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Enhancing the Utility of Preclinical Research in Neuropsychiatry Drug Development

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Abstract

Most large pharmaceutical companies have downscaled or closed their clinical neuroscience research programs in response to the low clinical success rate for drugs that showed tremendous promise in animal experiments intended to model psychiatric pathophysiology. These failures have raised serious concerns about the role of preclinical research in the identification and evaluation of new pharmacotherapies for psychiatry. In the absence of a comprehensive understanding of the neurobiology of psychiatric disorders, the task of developing “animal models” seems elusive. The purpose of this review is to highlight emerging strategies to enhance the utility of preclinical research in the drug development process. We address this issue by reviewing how advances in neuroscience, coupled with new conceptual approaches, have recently revolutionized the way we can diagnose and treat common psychiatric conditions. We discuss the implications of these new tools for modeling psychiatric conditions in animals and advocate for the use of systematic reviews of preclinical work as a prerequisite for conducting psychiatric clinical trials. We believe that work in animals is essential for elucidating human psychopathology and that improving the predictive validity of animal models is necessary for developing more effective interventions for mental illness.

Keywords

Animal models; Predictive validity; Psychiatry; Systematic reviews; CRF

1 Introduction

The recent withdrawal of big pharmaceutical companies from clinical neuroscience research, the dwindling pipeline of innovative treatments, and the large number of clinical failures have raised serious concerns about the future of psychiatric research [1, 2]. These trends have emerged due to poor understanding of the underlying psychopathology of common psychiatric conditions and the use of a classification system that lumps together heterogeneous etiologies that may require different treatment modalities [3–5]. Additional factors include the lack of objective markers to diagnose and monitor treatment response and frequent clinical failure of pharmacological treatments that showed initial promise in animal models [1, 6–9]. The ability of animal models to predict clinical outcomes in humans is referred to as *predictive validity*, a term closely related to *construct validity*, which is the ability of the model to recapitulate key aspects of the pathology [10]. In contrast to the slow progress in the development of new psychiatric treatments, the field of neuroscience has seen a rapid expansion of novel tools and approaches that allow us to address some of the

above challenges in ways that were not feasible before. The primary goal of this chapter is to discuss how these advances can improve the predictive validity of animal models in psychiatry.

Subheading 2 of this review examines the main obstacles and challenges that are responsible for the slow progress in generating new treatments for common psychiatric and neurological conditions. In Subheading 3, we discuss how key technological and conceptual advances have helped to overcome these traditional obstacles. Subheading 4 uses the translational failure of CRFR1 antagonists in clinical trials as a case study to examine common pitfalls and lessons learned about improving predictive validity of animal work.

2 Challenges and Obstacles

Most of the commonly used pharmacological treatments for psychiatric conditions were discovered serendipitously [1, 7, 11], and many of the drugs that were developed using animal models have failed to show efficacy in clinical trials [1, 6, 9, 12–15]. A major obstacle in drug development is the complexity of the human brain, which is comprised of 160 billion neurons, each of which connects to roughly 10,000 other neurons, establishing an overwhelming grid of roughly 1000 trillion synaptic connections that are dynamically monitored and maintained by a host of non-neuronal cells [16, 17]. This complexity coupled with the inaccessible and delicate nature of the human brain are responsible for our rudimentary understanding of how the brain retains and process information and how it generates emotions [4, 17, 18]. Unlike some neurodegenerative diseases such as Alzheimer and Parkinsonism where pathognomonic abnormalities provide possible clues for the underlying pathology, the gross morphology of common psychiatric conditions appears normal [4, 7]. In addition, the underlying microscopic abnormalities of psychiatric conditions are caused by complex interactions between environmental factors and abnormal function of many genes [18], making the search for a biological underpinning daunting.

In the absence of biological markers and a poor understanding of the underlying pathology, the American Psychiatric Association and the World Health Organization developed two classification systems for mental illness known as the *Diagnostic and Statistical Manual of Mental Disorders (DSM)* and the International Classification of Diseases (ICD), respectively. The original goal of these classification systems was the development of a common language to facilitate diagnostic consistency from clinician to clinician [3]. It was assumed that better inter-rater reliability would group individuals with similar pathology and promote the identification of underlying causes [3]. To qualify for a specific diagnosis, a patient has to endorse a certain number of complaints from a list of possible symptoms. For example, to diagnose major depression, a patient needs to endorse the presence of at least five out of the nine possible symptoms lasting for at least 2 weeks. Depressed mood or anhedonia is required to be present among the five core symptoms, with changes in appetite, sleep, energy, and concentration, a sense of worthlessness, or suicidal thoughts accounting for the rest of the symptoms needed to meet the full criteria for major depression. Importantly, an increase or decrease in either appetite, sleep, or energy level can be used to make a diagnosis of major depression. Thus, two individuals that share no common symptoms can be diagnosed with major depression [3, 19].

The DSM/ICD manuals managed to achieve adequate inter-rater reliability [19–22], but in doing so created heterogeneous classification systems that have hindered the development of effective new treatments in three major ways. First, the underlying heterogeneity has made it more difficult to identify a unique underlying pathology. Second, it made it more challenging to develop effective treatments that work across different pathologies. Finally, the somewhat arbitrary and subjective nature of the DSM/ICD classification systems has made the development of suitable animal models an arguably impossible task [4, 7].

3 The Opportunity: New Tools and Approaches

The development of new tools and conceptual approaches in the field of neuroscience has revolutionized our ability to study how the brain functions and has helped identify objective and measurable biomarkers of common psychiatric conditions. These advances are briefly reviewed and are discussed in terms of their implications for improving the predictive validity of animal models in psychiatric research.

3.1 Imaging

One of the most clinically significant advances in imaging has been the development of positron emission technology (PET) ligands to quantify amyloid burden in Alzheimer's patients [23, 24]. This technique can distinguish between Alzheimer's disease and other forms of dementia and has demonstrated the accumulation of amyloid plaques years before individuals show any signs of cognitive decline. This observation provides an important opportunity for early diagnosis and interventions that were not previously available [23, 24]. Novel PET ligands have also been used to diagnose neuroinflammation in live subjects providing novel insights into the role that neuroinflammation plays in the development of schizophrenia [25] and depression [26]. Animal models have played a critical role in the development and the refinement of these PET ligands [27–31] and will continue to guide new advances in this area [32, 33]. For example, reduced levels of the glutamate transporter, GLT-1, are a central biomarker of addiction in several animal models [14, 34], and the development of a GLT-1 PET ligand will help to clarify the construct validity of this finding in human addiction.

Resting state functional magnetic resonance imaging (rsfMRI) is another example of an imaging technique that has shown a great potential to improve our ability to diagnose, treat, and study psychiatric conditions [5]. rsfMRI provides information about the strength of the connectivity between different brain regions. Furthermore, it can generate connectivity maps that are stable over time and can be used as objective biomarkers of psychopathology. For example, Drysdale et al. [5] have used rsfMRI to define four different types of connectivity maps, or biotypes, in a large cohort of depressed individuals. Each of the four biotypes clustered in a different quadrant of an *X* and *Y* axis system in which frontostrialthalamic connectivity correlated with anhedonia and psychomotor retardation was represented on the *X* axis and fronto-limbic connectivity which is highly predictive of anxiety was aligned across the *Y* axis [5]. These biotypes were stable overtime, unaffected by age or medication use, and were used to successfully diagnose depression in 82% of the cases of an independent cohort [5]. Eighty-two percent of individuals that were characterized as biotype

1 responded to a 5-week treatment with repeated transcranial magnetic stimulation (rTMS) compared to only 25% and 29% response rates for biotypes 2 and 4, respectively. Connectivity pattern was a better predictor of response to rTMS compared to clinical presentation prior to treatment. Finally, 60% of patients diagnosed with generalized anxiety disorder were also classified as biotype 4, despite the fact that they did not meet criteria for major depression. In contrast, only 10% of patients diagnosed with schizophrenia showed connectivity patterns consistent with any of the four biotypes [5]. These findings demonstrate that rsfMRI can be used to partition individuals diagnosed based on the DSM classification system into four stable biotypes that seem to respond differently to treatment. Some of these biotypes are not specific for depression, as they are commonly seen in individuals with anxiety but no depression. This work highlights the utility of rsfMRI to better diagnose and treat individuals with psychiatric conditions. Moreover, recent advances have allowed the use of rsfMRI to generate connectivity maps in rodents and nonhuman primates [35, 36], providing an important translational tool to examine the mechanism by which these connectivity maps are established and how they contribute to anhedonia and anxiety-like behavior. For example, work from our group has recently shown that adult mice exposed to early life stress have increased fronto-limbic connectivity that is highly correlated with anxiety-like behavior [37], allowing for more mechanistic understanding of parallel findings in humans [38–44].

3.2 Additional New Technological Advances

Optogenetics and pharmacogenetics are novel technologies that use light or specific ligands to turn “on” and “off” specific population of cells or axonal terminals in behaving animals [45, 46]. These tools allow researchers to examine the contribution that a specific cell population of interest makes to complex behaviors such as anxiety, anhedonia, feeding, and drug-seeking behavior [46]. Although most of the work has been done in rodents, recent research has extended this field to nonhuman primates [46, 47]. Elegant optogenetic work in mice has recently demonstrated that activation of the corticotrophin-releasing factor receptor 2 (CRFR2) in the lateral septum is necessary and sufficient for promoting anxiety and hypothalamic-pituitary axis (HPA) activation in response to restraints [48]. These findings challenged previous assertion that activation of CRFR1, but not CRFR2, is responsible for inducing anxiety, providing a possible explanation as to why CRFR1 antagonists have failed to reduce anxiety in clinical trials (see also Subheading 4).

The availability of rapid and relatively inexpensive RNA sequencing and proteomics platforms allow for unbiased characterization of gene and protein expression in ways that were not available before. These tools, coupled with advances in viral vectors and novel molecular tools for editing the genome, allow for rigorous characterization of the role that specific transcripts play in modifying network function and complex behaviors. A good example of the utility of these approaches is the recent discovery that transient reduction in the expression of the orthodenticle homeobox 2 (Otx2) transcription factor in the ventral tegmental area of mice exposed to early life stress is responsible for the increased sensitivity of these mice to additional stress in adulthood [49].

Genetic work has also identified numerous genes such as Shank3, TSC1/2, DISC1, NLNG3, and CNTNAP2 that are implicated in the development of schizophrenia and autism spectrum disorders [4, 50, 51]. Manipulations in the expression of these genes in mice elucidated the role that these genes play in synaptic development and complex behavior [50, 51]. Advances in epigenetics and stem cell technology have allowed somatic cells, such as fibroblasts, to be reprogrammed directly into inducible neurons [52] or pluripotent cells (iPS) that can then be differentiated, in vitro, into a variety of cell types including neurons and glial cells [53, 54]. This technology allows for detailed characterization of neurons derived from individuals diagnosed with schizophrenia [55, 56], bipolar disorder [56], Alzheimer's disease [57], and many other psychiatric conditions [54] in a dish. This approach circumvents the challenge of characterizing molecular changes in the brain of living humans and will likely improve the construct validity of animal models by allowing for a direct comparison of findings obtained with human iPS and animal models [58].

Advances in artificial intelligence and machine learning have generated machines with a capacity to learn, process information, perceive, and strategize that resemble and even surpass the human brain [17]. The basic premise of this approach is that “the best way to understand a complex system is to build it from the ground up” and it does so by combining multiple disciplines including cognitive neuroscience, computational modeling, and statistics [17]. A fascinating example is the recent work by Testolin et al. showing that an unsupervised hierarchical generative network that was initially trained to recognize simple natural images can be efficiently trained to recognize letters in a highly sophisticated and accurate way [59]. Such a network could potentially be used to model different types of dyslexia in humans, bypassing the difficulty of studying this issue in animals.

3.3 New Conceptual Categorization Systems of Mental Illness

Concerns about the utility of the DSM/ICD classification systems in uncovering underlying pathology and guiding the development of new treatments have inspired the NIMH to develop an alternative approach known as the Research Domain Criteria (RDoC) [3, 60]. The RDoC differs from the DSM/ICD approach in several important ways. For example, the RDoC system places brain circuit dysfunction, rather than groups of symptoms, as the organizing principle for defining pathology. One example is the role of the prefrontal cortex, hippocampus, and amygdala in regulating fear conditioning and extinction [61, 62]. Pathology is also defined using quantifiable and continuous variables that are related to a circuit output (e.g., fronto-limbic connectivity assessed by rsfMRI as a proxy of anxiety). The notion that psychopathology is better represented as a continuous scale is conceptually different than the discrete diagnostic categories used by the DSM/ICD system [3, 63]. A third approach, recently advocated by the current NIMH director, Dr. Josh Gordon, is the use of unsupervised and unbiased bottom-up large data approaches to characterizing pathology [64]. His argument is that the DSM and the RDoC approaches make certain assumptions about pathology that are guided by partial knowledge and biases that can be avoided by allowing the data to sort itself into distinct patterns that are related to pathology. The work by Drysdale et al. provides a good example of the utility of this unsupervised approach [5]. As discussed above, these authors obtained rsfMRI connectivity maps from a large number of individuals diagnosed with major depression and then used unsupervised clustering

system to divide this population into four biotypes [5]. Individuals that are characterized as biotype 4 may present with generalized anxiety or major depression suggesting that a single pathology may manifest itself with different symptoms yet respond similarly to treatment. This is consistent with the observations that depression and anxiety respond to similar treatments and that other common conditions, such as diabetes, can present differently (e.g., retinopathy, neuropathy, renal failure) despite having a common underlying pathology and response to treatment. Moreover, “biotype 4 depression” is likely to respond differently to treatment compared to the “depression” of biotype 1 patients [5]. These findings demonstrate how the RDoC and unsupervised clustering systems can be used to refine the DSM/ICD diagnoses system in a manner that improves clinical outcomes. Identifying similar biotypes in rodents and nonhuman primates will likely improve the predictive and construct validity of animal models for the treatment of anxiety and depression.

3.4 Rating the Strength of the Preclinical Evidence

“Best practice guidelines” are a set of recommendations that help clinicians choose among treatment options based on the strongest available data. These clinical guidelines are based on systematic reviews of the literature followed by analysis that rates the quality of the evidence based on formalized criteria [65]. This evidence-based approach has improved clinical outcomes by addressing issues such as the placebo effect, different forms of biases in scientific research, and underpowered studies [65]. In a landmark publication, Sandercock and Roberts argued that a similar approach is needed to evaluate the strength of the preclinical data and should be a prerequisite for conducting clinical trials in humans [66]. As an example, they pointed to systematic reviews of clinical trials involving close to 7000 stroke patients that found no evidence to support the clinical use of nimodipine in reducing neurological sequelae of acute focal stroke [67]. Although these clinical trials were inspired by animal work, a systematic review of the preclinical data found no convincing evidence that nimodipine improved clinical outcomes in animals [68]. These findings support the notion that systemic reviews can improve the predictive validity of animal models, avoid unnecessary testing in humans, and reduce the costs associated with unjustified clinical trials [68] (for a more skeptical view on this issue, see ref. 9). Over the past two decades, several tools have been developed to formalize the evaluation process of preclinical studies [69]. Moreover, a recent systemic review of animal studies was instrumental for designing clinical trials that confirmed the utility of hypothermia in the management of acute ischemic stroke [70].

We were able to find only one example in which systematic review was used to assess preclinical data of psychiatric research [71] but suspect that this approach will improve the predictive validity of animal work. Such reviews should not only list all available research in order to avoid publication bias but also evaluate the strength of the evidence as it relates to clinical outcomes using a clearly defined set of assumptions. For example, reduced anxiety-like behavior after acute administration of a new anxiolytic drug using normal animals should not have the same predictive validity compared to studies showing that the drug is able to fully reverse stable anxiety-like behavior seen in animals exposed to early life stress (ELS). This is because ELS leads to robust increase in anxiety-like behavior across diverse mammalian species including rodents, nonhuman primates, and humans [4, 72]. Similarly,

when assessing the anxiolytic potential of CRFR1 antagonists in adult humans, reduced anxiety in constitutive CRFR1 knockout mice should have less predictive value compared to mice in which the CRFR1 was eliminated in adulthood (for more detailed discussion on this issue, see Subheading 4.7). Similar to the case with nimodipine [68], we suspect that thoughtful and systematic evaluation of preclinical work will raise questions about the rationale for conducting many of the failed clinical trials in psychiatric research and predict that this approach will help improve clinical outcomes in psychiatric research.

3.5 Success Stories

There are a few examples where animal work has played an important role in clarifying human psychopathology and has led to the development of new psychiatric medications. One of the most compelling examples is the discovery that abnormal expression of the orexin receptor is responsible for narcolepsy in a canine model of the disease [73]. This work, and preclinical work by others [74], uncovered an important role for the orexin system in regulating sleep-awake cycle and has led to the development of suvorexant, an orexin receptor antagonist that is now available to treat insomnia [75]. Elegant work in nonhuman primates has identified the alpha2A receptor in pyramidal neurons of the prefrontal cortex as a critical target for regulating working memory and attention [76, 77]. These findings paved the way for clinical trials showing that long-acting guanfacine, an alpha2A agonist, is an effective non-stimulant alternative for the treatment of ADHD in children [78]. Furthermore, the administration of myelin oligodendrocyte glycoprotein (MOG) to animals as a model of multiple sclerosis has highlighted important contribution of B cells and autoimmune antibodies to the demyelination process. This discovery led to the development of monoclonal antibodies to CD20 that depleted a specific population of B cells [79–81] and the recent approval of Ocrevus, a CD20 monoclonal antibody, for the treatment of primary progressive multiple sclerosis [82, 83]. These “success stories” demonstrate that animal work can advance psychiatric treatment and raise the question as to why these examples are relatively rare [1, 2, 12]. We chose the CRFR1 antagonists as an example of the challenges associated with this type of translational work and discuss possible ways to address these issues in the following sections.

4 CRFR1 Antagonists as a Case Study for Improving Predictive Validity of Animal Models in Psychiatry

4.1 The Corticotrophin-Releasing Factor (CRF) System

The CRF system is a complex set of four ligands, two receptors, and one modifier protein that coordinate endocrine, autonomic, immunological, and behavioral responses to stress in mammals [84, 85]. The four neuropeptide ligands include CRF and three urocortins (UNC1, UNC2, UNC3). These peptides are expressed in different brain regions and bind with different affinities to two highly homologous G-protein-coupled receptors, CRFR1 and CRFR2 [84–86]. The distribution of these two receptors is somewhat different, with CRFR1 highly expressed in the anterior pituitary, hippocampus, cortex, and amygdala, while CRFR2 is expressed in dorsal raphe nucleus (DRN), lateral septum (LS), periaqueductal gray (PAG), and choroid plexus.

CRF levels increase in several brain regions including the paraventricular nucleus (PVN) of the hypothalamus, amygdala, bed nucleus of the stria terminalis (BNST), and LC in response to threat [84, 86, 87]. The release of CRF in these brain areas promotes and coordinates the immediate “fight-or-flight” response as well as long-term adaptations to chronic stress [86–88]. Until recently, the prevailing dogma in the field has been that activation of the CRFR1 promotes a fight-or-flight response that includes the activation of the hypothalamic-pituitary-adrenal (HPA) axis, reduced appetitive behaviors, and increased heart rate, arousal, and anxiety [84, 86, 87]. In contrast, activation of the CRFR2 was thought to be important for terminating the acute stress response and to restore homeostasis [84–86]. This model is supported by a large body of work showing that CRFR1 knockout mice have blunted HPA reactivity and reduced anxiety-like behavior [6, 85]. Similarly, CRFR1 agonists promote anxiety, while CRFR1 antagonists reduce fight-or-flight responses [84, 85]. CRFR2 knockout mice show exaggerated HPA reactivity in response to stress and are more anxious compared to wild-type littermates, suggesting an important role for terminating the stress response [84–86].

Secretion of CRF from cells located in the PVN activate CRF1-positive cells in the anterior pituitary causing the release of adrenocorticotrophic hormone (ACTH) into the blood circulation followed by the secretion of glucocorticoids (corticosterone in rodents and cortisol in humans) from the adrenal gland [85, 88]. Elevated levels of glucocorticoids in turn activate the glucocorticoid receptor in a variety of tissues to induce metabolic, cognitive, and inflammatory changes that help the animal cope with threat [88–91]. Prolonged exposure to glucocorticoids causes multiple metabolic, immunological, and behavioral abnormalities. Therefore, multiple mechanisms have evolved to ensure efficient termination of this response [88–91].

Another important hub of the CRF response to stress is the central nucleus of the amygdala (CeA) [87]. CRF-positive cells in the CeA are activated in response to multiple types of threats to stimulate a broad network of autonomic, cognitive, and behavioral responses that is independent of CRF activation of the HPA [87, 88, 92, 93]. Under low levels of stress, CRFR1 stimulation depresses glutamatergic transmission, but activation of CRFR1 enhances glutamatergic transmission in the CeA under high levels of stress [86]. These findings explain why blockade of CRFR1 in the CeA had no effect on basal anxiety levels but attenuated anxiety under stressful conditions [86].

Stress-reactive CRF-positive cells located at the BNST and the CeA innervate dopaminergic neurons in the nucleus accumbens (NAc) where they modulate appetitive behaviors such as social interaction and exploration of a novel object [87, 94]. Dopaminergic neurons in the NAc express both CRFR1 and CRFR2, and incubation of NAc slices with CRF increased the release of dopamine in a dose-dependent manner that requires the co-activation of CRFR1 and CRFR2. Intra-NAc administration of CRF promoted conditioned place preference and enhanced exploration of a novel object, while administration of nonselective CRF receptor antagonist into the NAc blocked novel object exploration. Together these findings reveal an important role for CRF in driving appetitive/rewarding behaviors. Interestingly, exposure to repeated swimming induced helpless behavior and blocked the natural tendency to explore a novel object. This stress-mediated anhedonia persisted 90 days after the initial exposure to

stress and caused abnormal dopaminergic response to CRF. For example, in animals that were exposed to repeated swimming, CRF was no longer able to induce dopamine release and triggered an aversive response in the conditioned place preference [94]. These findings show that CRF causes appetitive response in naive animals and aversive responses in animals exposed to severe stress. Similarly, rats that were exposed to early life stress show reduced sucrose consumption and low levels of social play later in life. These anhedonia-like behaviors were associated with abnormal activation of CRF-positive cells in CeA and were reversed by viral-mediated CRF knockdown in the CeA [93].

4.2 CRF Activation in Early Life Causes Long-Term Changes in Stress Reactivity, Cognition, and Appetitive Behaviors

The number of CRF-positive cells in the hippocampus reaches a developmental peak at around postnatal day 18 (P18) after which the number of these cells declines significantly to levels seen in adulthood [95]. Adult rats that were exposed to stress during the postnatal period have increased number of CRF-positive cells in the hippocampus [96]. Chronic elevation of CRF in the hippocampus of ELS animals has been shown to reduce spine density and to simplify dendritic arborization in the hippocampus. These structural abnormalities appear to be responsible for the poor hippocampal-dependent memory seen in adult animals that were exposed to ELS [97]. These assertions are supported by work showing that exposure of organotypic slices to CRF causes simplification of dendritic arborization and the retraction of postsynaptic spines, changes that resemble those seen in animals exposed to ELS [98]. Transient expression of CRF in forebrain neurons during the first 3 weeks of life, using the doxycycline Tet-Off system, causes increased anxiety- and depression-like behaviors in adulthood [99], and intracerebroventricular injection of CRF to newborn pups leads to cognitive deficits in adulthood [100]. In addition, administration of CRFR1 antagonist early in life reversed the dendritic abnormalities and the cognitive deficits seen in rats exposed to early stress [96] with similar findings reported in mice in which CRFR1 was knocked out in glutamatergic forebrain neurons [101, 102]. Importantly, administration of CRFR1 antagonists in adulthood appears to be less effective in reversing the cognitive deficits associated with early life stress [97]. Thus, CRF activation early in life leads to changes in synaptic connectivity, increased anxiety, and anhedonia that persist into adulthood [97].

4.3 Back to the Drawing Board: Lessons Learned from Clinical Failures

The observations that stress increases CRF levels in circuits that regulate anxiety and appetitive behaviors and that stress-related behavioral changes could be blocked by CRFR1 antagonists suggested a central role for CRFR1 activation in both the acute and long-term consequences of stress. This assertion was further supported by clinical studies showing increased CRF protein levels in the cerebrospinal fluid of depressed patients and individuals with severe PTSD [84, 86]. Increased CRF mRNA levels were also reported in the PVN and LC of postmortem tissue obtained from depressed patients and cortical areas of suicide victims [84]. Additionally, single nucleotide polymorphism (SNP) within the CRF gene and the CRF1 receptor was associated with increased risk for depression and PTSD [84, 88].

These findings inspired the development of several CRFR1 antagonists that were then tested in randomized control trials for the treatment of major depression, generalized anxiety, social anxiety, PTSD, irritable-bowel syndrome, and alcohol dependence. The results were consistently negative across all clinical trials [13, 84]. These disappointing outcomes raised the question as to why CRFR1 antagonists appeared so promising in preclinical studies but failed to show efficacy in clinical trials (for additional commentaries on this issue, see refs. 6, 13, 84). We address this question by highlighting common pitfalls associated with preclinical work and suggest experimental approaches to address these challenges.

4.4 Underutilization of Animal Models with Robust and Stable Anxiety- or Depression-Like Phenotypes

A closer look at the preclinical work indicates that the vast majority of the studies focused on the ability of acute administration of CRFR1 antagonist to modify anxiety- or depression-like behavior in normal male rodents or nonhuman primates [6]. This approach has little in common with clinical trials in which chronic administration of CRFR1 antagonist is used to reverse a stable and highly entrenched psychopathology such as anxiety, PTSD, depression, or substance abuse. Conspicuously missing from the preclinical work are studies examining the ability of chronic and systemic administration of CRFR1 antagonists to reverse stable and clinically relevant anxiety- or depression-like phenotypes in animals. For example, repeated exposure to forced swimming leads to long-term deficits in appetitive behaviors that are associated with abnormal dopaminergic response to CRF [94]. It would be important to know whether chronic administration of CRFR1 antagonist after exposure to repeated swimming can reverse the appetitive deficits (i.e., novel object exploration, helpless behavior, and reduction in CRF-mediated dopamine release). Similarly, exposure to ELS is a significant risk factor for the development of anxiety and anhedonia across a broad range of mammalian species, including rodents, nonhuman primates, and humans [72, 103]. As discussed above, ELS induces a stable anhedonic state in male rats that can be reversed by CRF knockdown in the CeA [93]. Therefore, it would be informative to know whether chronic treatment with CRFR1 antagonist could reverse the deficits in sucrose preference and social exploration seen in adult rats that were exposed to ELS. Note that systemic administration of CRFR1 antagonist may not recapitulate the behavioral outcomes seen with localized knockdown of CRF for several reasons. First, CRF activates both CRFR1 and CRFR2, and therefore blocking CRFR1 alone may not be sufficient to reverse the anhedonia. Moreover, deletion of CRFR1 in dopaminergic neurons increases anxiety, while deletion of CRFR1 in glutamatergic neurons reduces anxiety [104], suggesting that CRFR1 antagonists have opposing effects depending on the cell population they target. Similarly, CRFR1 activation in the amygdala increases anxiety, while CRFR1 activation in the globus pallidus is anxiolytic [86]. These findings highlight the complexity by which the CRF system modifies anxiety and depression and the challenges of using systemic and chronic CRFR1 blockade to reverse stable anxiety- and depression-like phenotypes in animals.

In summary, the overreliance on behavioral outcomes seen after acute administration in animals with normal levels of anxiety or anhedonia is an important reason for the “translational failure” of CRFR1 antagonists and many other pharmacological interventions. Similar shortcomings are seen in animal models of addiction in which the efficacy of new

compounds to block drug-seeking behavior is tested in “normal animals” [34, 105–107] and not in a subpopulation of animals that show compulsive drug use [105, 108]. We therefore advocate testing the efficacy of new compounds in animal models with robust and clinically relevant abnormalities and consider this type of work as stronger evidence when conducting systematic reviews of preclinical work.

4.5 The Need to Assess Outcomes Using Chronic Administration

As stated above, most of the animal work with CRFR1 antagonists tested the effects of acute administration of the drugs on stress reactivity. Consistent with the poor outcomes found in human clinical trials, the few examples in which chronic and systemic treatment with CRFR1 antagonists were used to reverse stable or semi-stable anxiety-like phenotype have not been particularly encouraging. For example, exposure to repeated social defeat leads to long-lasting changes in anxiety and appetitive behaviors that are reversed by chronic administration of antidepressants [109]. However, chronic and systemic administration of the CRFR1 antagonist, GSK876008, was not effective in reversing deficits in sucrose preference nor in reversing helpless behavior in the forced swim test [110]. Moreover, chronic treatment with the CRFR1 antagonist antalarmin failed to reverse most of the acute and chronic anxiety-like behaviors induced by 14-day social separation in male rhesus macaque monkeys [111]. In contrast, an acute oral dose of antalarmin was able to reduce HPA activation and anxiety-like behavior in male rhesus macaques exposed to social intruder stress [112]. The different outcomes seen following chronic versus acute administration suggest that chronic administration of CRFR1 antagonist may lead to compensatory changes that reduce the efficacy of this intervention. Assessing the ability of chronic blockade of CRFR1 to reverse stable anxiety-like phenotype in animals will likely improve the predictive validity of this approach in clinical trials. Additional work is also needed to test whether injecting CRFR1 antagonists immediately after a traumatic event, such as social defeat, can prevent the development of PTSD-like symptoms or anhedonia in animals. In other words, animal studies can help clarify whether CRFR1 antagonists are more effective in *preventing* stress-induced psychopathology versus *reversing* it.

4.6 Unanticipated Complexity

Another reason for the translational failure of CRFR1 antagonists is the oversimplified assumption that over-activation of CRFR1 in adulthood is critical for inducing mood and anxiety symptoms. This is not likely to be the case for several reasons. For example, systemic administration of CRFR1 antagonist inhibited light-mediated startle but slightly enhanced fear-mediated startle response [113]. These opposing outcomes are likely due to the different circuits by which light and shock induce startle [113]. Recent work has shown that CRFR2 stimulation also plays a role in increasing anxiety-like behavior and HPA activation [48], suggesting that blockade of both CRFR1 and CRFR2 might be necessary for anxiolytic effects. In addition, higher levels of CRFR2 are found in the amygdala of humans and nonhuman primates compared to rodents underscoring the importance of using nonhuman primates to study this issue [111]. Additional work is therefore needed to test whether nonselective CRF antagonists can reverse stable anxiety- and depression-like phenotypes in rodents and nonhuman primates.

4.7 Lack of Developmental Consideration

Much of the translational work has been based on the observation that CRFR1 knockout mice show a robust and reproducible reduction in anxiety [6]. It is unclear, however, whether deleting CRFR1 early in development and/or in adulthood is responsible for the anxiolytic phenotype. In fact, in several cases where this issue was examined [114, 115], it was found that deletion during development, and not in adulthood, is responsible for the anxiety-like phenotype. As discussed in Subheading 4.2, transient alterations in the levels of CRF early in the development causes long-term changes in cognition-, anxiety-, and depression-like behavior [99, 100], and blocking this response early in life with CRFR1 antagonists or CRFR1 deletion reverses the long-term effects of ELS on anxiety and cognition [96, 102]. Finally, CRFR1 antagonists used early in life appear to be more effective in reversing the cognitive deficits associated with ELS compared to interventions that block CRFR1 in adulthood [97]. These preclinical findings raise the possibility that CRFR1 antagonists might be more effective in treating childhood anxiety as opposed to adult anxiety.

5 Conclusions

Significant progress in neuroscience research has provided new tools to characterize and investigate the mammalian brain in ways that were not available two decades ago. These rapid changes coupled with new conceptual approaches to diagnose mental illness and the use of systematic reviews to evaluate the strength of the preclinical data should improve the predictive validity of animal work in psychiatric research.

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