicide or show “feelings of worthlessness or excessive or inappropriate guilt”. Patients can share a substantial sample of their feelings and thoughts via verbal communication with a psychiatrist; this highly differentiated way of communication, however, is not possible between animals and experimenters. Consequently, various test systems are used in preclinical studies as surrogates to overcome this problem. Some tests have become so popular that laboratories employ them as first-choice tests, despite the fact that their utility has been repeatedly questioned. For example, the test that is used most commonly to demonstrate a depressive-like behavioral phenotype in mice or rats is the forced swimming test, together with its counterpart, the tail suspension test. However, these tests have never been intended for such use; rather, they were developed to screen drugs. Tricyclic antidepressants, SSRIs, and SNRIs show clear efficacy in these tests, or at least change the behavior of the animals; however, these tests are not appropriate for animal models of depression. It is also uncertain whether these tests can detect the antidepressant efficacy of a compound with a completely different acting mechanism compared with tricyclic antidepressants, SSRIs, or SNRIs.

4.4. Behavioral tests are not trouble free

The use of transgenic mouse lines has become widespread, which also means that many laboratories with little behavioral research experience need to perform behavioral tests to prove the desired phenotype of their knockout/knockin lines. Compared with the technical difficulties of generating a new transgenic line, the application of most behavioral tests may seem ludicrously simple; however, in fact, many things can go wrong. Working with mice is especially difficult, as most of the tests have been developed for rats, which are much more ‘intelligent’ animals. Many laboratories do not consider that their transgenic animals may have sensory deficits when they assess them using behavioral tests (Bailey et al., 2006; Crawley, 2007; Glinka et al., 2012). Many of us working in this field disregard the evidence of significant baseline differences in the behaviors of the inbred mouse strains that are used to generate targeted mutant mouse lines (Bailey et al., 2006; Crawley, 2007). It is well known that the environment plays an important role in determining behavior and that the lighting conditions and familiarity of the experimental settings, for instance, have a profound impact on the behavior of an experimental animal (Bailey et al., 2006; Crawley, 2007). The fact that laboratory rodents are nocturnal and, thus, are generally quiescent during the light phase of the day is frequently ignored. Therefore, in rodents, the determination of the effect of psychotropic drugs on natural action patterns of behavior should be performed during the dark phase of the light–dark cycle. This means that animals must be housed under a reversed light–dark schedule (Mitchell and Redfern, 2005).

4.5. Issues with typical drug testing protocols

Many “screens” try to detect antidepressant-like activity quite quickly, within minutes or hours, and the drugs are given prior to the testing, thus producing a behavioral alteration rather than preventing a disease-induced type of behavior. It is obvious that such an approach bears no similarity to the clinical situation, in which drugs are administered only after disease symptoms have appeared and in which a delayed onset of therapeutic effects of at least 2–3 weeks is expected. In light of such data, we suggest that one important characteristic factor
in the development of animal models with predictive validity is the reproduction of a time course of the “therapeutic effects”. Thus, the ideal model should respond to chronic, but not acute, treatment with conventional antidepressants. The importance of this feature should not be underestimated, as it is only possible to detect the actual time point of the therapeutic onset of a drug when a model shows a gradual response that reflects the drug’s gradual onset of action. Models in which the clearest evidence of gradual onset of action has been obtained include the chronic mild stress model, the social stress/resident–intruder paradigm in rats, and the chronic psychosocial stress model in tree shrews.

A further complicating issue is the increasing use of mouse lines, especially the various genetic models. These mice are behaviorally characterized in tests that have been originally developed for rats (e.g. the Morris water maze, the elevated plus maze and the open field). In such cases scientists make the assumption that mice are just like “small rats”, but in fact they are not, and often behave very differently.

Another critical issue is the route of drug administration. In most cases animals receive drugs via i.p. injection, whereas patients typically take drugs orally in the clinical settings. For a better translation of results oral administration should be used in preclinical settings because it provides several advantages: i) it mimics the clinical situation, as most patients take drugs orally, ii) drugs taken orally produce metabolite concentrations that differ from those obtained after i.p. or i.v. administration, and iii) it minimizes the uncontrollable acute stress effects of injections.

To date, little attention has been paid to potential species-specific differences in the metabolism of the applied drugs and their dosages. Currently, antidepressant drugs are typically tested in mice and rats assuming that their drug metabolism is similar to humans. But this is not always the case and drug metabolism should be evaluated more carefully, before running a drug test. To exclude the effects of sub- or supraeffective doses, there is an urgent need to monitor the concentrations of circulating antidepressants and their pharmacologically active metabolites in the animals under investigation. The demonstration that the drug effects are detected at clinically relevant doses that do not produce other, potentially confounding physiological and behavioral effects is equally important (Mitchell and Redfern, 2005).

4.6. Factor age

The majority of animal studies are performed in young adult animals. However, the incidence of MDD is significantly higher in adolescence and old age (Kessler et al., 2007). This issue has not received much attention in preclinical research. It should be acknowledged that antidepressants are often less effective in elderly patients, as these individuals may take longer to respond to medication, are more prone to the side effects of the antidepressant drugs, and experience greater difficulties in tolerating doses that are in the therapeutic range (Parikh, 2000).

Fig. 5. Neuropathology of depression: cellular changes that may contribute to the hippocampal volume decrease of depressed patients. Animal models for depression, especially the chronic stress models have provided useful tools to understand the stress-induced neuropathological changes. Prominent examples are depicted here. The stress-induced reorganization of the dendritic architecture is well documented in animal models and likely to contribute to the volume decrease. Notably, a similar phenomenon has been shown in post mortem brain samples from depressed patients. Reduced adult neurogenesis has been shown both in humans and experimental animals. Because of low incidence its contribution to the volume decrease is questionable. Changes of glial numbers and cellular morphology have been shown both in humans and animal models; therefore, these cellular changes are likely to contribute to the volume decrease. We have shown that stress results in reduction of hippocampal capillarization, but whether this is also present in depressed patients is yet unknown. Importantly, contrary to earlier assumptions, there is no evidence that significant amount of neuronal cell death is present in the hippocampi of depressed patients, or in animals subjected to chronic stress.
4.7. The sex issue

Epidemiological studies clearly demonstrate that women are much more vulnerable to stress-related psychopathologies and that depression occurs at least twice as commonly in women as in men; however, most preclinical research modeling these disorders is conducted mainly using male animals (except e.g., Herzog et al., 2009; Willard and Shively, 2012). It remains unclear whether this prominent sex difference in the prevalence of depression is caused by cultural aspects or is based on biological differences between the sexes. As this sex difference develops during adolescence, it has been suggested that, in women the sex steroids and the sexual differentiation of the brain contribute to a female-specific etiology (Naninck et al., 2011); in contrast, other authors have concluded that there are minimal sex differences in the etiology of major depression (Kendler et al., 2002, 2006).

5. Advantages of the animal models: understanding the pathophysiology of depression

From the viewpoint of drug development the usefulness of animal models has been repeatedly questioned however, the same models helped us to understand better the underlying pathology of the disease (see e.g. MacQueen and Frodl, 2011; Price and Drevets, 2012). For example, from the stress-based models we gained plenty of insight on the cellular alterations that could contribute to the hippocampal volume decrease in depression (see Fig. 5 and Czeh and Lucassen, 2007). The stress-induced reorganization of the dendritic architecture is a well-documented phenomenon in animal models (e.g. Magarinos et al., 1996; reviewed by e.g. Lucassen et al., 2014) and similar changes have been demonstrated in the brains of depressed patients (Hercber et al., 2010; Soetanto et al., 2010). Likewise, both experimental and human data demonstrate that stress/depression and antidepressant treatment regulate adult neurogenesis (e.g. Czeh et al., 2001; Boldrini et al., 2009). Importantly, contrary to earlier assumptions, there is no evidence that significant amount of neuronal cell death is present in the hippocampi of depressed patients (Lucassen et al., 2001a, 2006, 2014; Stockmeier et al., 2004; Cobb et al., 2013) and that is also true for animals subjected to chronic stress (Vollmann-Honsdorf et al., 1997; Lucassen et al., 2001b, 2004). On the other hand, astrocytes seem to be affected by depression and stress as documented in humans (Rajkowska and Stockmeier, 2013) and in animal models (Czeh et al., 2006, 2013). Lately, we have shown that stress results in reduction of hippocampal capillarization (Czeh et al., 2010) and affect GABAergic interneurons (Hu et al., 2010; Czeh et al., 2015), and comparable neuro-pathological findings have been reported in the brains from depressed patients (Torrey et al., 2005; Surtees et al., 2008; Konradi et al., 2011).

6. Summary and future directions

The development of animal models for depression has been largely influenced by the needs and goals of the pharmaceutical industry. Their principal objective is the discovery of drugs that are more effective and/or safer than existing therapies. These aims impose pragmatic constraints on the design and validation of models, such as the ability to screen for the activity of a large number of compounds rapidly (Howard, 1989). In contrast, academic research is also interested in the underlying pathophysiology of the disease and has developed genetic and chronic-stress models; these models are labor demanding, which limits their industrial application (Nestler et al., 2002). The crucial role of psychosocial stress and chronic stress in general, at least in a subpopulation of patients with MDD (Kendler et al., 1999; Paykel, 2001), will encourage the development of behavioral stress models that reproduce the biobehavioral consequences of these factors.

In the future, rodents will remain the main experimental animals. However, experimental animals that have a receptor pharmacology that is closer to that observed in humans should be more widely used for the behavioral and neurochemical analysis of the effects of novel antidepressants. The current models have significant limitations; for example, none of the models can replicate the relapsing–remitting nature of the disease, and very few attempts have been made to develop animal models specifically in female animals, or to scrutinize drug treatments that are sensitive to sex differences. Depressed patients have strong cognitive biases in attention, memory, and interpretation (Matthews and MacLeod, 2005), an issue that is rarely addressed in preclinical research. From the clinical point of view, prevention is the best approach to “treating” any illness; however, this requires early diagnos- is and screening methods for the identification of individuals at high risk. This approach is also underrepresented in preclinical studies.

The models available currently are being continuously refined. New mutant mouse models and gene targets of interest are constantly being developed and investigated. In the future, theory-based approaches as well as unbiased genome-wide association studies (GWASs) will identify new candidate genes. Although the generation of new transgenic mouse lines is a continuously evolving technique and often represents a technically elegant approach, based on what is currently known regarding the genetic background of the disease, this approach is unlikely to bring any real breakthrough in this field. The major obstacle here is that we know that there is no single depression-causing gene. Rather, a combination of several genes may constitute a vulnerability to the disease, each having little or no effect on its own. From the behavioral neu- roscientific perspective, the most significant contribution of the genetic models has been that they have led to the emergence of the optogenetic toolkit. The recent rapid developments observed in this new field of research hold the promise of developing significantly better models in the near future.

Animal models based on stress exposure will also prevail. In the past and in the future they were and will be instrumental to understand the neuropathological changes contributing to the pathophysiology of MDD. The continuous rise in the incidence of depression, which has been documented every year since the early 20th century, cannot be explained by the spreading of genetic susceptibility factors, or the emergence of new mutants. Rather, it is caused by the rapid and drastic socioeconomic changes observed in this period, which are po- tent stressors for individuals. For this reason, models that are based on environmental or social stressors will remain highly relevant in research aiming to understand the underlying pathophysiology of major depression.

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