Mini-review: Translational Value of Rodent Models in Behavioral Neuropsychiatry

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Model organisms have become the transformative tools for biological discovery of the cellular and molecular processes that underlie human disease pathogenesis. Here, we discuss the use and translational value of rodent models, and highlight their strengths and caveats in the context of human psychiatric illnesses such as depression, anxiety, and posttraumatic stress disorder. Human genetic association studies and epidemiological analyses cannot detect causative biological mechanisms that control behavior; however, we can use nonhuman animal models to validate and evaluate the function, and potential contribution, of likely candidate genes that were previously tied to differences in disease-relevant endophenotypes. Although animal experimentation allows rigorous control of both genetic and environmental variables, all model organisms have limited biological validity and will never fully capture the broad diagnostic criteria for human psychiatric illness, arguably the most complex of all human disorders. With this in mind, we evaluate the utility and limits of translating rodent psychiatric illness-relevant behavioral assay (elevated-zero maze, forced-swim test, sucrose preference test, and tail suspension test) data to humans. Shortcomings also may include deficiencies in development of models, poor experimental design, inadequate methodologies, physiological differences between species, and problems with the interpretation of results. Research on animals will continue despite these limitations, and innovative translational approaches to neuropsychiatric disorders such as the automation of behavioral assays, neural imaging in awake animals, and epigenetic profiling will elucidate the neurocircuitry of behavioral dysregulation. Optimizing the translational value of model organisms could contribute to an improved understanding and treatment of mental illness.

Abbreviations: ADHD – attention deficit/hyperactivity disorder; ASD – autism spectrum disorder; EPM – elevated-plus maze; EZM – elevated-zero maze; fMRI – functional magnetic resonance imaging; FST – forced-swim test; GWAS – genome-wide association studies; HPA – hypothalamic-pituitary-adrenal axis; MDD – major depressive disorder; OCD – obsessive-compulsive disorder; ofMRI – optogenetic functional magnetic resonance imaging; PTSD – posttraumatic stress disorder; SCZ – schizophrenia; 5-HT – serotonin; SNP – single nucleotide polymorphism; SPT – sucrose preference test; TST – tail suspension test

Keywords: animal models; anxiety; behavior; behavioral neuroscience; depression; neuropsychiatry; phenotype; posttraumatic stress disorder; rodent models

Introduction

The global spread of smallpox, a vicious infectious disease, devastated mankind for millennia. Smallpox is caused by infection with the variola virus, and the use of “variolation”—inoculation with cowpox (i.e., vaccination)—led to its eradication. The world’s first vaccine used to combat smallpox would not have been possible without experiments with cows (Barquet, 1997). Research in nonhuman animals such as mice, rats, chickens, rabbits, cats, dogs, pigs, goats, sheep, cattle, and monkeys (Table 1) has
been and continues to be instrumental in the development of life-saving and life-improving medications (Notter, 2000; Prichard, 1982; Jackson et al., 1993; Tunstall-Pedoe et al., 1999; Coombes et al., 2007; Piccart-Gebhart et al., 2005), vaccines (Spiegel, 1947), antibiotics (Salisbury, 1996), anesthetics (Saint André et al., 2002), and surgical procedures (Rushman, 1966; Desmoulin-Canselier & Moutaud, 2019). This list is certainly not exhaustive. Notably, rodent models account for 95% of all nonhuman animal models used in biomedical research (Hickman et al., 2017). Ease of maintenance, minimal housing requirements, and a short generation time make rodents ideal for study (Hickman et al., 2017). Indeed, the use of model organisms in biomedical research, such as rodents, extends to all human disease conditions, including psychiatric illness (Kaffman et al., 2019; Kaffman et al., 2012; Stanford, 2020). Given that the body’s most complex functions such as thought, personality and emotion lie in the brain, psychiatric disorders are some of the hardest to model (Kaffman et al., 2012). Moreover, the overlapping biological substrates (e.g., neurotransmitter systems) underpin different psychiatric conditions, and the function of each separate neurotransmitter system may modulate many other chemical systems simultaneously (Kaffman et al., 2012). These factors, in part, contribute to the repeated failure of model organism research to yield findings that translate into humans (Stanford, 2020).

### Human Genetic Approaches to Psychiatric Disease

The genetic underpinnings of psychiatric illness become more apparent, and for this reason the essence of what it means to be a healthy human being versus mentally ill is increasingly defined in biological terms. (Deacon, 2013; Pescosolido et al., 2010). Indeed, a growing body of evidence, collected over many years, has helped identify putative genetic markers of various psychiatric disorders (Deacon, 2013). This scientific progress is exemplified by studies initiated by psychiatrists a few decades ago that demonstrated a predisposition to schizophrenia (SCZ) given a certain genetic makeup (Kety et al., 1976, 1978). Since that time, the genome-wide association studies (GWAS) (Bush & Moore, 2012)—an established method used to identify multiple common genetic variants, or single nucleotide polymorphisms (SNPs), that influence the risk of disease—have grown in popularity. Crucially, the GWAS became a much-discussed approach for identifying genetic factors contributing to the etiology, or cause, of many different complex neuropsychiatric disorders. Based on such an analysis, for example, the estimates of heritability of risk for SCZ (Visscher, 2017), alcohol use disorder (Hart & Kranzler, 2015), and posttraumatic stress

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<thead>
<tr>
<th>Animal Model</th>
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<tr>
<td>Mice</td>
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<td>Rats</td>
<td>Bazell, 1998</td>
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<td>Chicken</td>
<td>Rous, 1911</td>
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<td>Rabbits</td>
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<td>Snakes</td>
<td>Ferreira et al., 1970</td>
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<td>Cats</td>
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<td>Dogs</td>
<td>Weil, 1915; Goonetilleke et al., 2003</td>
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<td>Pigs, Goats, Sheep</td>
<td>Paton, 1972</td>
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<td>Cattle</td>
<td>Barquet, 1997; Langworthy et al., 2000</td>
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<td>Monkeys</td>
<td>FDA, 1998; Rausch et al., 1999; Clarke, 1906</td>
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disorder (PTSD) (Nievergelt et al., 2018) are approximately 70%, 50%, and 40%, respectively.

As cheaper, faster, and more accurate sequencing methods emerged, they have been revolutionary in our search for genetic variants that influence the risk for psychiatric illness (Biesecker & Peay, 2013). These technologies have produced large volumes of data about the genetics of human disease, but they come with drawbacks. For example, the relative success of these genome-wide approaches in detecting potentially causal variants in highly polygenic disorders such as SCZ relies on a sufficiently large sample size (Need et al., 2012). Further, individual SNPs exert a relatively small effect on overall disease risk (10—20%), and the SNPs associated with substantial risk for SCZ, for example, are not restricted to this disorder (Visscher, 2017). Another major disadvantage of human genetic association studies and epidemiological analyses is their inability to detect causative biological mechanisms that control behavior (Biesecker & Peay, 2013). What does the presence or absence of a given allele or SNP really mean, in terms of what has gone wrong?

Model Organisms in Translational Neuropsychiatry Research

Appreciating the above-mentioned limitations informs the choice of an experimental approach and method used in examining any psychiatric disturbance. Reflecting this, one of the useful alternatives is to engineer nonhuman genetic, pharmacological, and lesion/ablation models of mental illness. Nonhuman animal models can be useful, however, the anatomic and cellular complexity and diversity of the brain, as well as the dynamic nature of brain development—a particular genetic makeup (i.e., genotype) can lead to multiple different observable traits (i.e., phenotypes)—creates many challenges in the field of translational neuroscience (Nestler & Hyman, 2010; Fernando & Robbins, 2011). Given that it is difficult to cover every aspect of all animal models of neuropsychiatric disorders, our approach has been to evaluate the suitability and limitations of rodent models in the context of human psychiatric illness. More specifically, we discuss the use and translational value of rodent models, and highlight their strengths and caveats, mainly in terms of behavioral dysregulation relevant to major depressive disorder (MDD), anxiety, and PTSD.

The practice of using nonhuman animals (referred to henceforth as animals) for scientific purposes has played a central role in medical progress throughout history (see Table 1). Scientists continue to foster the development of model systems to deliver much-needed life-saving therapeutic strategies, and the importance and clinical relevance of animal models across various disciplines including psychiatry cannot be understated (Nestler & Hyman, 2010; Fernando & Robbins, 2011). For example, rodent models were used to identify the importance of D2 dopamine receptor dysregulation in SCZ, and allowed for the development of antipsychotic drug treatments (Nestler & Hyman, 2009).

Currently, there are animal models of virtually every common behavioral, cognitive, affective and stress-related disorder (Nestler & Hyman, 2010; Fernando & Robbins, 2011). Examples include, but are not limited to, anxiety, attention deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), bipolar disorder, MDD, obsessive-compulsive disorder (OCD), PTSD, SCZ, and substance abuse/addiction. Each of these preclinical models comes with its own set of limitations but has tremendous value in fostering much-needed life-saving therapeutic strategies (Notter, 2000; Prichard, 1982; Jackson et al., 1993; Tunstall-Pedoe et al., 1999; Coombes et al., 2007; Piccart-Gebhart et al., 2005; Spiegel, 1947; Salisbury, 1996; Saint André et al., 2002; Rushman et al., 1966; Desmoulin-Canselier & Moutaud, 2019; Franco, 2013; Assary et al., 2018; Lezak et al., 2017). For example, spontaneously hypertensive rats are used to model ADHD and evaluate the therapeutic utility of psychomotor stimulant drugs (Wultz et al., 1990). Indeed, the underlying molecular and cellular mechanisms that result in human pathology can be recapitulated using model organisms, and pharmacological interventions that were developed and evaluated in animal models are effective for the illness in humans. However, it is clear that nonhuman
animal cognition is dissimilar to the higher cognitive ability of humans (Maclean, 2016).

One of the key aspects of mental illness that behavioral biologists and clinical neuroscientists have grown to appreciate is the complex bidirectional interplay between genes and environment in shaping behavioral outcomes. It is now widely accepted that brain functioning and behavior in typical and diseased individuals is caused by an interaction between genetic variants and potentially wide-ranging environmental components. Because animal experimentation allows rigorous control of both genetic and environmental variables, these studies are essential to address the neurochemical basis of psychopathology and abnormal behavior. For example, researchers can use genetic or pharmacological techniques to manipulate animal behavior. Thus, this experimental approach is likely to remain an important translational tool for exploring a variety of neural and behavioral correlates that may serve as approximate markers for mental illness in humans. By using animals, we can validate and evaluate the function and potential contribution of genetic candidates that have been previously tied to differences in disease-relevant endophenotypes. Furthermore, much of the recent success in identifying cellular/molecular and circuit-level mechanisms and factors that underlie behavioral disorders in the human population is owed to the study of animal models that allow for invasive experimentation (e.g., genetic or pharmacological manipulation, experimental surgery) unthinkable in humans (Assary et al., 2018; Lezak et al., 2017; Maclean, 2016).

Without question, prior experimentation has demonstrated that we can predict human response to disease by using animals (Nestler & Hyman, 2010; Fernando & Robbins, 2011; Franco, 2013; Robinson et al., 2019). However, this is not easily done, in part, because neuropsychiatric phenotypes such as PTSD are complex and difficult to assess in model organisms (Verbitsky, 2020). Other shortcomings may include deficiencies in development of models, poor experimental design, inadequate methodologies, physiological differences between species, and problems with the interpretation of results (Robinson et al., 2019; McGonigle & Ruggeri, 2014; Maximino & van der Staay, 2019). Another important consideration relates to choosing the appropriate model system (McGonigle & Ruggeri, 2014; Maximino & van der Staay, 2019). For example, rodents have been the primary model organism for mechanistic studies of MDD and pharmacotherapy with antidepressants in humans since the mid-twentieth century (Schildkraut, 1965). These basic research efforts furthered our understanding of the neurobiology and pathophysiology of MDD, as well as complex biochemical mechanisms of drug action, but antidepressants are efficacious in 30%–40% of patients suffering with MDD (Blackburn, 2019). As of the writing of this, many different preclinical models of mental illness have been generated, ranging from nonhuman primates, large mammals, rodents, fruit flies, and zebrafish (Need et al., 2012; Nestler & Hyman, 2010; Franco, 2013). Clearly, the brains of nonhuman primates and larger mammals (e.g., pigs, sheep) give the best approximation to human condition, and might therefore be of larger translational value (Nestler & Hyman, 2010; Fernando & Robbins, 2011; Franco, 2013). However, even though the brains of smaller animal species (e.g., mice, rats) show lesser biological relatedness to humans in terms of their resemblance to the human brain, due to various practical and methodological reasons, rodents are used in the large majority of basic research studies (Nestler & Hyman, 2010; Fernando & Robbins, 2011; Robinson et al., 2019).

Behavioral Phenotyping in Neuropsychiatry Research

Regardless of their behavioral repertoire or cognitive ability, all animal models have limited biological validity and will never fully capture the broad diagnostic criteria for human psychiatric illness, arguably the most complex of all human disorders (Nestler & Hyman, 2010; Fernando & Robbins, 2011; Maclean, 2016; Maximino & van der Staay, 2019). The use of clinically relevant models for target identification and validation provides ways to explore dimensions (i.e., well-understood
symptoms or symptom clusters) of neuropsychiatric illness in order to identify the neurochemical underpinnings and behavioral correlates. However, given the subjective nature of many psychiatric clinical symptoms, scientists are unable to model entire disorders such as MDD or anxiety. Indeed, certain symptoms of MDD such as guilt, sad mood, or suicidality are likely uniquely human conditions (Maximino & van der Staay, 2019). Related, high comorbidity rate of MDD and anxiety disorders presents another great clinical and preclinical challenge (Belovicova et al., 2017; Gaspersz et al., 2018). Indeed, MDD and anxiety in humans frequently occur together, share clinical symptoms and causes, and have similar treatments (Gaspersz et al., 2018; Melton et al., 2016). Similarly, depressive-like behavior and anxiety-like behavior can prove tremendously difficult to distinguish in rodents, especially since similar symptoms underlie both conditions, and because both types of behavior change following treatment with antidepressant drugs (Ramaker & Dulawa, 2017). Also, stress is a risk factor for MDD, but most methods of stress-induction in rodent models produce general symptoms of anxiety such as immobility, as opposed to any differentiating MDD-like symptom (Gururajan et al., 2019). In addition, sex differences add another layer of complexity when modeling human psychiatric illness. For example, stress-related disorders are more prevalent in women compared with men, and there are differences in serotonergic systems between sexes that influence sensitivity to serotonin (5-HT; Dalla et al., 2010).

On the other hand, we can approximate other domains of human MDD such as behavioral despair or anhedonia (i.e., the desire/lack of desire to seek pleasurable stimulation) by observing depressive-like behavior (Schildkraut, 1965) in a variety of model systems, including manipulation with pharmacological agents, and environmental factors as well as gene editing (deletion or overexpression), and lesion/ablation of brain areas (Maximino & van der Staay, 2019). Below, we discuss in more detail behavioral screening assays in rodent models of neuropsychiatric disorders including MDD, anxiety and PTSD, though the following list is not exhaustive and is only intended to illustrate some of the challenges in interpretation of test results.

Interpretation of Behavioral Test Results in Rodents

**Depression: Forced-swim Test, Tail Suspension Test, Open Field Test, and Sucrose Preference Test**

As mentioned above, depression is difficult to model in rodents due to subjective symptoms of sadness, guilt, or suicidality. Conversely, somatic symptoms such as behavioral despair, anhedonia, fatigue, and psychomotor dysfunction can be approximated (American Psychiatric Association, 2013; Anyan & Amir, 2018). Scientists who use preclinical models of MDD use a battery of behavioral paradigms designed to measure preclinical models of MDD use a battery of behavioral paradigms designed to measure depressive-like behavior (Anyan & Amir, 2018).

Figure 1: Porsolt forced-swim test.

The Porsolt forced-swim test (FST), a measure of behavioral despair, for example, is the *sine qua non* of depressive-like behavior testing in rodents [along with the tail suspension test (TST)] (Belovicova et al., 2017; Anyan & Amir, 2018; Porsolt & Le Pichon et al., 1977; Porsolt & Bertin et al., 1977). Briefly, each rodent is placed in a cylinder filled with water, and the animal will exhibit escape-directed behaviors such as swimming and climbing. Enhanced immobility (i.e., a floating posture) is interpreted as depressive-like behavior, since naïve, control rodents placed into water swim and struggle to escape (Anyan & Amir, 2018; Porsolt & Le Pichon et al., 1977; Porsolt & Bertin et al., 1977). Although this interpretation is now being reconsidered (Estanislau et al., 2011; Campus et
al., 2015; Molendijk & de Kloet, 2015), typically, rodents that stop swimming and struggling sooner are judged as showing greater ‘despair’ (Belovicova et al., 2017; Porsolt & Le Pichon et al., 1977; Porsolt & Bertin et al., 1977). In addition to the FST,

![Tail suspension test.](image)

neuroscientists use the tail suspension test (TST) to measure behavioral despair. In the TST, enhanced immobility (i.e., a hanging posture), when the animal is suspended by its tail, is interpreted as depressive-like behavior. Many researchers interpret the FST and TST data in the context of depressive-like behavior because the majority of clinically used classical antidepressants, as well as experimental pharmacological agents with fast-onset antidepressant effect, have been shown to decrease the duration of immobility (Ramaker & Dulawa, 2017; Anyan & Amir, 2018; Porsolt & Le Pichon et al., 1977; Steru et al., 1985; Steru et al., 1987; Stukalin et al., 2020). Some, however, claim that the FST and TST measure anxiety-like behavior because they found an inverse relationship between depressive-like and anxiety-like behavior in rodent models of comorbid anxiety and depression (Anyan & Amir, 2018; Petit-Demoulierev et al., 2005). Also, rats treated with an anxiogenic (anxiety-inducing) compound display increased escape-directed behaviors, whereas anxiolytic (anxiety-reducing) compounds decrease these behaviors (Nishimura et al., 1989).

Additionally, it is necessary to account for motor disturbances/activity levels because some species of mice can swim longer than others (Molendijk & de Kloet, 2015). To assess possible non-emotional confounding effects such as motor dysfunction, researchers commonly use the open field paradigm to measure locomotor and exploratory behavior, and sometimes anxiety or emotionality (Seibenhener & Wooten, 2015). Briefly, the animal is placed into an unfamiliar chamber devoid of any items, and photocells imbedded in the wall emit an infrared beam in a grid-like pattern. The animal is allowed to move about for a single 20-minute session, and infrared photocells track movement in the X, Y, and Z (i.e., vertical activity or rears) axes, as well as stereotypies. Measures recorded include distance, speed, and place preference within the chamber (i.e., basic activity) (Seibenhener & Wooten, 2015). Crucially, these measures provide a variety of behavioral information regarding the emotionality of the rodent.

Aside from the FST and TST, since its introduction, the sucrose preference test (SPT) has become one of the most widely used assays for measuring the features of anhedonia (i.e., the desire/lack of desire to seek pleasurable stimulation) in rodents (Scheggi et al., 2018). More specifically, if mice do not show a preference for sugar water over plain water, this loss of interest in the pleasurable experience of sucrose is interpreted as depressive-like behavior (Scheggi et al., 2018). And similar to their effects on behavioral despair seen in the FST and TST, various antidepressants reverse decreased sucrose preference in many different models of depression (Ramaker & Dulawa, 2017; Scheggi et al., 2018).

Clearly, the FST, TST, and SPT are used to reproduce relevant attributes of MDD such as despair (Nafkai et al., 2018) or anhedonia (Scheggi et al., 2018), but each of these assays is used to assess specific behavioral domains rather than a complete phenotypic aspect of depression (Maximino & van der Staay, 2019; Porsolt & Bertin et al., 1977; Scheggi et al., 2018). Last, similar to all neuropsychiatric disorders, MDD is characterized by sex differences in terms of prevalence, clinical presentation, and antidepressant response (Eid et al., 2019; LeGates et al., 2019). In preclinical animal models of depression, researchers also observed sex-specific effects in depressive-like behavior and neurochemical responses following stress or drug treatment (Dalla et al., 2010; Eltokhi et al., 2020). Thus, behavioral studies should include both sexes and, in female animals, behavioral experiments should be conducted across different...
stages of the estrous cycle to assess neuropsychiatric phenotypes (Eid et al., 2019; LeGates et al., 2019; Dalla et al., 2010; Eltokhi et al., 2020).

**Anxiety: Elevated-zero Maze**

Certain dimensions of anxiety such as uncontrollable and excessive worrying or rumination may be uniquely human experiences, but other signs of anxiety including increased heart rate, aberrant sleep, or cognitive difficulties can be recapitulated in rodent models (American Psychiatric Association, 2013). With regard to putative models of anxiety-related disorders, a variety of ethologically relevant (i.e., unconditioned) behavioral assays are used to study anxiety-related processes (Assary et al., 2018).

![Elevated-zero maze apparatus](image)

Figure 3: Elevated-zero maze apparatus

The focus is typically on studying approach-avoidance behaviors, baseline vigilance, or defensive behaviors. The elevated-zero maze (EZM), for example, is one standardized test of approach-avoidance behavior, and is a modification of the classic elevated-plus maze (EPM; Walf & Frye, 2007). The EZM lack the plus shape of the EPM, which eliminates ambiguous interpretation of results when the animal spends time in the central square of the EPM (Shepherd et al., 1994). The EZM is a conflict test, as rodents have a natural tendency to actively explore a new environment, yet they show aversion toward an elevated open runway. The rodent is given the choice of spending time in open maze arm or enclosed maze arm, elevated approximately one meter from the floor. Animal movements are recorded using a computerized video-tracking system for at least five minutes. In general, rodents exhibiting lower amounts (both of entries and percent time spent) of exploration of the open runways, shorter total distance traveled, fewer head-pokes over the side of the maze, longer latencies to move when initially placed on the maze, and more fecal pellets are thought to be more anxious. Certainly, however, some dependent measures are more useful than others (e.g., entries into open runways are more predictive of anxiety than defecation; Walf & Frye, 2007).

The EZM as a behavioral assay of anxiety-related behavior in rodents is used extensively as part of preclinical development and testing of psychotropic drugs for anxiety disorders in humans (Grillon et al., 2019). Translational models of anxiety play a central role in drug development (Borsini et al., 2002; Kulkarni et al., 2007) but the altered anxiety-like behavior does not necessarily indicate an anxiolytic or anxiogenic effect of the drug per se (Shepherd et al., 1994). Indeed, the interpretation of the EZM results may be ambiguous during profiling of pharmacological agents because prior experience such as handling of animals before testing, pre-exposure to a novel environment before testing, timing of testing, and circadian rhythms can influence behavior of rodents (Kafkafi et al., 2018). These confounds make behavioral phenotypes less reproducible and replicable, which, consequently, reduces the translational value of this assay in drug discovery (Grillon et al., 2019; Von Hohenberg et al., 2018; Kulkarni et al., 2007; Shepherd et al., 1994). It is important to note, however, that animal models in the field of behavioral pharmacology represent a great translational opportunity for target identification and validation, as well as the development of safe and effective therapeutics such as 5-HT4 receptor agonists for the treatment of depression with comorbid anxiety (Mendez-David et al., 2014; Murphy et al., 2020).

**Posttraumatic Stress Disorder: Marble Burying**

PTSD is unique among stress-related psychiatric illnesses in that exposure to a traumatic event (e.g., war, natural disaster, crime victimization, etc.) is a required component of the diagnosis (American Psychiatric Association, 2013). In rodent models, fear conditioning is commonly employed as an environmental stressor because of its etiological and ethological
validity (in this paradigm, rodents are trained to associate a tone with a mild electric foot shock) (Mahan & Ressler, 2012; Gafford & Ressler, 2011). Studies that examine the pathological consequences of environmental stress in rodents are instructive, but, in a truly valid model of the human condition, PTSD-related symptoms should emerge following traumatic stress in a genetically vulnerable population (Nievergelt et al., 2018; American Psychiatric Association, 2013; Mahan & Ressler, 2012; Gafford & Ressler, 2011). Furthermore, studies of rodent models of stress vulnerability have identified female-specific effects, and indicate that the hypothalamic-pituitary-adrenal (HPA) axis may be a substrate for sexual differences in the stress response (Cohen & Yehuda, 2011). These findings justify further examination of both sexes (Cohen & Yehuda, 2011; Ressler et al., 2011). Still, despite ample evidence that the prevalence of PTSD among females is roughly twice that of males, the number of studies that use female animal models is limited (Cohen & Yehuda, 2011).

PTSD is a complex phenotype and, in humans, is characterized by three symptom clusters: intrusive recollection of traumatic memories, hyperarousal and avoidance behavior (other symptoms include generalized anxiety and aberrant sleep patterns) (American Psychiatric Association, 2013). Rodent studies use a variety of tasks to assess the presence or absence of PTSD-like symptoms such as hyperarousal that is characterized by hypervigilance and exaggerated startle response (American Psychiatric Association, 2013). For example, marble burying is a rodent experimental paradigm commonly used to examine hyperarousal, one of the core symptoms of PTSD in a human population (American Psychiatric Association, 2013; Mikics et al., 2008). It is a clinically relevant behavioral assay of hypervigilance (rodents will bury objects in their home-cage bedding), and rodents exposed to traumatic stress bury more compared with naïve control animals (Mikics et al., 2008). This standardized paradigm, however, is not used only in models of PTSD, and is another example of cross-domain modeling of symptoms (Kedia & Chattarji, 2014). More specifically, it has been argued that this task may be more relevant to anxiety-like behavior, since anxiolytic drugs decrease marble burying activity (Nicolas et al., 2006). Additionally, this species-specific behavior is related to digging behavior and considered to be an indicator of repetitive digging. Crucially, repetitive burying is also used as a proxy for repetitive behaviors (Cohen & Yehuda, 2011), a behavioral phenotype relevant to both ASD and OCD (American Psychiatric Association, 2013; Thomas et al., 2009; de Brouwer et al., 2019).

**Recent Progress and Future Prospects**

One of the objectives of neuroscience is to identify the brain regions and neural circuits that underlie the pathogenesis of symptoms in individuals who are affected by psychiatric conditions (Van Den Heuvel et al., 2019). Standard neuroimaging techniques such as functional magnetic resonance imaging (fMRI) have been adopted by translational neuroscientists and are now frequently used in animal models as well (Mandino et al., 2019). Certainly, these methods are providing a window into the neurobiology of psychiatric illness such as addiction (Jupp & Dalley, 2014), ASD (Ellegood & Crawley, 2015), mood disorders (Jonckers et al., 2015), and SCZ (Kannan et al., 2013). Innovative technology is redefining modern brain research and is perhaps the single most powerful catalyst with the potential to greatly affect peoples’ lives. For example, one novel method for examining functional network connectivity in the brains of animals is optogenetic fMRI (ofMRI) (Lee et al., 2010). Traditional “optogenetic” tools, a groundbreaking invention in its own right (Beydon et al., 2005), enable light-based modulation of genetically defined neurons and temporally precise stimulation of neural circuit activity. On the other hand, standard fMRI has become a popular technique for visualizing functional connectivity patterns of the brain (Mandino et al., 2019). Thus, the ofMRI approaches will undoubtedly help illuminate the brain circuit mechanisms and neuroanatomical correlates that underlie cognitive, affective and
behavioral dysfunction that is observed across various psychiatric disorders.

Indeed, only as recently as 2018, researchers have generated data using ofMRI in a rat model of depression that suggest a causal link between lateral habenula downregulation and reduction in default-mode network connectivity (Von Hohenberg et al., 2018). As a mediator of negative motivational behavior, the lateral habenula is oppositional to the brain’s reward center, decreasing reward-driven behavior and plays a role in decision making. The default mode network (DMN) consists of interrelated brain regions that display increased activity during periods of resting wakefulness and inattentiveness, while displaying downregulation during conscious cognitive processing (Von Hohenberg et al., 2018). It was previously known that patients with depression display hyperactivation of the default mode network, disrupting cognitive processing (Brakowski et al., 2017; Von Hohenberg et al., 2018), and that the aberrant activity of the lateral habenula is associated with symptoms of depression (Yang et al., 2018). These recent findings, therefore, reinforced prior work showing brain activity and connectivity differences between patients with depression and healthy control participants. By using ofMRI, however, these new results extended earlier work to demonstrate causality (Lee et al., 2010) where there was previously only correlation.

Animal models also have translational relevance to behavioral epigenetics, and research in this field helps us understand how environmental conditions modify the expression of genes, primarily through biochemical modifications, without changing the DNA sequence (Cavalli & Heard, 2019). It is now widely recognized that epigenetic dysregulation is linked with neuropsychiatric illness (O’Donnell & Meaney, 2020; Smigielski et al., 2020; Kuehner et al., 2019), and rodent models are providing insights into a central role of epigenetic mechanisms in behavioral and cognitive abnormalities (Phillips & Roth, 2019; Bountra et al., 2011) that are evident in MDD (Sun et al., 2013), anxiety (Zovkic & Sweatt, 2013), and PTSD (Zovkic & Sweatt, 2013). As but a single example, the exposure to trauma such as neglect and abuse during the early childhood period has been associated with later development of MDD, anxiety, and PTSD (Copeland et al., 2018). Moreover, modeling the effects of early postnatal exposure to traumatic stressors in rodents [Schmidt et al., 2011; e.g., repeated/daily maternal separation, environmental deprivation, administration of high doses of synthetic glucocorticoids (e.g., cortisone or prednisone that help fight off inflammation; Doherty & Roth, 2016; Jawahar et al., 2015), chronic exposure to bacterial mimetics (e.g., lipopolysaccharide), revealed long-term, permanent increases in depressive-like and anxiety-related behaviors when offspring were tested as adults (Holmes et al., 2005; Babicola et al., 2021). Notably, these behavioral sequelae are, in part, driven by epigenetic mechanisms such as DNA methylation and histone posttranslational modifications (Jawahar et al., 2015; Doherty & Roth, 2016). Animal model research of epigenetic modifications as an underlying mechanism for aberrant behavior has its own limitations but is significant in our improved understanding of the etiology, prevention, and treatment of neuropsychiatric illness (O’Donnell & Meaney, 2020; Kular & Kular, 2018). Indeed, both positive/protective and negative/risk factors jointly influence and shape phenotypic outcomes such as stress vulnerability or resilience (Santarelli et al., 2017; Murthy & Gould, 2018), which may limit the translational value of early life adversity rodent paradigm in the field of epigenetics (Singh-Taylor et al., 2018). On the other hand, some epigenetic modifications such as DNA methylation changes are persistent and could be detected in individuals for years after they were exposed to trauma (Heijmans et al., 2008; Perroud et al., 2014; Walton et al., 2017). Notably, these epigenetic marks may potentially be valid biomarkers for prediction of mental illness, as well as treatment response, in patients suffering with neuropsychiatric disease including MDD (Lisoway et al., 2018), borderline personality disorder (Perroud et al., 2013), PTSD (Yehuda et al., 2013), SCZ (Melas et al., 2012), and ADHD (Adriani et al., 2018).
Conclusion

Animal models of human neuropsychiatric phenotypes will continue to be an important area of research, despite their inherent shortcomings (Baker et al., 2020; Ericsson et al., 2013; Lloyd et al., 2016; Rizzo & Crawley, 2017). Non-human animal models that use genetic, pharmacological, and/or ablation procedures will remain integral in the study of psychiatric illness. Physiological differences between species and non-standardized interpretation of test results is a limitation, but does not diminish the value of model system research. Continued development of new genetic models, the refinement of behavioral assays relevant to the symptoms, and improved data interpretation (Reardon, 2019) are critical to furthering our understanding and treatment of neurobehavioral disorders (Cryan & Mombérau, 2004; Soderlund & Lindskog, 2018). Innovative approaches in translational neuroscience such as automated continuous behavioral monitoring (Volkar & Gaburro, 2020; Bikovski et al., 2020; Schalla et al., 2020), neural imaging in awake animals (Zhang et al., 2019), and targeting of different epigenetic regulators (Kuehner et al., 2019) represent powerful tools for improving the behavioral assessment and providing insights into the neurocircuitry of behavioral regulation.

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