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Increasing the validity of experimental models for depression

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Major depressive disorder (MDD) is a central nervous system disorder characterized by the culmination of profound disturbances in mood and affective regulation. Animal models serve as a powerful tool for investigating the neurobiological mechanisms underlying this disorder; however, little standardization exists across the wide range of available modeling approaches most often employed. This review will illustrate some of the most challenging obstacles faced by investigators attempting to associate depressive-like behaviors in rodents with symptoms expressed in MDD. Furthermore, a novel series of depressive-like criteria based on correlating behavioral endophenotypes, novel *in vivo* neurophysiological measurements, and molecular/cellular analyses within multiple brain are proposed as a potential solution to overcoming this barrier. Ultimately, linking the neurophysiological and cellular/biochemical actions that contribute to the expression of a defined MDD-like syndrome will dramatically extend the translational value of the most valid animal models of MDD.

Keywords: mouse; major depressive disorder; endophenotypes, models

Introduction

Major depressive disorder (MDD) is diagnosed when a culmination of both cognitive and/or behavioral affective symptoms emerge.[1,2] On average, a striking 4% of the U.S. population is affected by MDD each year, while another 17% are likely to experience symptoms of MDD in their lifetime.[3,4] Importantly, this disorder is associated with significant rates of morbidity and mortality, as well as rampant global expansion. Current estimates project that MDD will become the second leading cause of disability worldwide by 2030.[5]

MDD is a highly complex disease associated with a heterogeneous presentation of symptoms. Multiple distributed brain circuits are likely to underlie the vast array of behavioral endophenotypes characteristic of the disorder, and alterations across both cortical and subcortical brain areas have been observed in MDD.[6,7] The fundamental challenge of all research initiatives in biological psychiatry is identifying neural determinants of abnormal emotional events. Likewise, pinpointing predispositions across a population of individuals at the genomic level that confer responses to environmental conditions and inevitably determine patterns of activity across larger-scale brain networks responsible for emotional and cognitive processing is urgently needed. Clinical studies, however, are quite limited in their ability to fully elucidate the functional role of neural mechanisms in psychiatric diseases when considering the numerous ethical issues often times associated with probing such questions. This common clinical obstacle continues because of the high prevalence of comorbid or developmental influences associated with any given psychiatric disorder, and the current limitations inherent to each of the primary noninvasive modalities used to study human brain function with a sufficient degree of spatial and temporal resolution (i.e., functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and positron emission tomography[8]). Thus, investigators have sought to overcome these issues

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by utilizing animal models of MDD in order to test critical hypotheses concerning the function of brain mechanisms.

Even still, no criteria are currently available to make a confident assessment of the face, construct, and/or predictive validity achieved in animal models, even when using the wide range of behavioral approaches available to measure depressive-like behaviors. Here, we discuss many of the challenges associated with examining MDD in rodent models, and further propose that a criteria be established in future validations of these models of MDD, such that they begin to take into account patterns of neural circuitry along with the concomitant expression of behavioral events. We will focus our discussion on mice, since they offer the most complex range of affective-like behaviors, in combination with the largest range of genetic and molecular tools to study the functional role of particular neural mechanisms in a cell-type specific manner. By establishing detailed criteria based on the orchestration of both neural circuit activity and the expression of distinct behaviors, we hope to build upon current recommendations, such as that recently proposed by Nestler *et al.*[9]

What makes a good model good?

The goal of disease models is to recapitulate salient aspects of a disorder in a manner that allows for hypothesis testing. Certain types of validity for any particular model can judged along at least three primary domains: *face validity*, *predictive validity*, and *construct validity*. Face validity refers to the extent to which an animal model recapitulates important anatomical, biochemical, neurophysiological, or behavioral features of a human disease.[9,10] Unfortunately, achieving face validity in animal models of neuropsychiatric disorders is a daunting task. First, little is clinically known about the anatomical, biochemical, and neurophysiological features of these disorders. Second, the behavioral manifestations observed in these disorders often occur across multiple behavioral domains (including homeostatic regulation, arousal systems, and cognitive function). Since dysfunction across these behavioral domains is often observed outside of the context of neuropsychiatric illnesses, isolated dysfunction across any single domain is not sufficient to meet criteria for a given disorder. As such, a putative animal model of depression with isolated dysfunction across a single domain (i.e., insomnia) should probably not be considered as having a high degree of face validity, particularly when regarding all of the numerous and potential symptoms that may, or may not, be present in clinical depression. At the very least, conclusions from measurements of a single behavioral event, such as appetite suppression in rodents, should be considered lightly, and not to be discussed beyond the implication that patients diagnosed with isolated decreases in appetite also have major depressive disorder. On the other hand, if a rodent model of a neuropsychiatric illness reasonably encompasses face validity by capturing the same aspects of several operationally defined measures similar to what patients exhibit (i.e., animals mimicking a behavioral syndrome characteristic of the psychiatric disorder), the experimental model may still, nonetheless, be lacking the level of construct validity that would be required for asserting a reliable interpretation of behavioral results. In essence, construct validity is obtained by integrating the most relevant and observable features associated with the onset and progression of a psychiatric illness into the original design of an experimental model. For instance, it remains unclear how well the behavioral responses on the forced swim test, tail suspension test, or sucrose preference test truly reflect a lack of motivation or anhedonia, two frequently reported symptoms of depression.[11] Predictive validity refers to the extent to which various models respond to treatments in a way that corresponds with the effects produced by those same treatments in human. While models with predictive validity have been successfully utilized in many preclinical trials to screen novel therapeutics, these models do not necessarily provide any insight in the neurobiological underpinnings of neuropsychiatric disorders.

These weaknesses in validity apply to nearly all currently established behavioral models for psychiatric disorders. Most behavioral responses measured within animal models are merely presumed to be driven by the same biological mechanisms underlying any given psychiatric disorder. This is of major consequence, since the lack of validity in experimental models has dramatically hampered attempts to gain a comprehensive understanding of the neural mechanisms controlling psychiatric disorders. For example, the administration of cocaine at a range of doses routinely increases motor behavior and alters impulse control in rodents

(i.e., an effect also observed across most mammalian species).[12,13] These behavioral manifestations closely resemble many salient clinical manifestations observed in mania. Likewise, an acute administration of phencyclidine (i.e., PCP) generates a behavioral response also shared by a variety of animal species that closely resembles a behavior prominently expressed in schizophrenia.[14] Still, it remains a challenge to determine experimentally the neural underpinnings of these psychiatric disorders even when given an impressive strength in face validity.[15] The mouse treated with cocaine displays face validity with regards to bipolar disorder, while significantly lacking any construct validity.

Overcoming deficiencies in the validity of animal models is long overdue, as evidenced by an absence of having identified definitive neurobiological mechanisms underlying most of the complex and vastly heterogeneous symptoms attributed to psychiatric disorders. Here, a novel perspective is proposed that offers a unique and great potential for understanding individual differences through the functioning of multiple neural circuits within an individual *in vivo* (animal or human) that correspond temporally with behavioral responses. In addition, postmortem analyses of specific patterns of molecular and cellular events can be used to confirm potentially novel therapeutic targets spanning broad neural circuits; allowing for treatments that are tailored to an individual's symptomatology. Ultimately, the establishment and introduction of new experimental models with high levels of predictive, construct, and face validity will dramatically increase the potential to unveil the neurobiological mechanisms of exceedingly complex brain disorders.

Seeking construct validity in murine models of MDD

The diagnosis of MDD is useful in that it categorizes and groups together symptoms that co-occur across human populations (see Fig. 1); however, a shared diagnosis of MDD does not necessarily equate to common neurobiological dysfunction between two individuals.[16] This is further complicated by the fact that the current definition of MDD, as is designated in the DSM-IV-TR,[2] fully allows for two individuals that are diagnosed with MDD to not share even one overlapping symptom (see Fig. 2). This single issue illustrates perhaps the most challenging obstacle for researchers to overcome when defining MDD across the human population,[16] let alone in rodents. Along similar lines, MDD is based on domains of behavioral dysfunctions that are in most cases expressed solely in humans, making it impossible for a mouse to actually have MDD (by definition). Measurements of mood, guilt, or suicidal thinking in animals is simply not feasible, whereas quantifiable assessments of such constructs can be derived from self-report questionnaires by humans.

Major Depressive Disorder

Diagnostic Criteria
Poor mood
Decreased interest or pleasure in activities (anhedonia)
Feelings of worthlessness or excessive guilt
Insomnia or hypersomnia
Decreased concentration or indecisiveness
Changes in appetite or significant weight change
Fatigue or loss of energy
Psychomotor retardation or agitation
Recurrent thoughts of death or suicidal behavior

Figure 1. **Criteria for major depressive disorder.**

Researchers have sought to overcome these challenges by using at least three general approaches within animal models of depression. The first approach is based on designing and testing models based on their ability to mimic the behavioral effects of current antidepressants.[17] For instance, mice that are administered a potentially novel antidepressant would be subsequently examined for depressive-like behaviors already established as being sensitive to conventional antidepressant treatments (i.e., tail suspension task, forced swim task, to be described subsequently) like that of the selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), and serotonin-norepinephrine reuptake inhibitors (SNRIs). There are, however, several

	Patient 1	Patient 2
Symptoms	Poormood	Anhedonia
	Hypersomnia	Insomnia
	Feelings of worthlessness	Decreased concentration
	Psychomotor retardation	Significant weight loss
	Fatigue	Suicidal thoughts

Figure 2. **Examples of nonoverlapping symptoms in two different patients with MDD.**

major drawbacks to this approach. First, studies that fall into this line typically characterize behavioral changes in rodents following the acute administration of a potential antidepressant. In reality, conventional antidepressants demonstrate no clinical benefits when administered acutely; it actually takes weeks of their administration before any remission of symptoms occurs in patients suffering with MDD. Secondly, even after their chronic administration, these agents only ameliorate depressive symptoms in 25–40% of patients with MDD.[18] As such, the argument could be raised that a more appropriate model of "antidepressant-like" behavioral effects produced by these compounds should be minor, or even altogether lacking. Along this same critical line, conventional antidepressants (in particular, SSRIs) exhibit clear clinical benefits in other neighboring psychiatric disorders, including posttraumatic stress disorder, obsessive–compulsive disorder, and generalized anxiety disorder. Thus, the MDD-like behaviors that are modulated by antidepressants may actually be better linked to the anxiolytic, antiobsessive, or antihypervigilant-like effects of certain agents across the aforementioned spectrum of human neuropsychiatric disorders. Together, these limitations likely underlie the poor utility of this approach in investigating the neurophysiological basis of MDD.

Third, a recent molecular approach makes empirical determinations about the action of specific genes/proteins on behavioral responses. Exploring the role of specific gene targets holds a great deal of promise in models of MDD, albeit these studies are almost exclusively used in mice. Transgenic mouse lines enable the targeted overexpression or conditional knockdown of specific candidate genes in MDD including tryptophan hydoxylase-2, and brain-derived neurotrophic factor.[8,19] Undoubtedly, this approach will contribute significantly toward identifying novel targets to pharmacologically treat clinical depression. In the technical sense, these mice exhibit genetic construct validity with MDD; however, there are significant limitations associated with this approach. MDD is a heterogeneous disorder and many different candidate genes (and gene combinations) are likely to underlie the illness.[20] Furthermore, there is a clear environmental contribution to MDD,[21,22] and gene alterations alone are not sufficient to cause MDD in the majority of individuals.[23] As a result, specific genetic mutations are only likely to be present in a fraction of human populations with MDD, and various genetic mouse models of MDD are unlikely to recapitulate the full range of brain and behavioral alterations observed in the human disorder.

To date, the role of no single protein has been causally attributed to depression in humans. Therefore, creative approaches to modeling one of the most significant triggers of clinical depression in an animal model have focused on exposing normal rodents to different forms of chronic stress. Humans are a unique mammalian species being capable of sustaining a distressed emotional response over long periods of time.[24] This allows for the consequences of chronic stress to ensue as a result of allostatic load.[25] While the validity of any animal model should always be carefully considered, there are rodent models like those described below that demonstrate etiologic validity, along with important neurobiological changes, some of which are correspondingly present in postmortem tissue derived from depressed humans.[9]

Exposure of rodents to chronic stressors, including chronic mild stress,[26,27] or repeated social stress, can modify the activity, transcriptional state, and morphological profile of neurons across cortical and limbic brain areas.[28–34] Future work needs to elucidate the mechanisms through which initial social conflicts eventually come to result in maladaptive outcomes. Common features of several types of chronic social stressors, ranging from prolonged maternal separation during infancy to persistent social subordination as adults, is a loss over control of the threatening stimulus, and not being able to predict their later occurrence.[35,36] In fact, controllability and predictability are known as being highly important characteristics for determining the outcome of stress as these characteristics are significant risk factors for engendering pathological effects, as identified early on using rats that were exposed to inescapable electric shock.[37–40] Likewise, social isolation and anticipation about potential confrontations serve as considerably uncontrollable threats with significant emotional value.

In short, experimental conditions of chronic social stress in most animals are followed by an ethologically relevant behavioral "syndrome" that mimics several of the cardinal features of clinical depression, including anhedonia, social avoidance, a reduced ability to cope with environmental

challenges, and anxiety-like behaviors.[28] Mice, for example, that experience chronic bouts of social defeat stress develop these aforementioned behaviors, which are reversed by chronic, but not acute, treatments with standard antidepressants, including imipramine and fluoxetine.[41]

Measuring face validity in murine models of MDD

Several behavioral tests have been created and used to quantify the face validity of various models of MDD. Among the most widely used tasks for modeling MDD-like behavior in mice are the forced swim (FST) and the tail suspension tests (TST).[42] These tests are fundamentally identical, and based on the observation that antidepressant treatment increases the extent to which an animal attempts to escape an environmental stressor (a water tank in the FST, and hanging by their tail in the TST). Since the time an animal spends trying to escape the environmental stressor increases with antidepressant treatment, these tests have been interpreted as measurements of "learned hopelessness."[43,44] Unfortunately, both of these widely used tests and the interpretation of the observations made using these tests as models of MDD have considerable limitations. First, acute treatment with antidepressants increases the time animals spend trying to escape the task. However, as previously discussed, acute antidepressant treatment exhibits little clinical efficacy in patients with depression. Second, genetic mutations that enhance locomotor behavior also have the potential to decrease immobility time during the FST and TST tests.[45] Investigators typically attempt to control for this confound by demonstrating that various genetic models that exhibit deficits on the FST or TST tasks do not display altered behavioral profiles in the open field. The major problem with this approach is that this strategy does not account for the central observation that antidepressants themselves increase motor activity in mice.[46] As such, the effect of acute antidepressant treatment observed during the FST and TST (the primary basis after which the task was modeled) may be partially due to the psychomotor activating properties of the agents and not a specific decrease in hopelessness. Perhaps the biggest criticism that can be raised regarding these tasks is that they are typically run under conditions in which it is impossible for mice to escape the environmental stressor. Thus, the argument could be raised that it is significantly more adaptive (and evolutionarily advantageous) for mice subjected to the TST or FST to simply float or hang quietly and conserve their metabolic reserve, given that their efforts to escape will never translate to an improved behavioral outcome. This possibility becomes even more important to consider in cases where animals are subjected to the FST or TST over multiple occasions. In these instances, animals may learn that exposures to the inescapable environmental stressors are time limited (further increasing the relative benefit of conserving their metabolic reserve). Finally, and most importantly, hopelessness is not part of the diagnostic criteria for MDD in humans. The foot shock escape (FSE) task is another behavioral test that has been proposed as a model of learned hopelessness in mice.[47] Unlike the FST and TST, the foot shock escape task is run under conditions in which an animal can actually escape an environmental stressor.[48] Nevertheless, the foot shock escape task still shares many of the same criticisms levied toward the FST and TST. Similar to these tasks, results derived from this escape task are interpreted as a model of learned hopelessness based on the acute effect of the antidepressant agent. In light of these issues, immobility time on the FST, TST, and FSE tasks should be regarded as tentative when being used as behavioral indices of MDD-like behavior in rodents until further neurobiological justification is provided.

The open-field task is a standard test used to quantify locomotor behavior in rodents. While this test is also widely used to quantify behavioral correlates of psychomotor activation (or retardation) in murine models of MDD, it is critical to note that many behavioral variables, including hunger, novelty, and fear modulate the drive of animals to explore a novel environment.[49,50] Given that several of these behavioral indices are altered in MDD, we propose the following recommendations for measuring psychomotor profiles in murine models of MDD. First, mice should be tested while they are in their home cage, and the locomotor profiles of mice should be quantified across the entire dark cycle (since mice are more likely to explore during this period). Finally, locomotor profiles should be normalized to the total time each mouse spends awake during the dark cycle in order to avoid potential confounds that could be introduced due to changes in sleep–wake patterns that may be present in MDD mouse models. Anhedonia, defined as an

inability to experience pleasure, is a hallmark endophenotype of MDD. Common behavioral tests used to quantify anhedonia in murine models of MDD are the sucrose preference test and intracranial self-stimulations (ICSS).[51–53] The sucrose preference test is based on the idea that murine models of MDD will derive less pleasure from sucrose, and will thus choose to consume less over a given time period compared with normal mice. The test is normally run under conditions of free access to sucrose and water, and anhedonia is quantified based on the ratio of sucrose to water consumption over that time. While this test may indeed serve as an accurate behavioral measurement of anhedonia, several important factors must be considered prior to interpreting behavioral differences in murine models of MDD as a loss of pleasure. First, sucrose carries caloric value. Thus, decreased sucrose consumption during periods of free access may reflect a decrease in the overall caloric requirement in given mouse models of MDD. This is an especially important confound that must be controlled since changes in food consumption (i.e., appetite) are also hallmark features of MDD. Second, novel genetic manipulations that induce MDD-like behaviors in mice may have the potential to alter taste signaling (this concern is largely limited to transgenic mouse models of MDD). Thus, the decreased sucrose preference observed in these models may actually reflect their inability to register sweetness, rather than anhedonia.

ICSS serves as a promising and perhaps underutilized approach to characterize brain reward systems in murine models of drug taking behavior and MDD.[54–56] Several ICSS approaches, including electrical stimulation of basal forebrain[57] and optogenetic stimulation of VTA,[58] have been shown to drive/reinforce motivated behaviors. Since these approaches ultimately allow for direct quantification of the influence of the brain reward systems on behavior, ICSS could readily be used to determine if the brain reward systems lose their ability to drive motivated behavior (i.e., a correlate of anhedonia) in various murine models of MDD. Unfortunately, these ICSS-based approaches are technically challenging and not necessarily suitable for high-throughput behavioral characterization. Sexual behaviors have also been used to quantify anhedonia.[59,60] While this behavior has historically represented an ethologically viable approach in rats, it may also have utility in quantifying anhedonic-like behavior in genetically modified mice.[61]

Multiple classic behavioral tasks, including the t-maze working memory task, the Morris water maze, the radial arm maze, and the delayed 5-hole nose poke (delayed-5NP) task can be used to quantify cognitive function (i.e., working memory/attention) in mice.[42,62] Nevertheless, there are several challenges to using these tasks to quantify cognitive deficits in murine models of MDD. The main limitation is that each of these tasks requires that mice perform motivated behaviors. For instance, the behavioral output used to measure cognitive function in the Morris water maze task is motivated by the desire of mice to escape a tank filled with water, and food rewards are typically used to motivate behavioral output in the radial arm maze, the t-maze, and the delayed-5NP tasks. Thus, alterations in behavioral indices that reflect cognitive function in normal mice may actually reflect alterations in hedonic processing (or other affective processes) in murine models of MDD.

Profound changes in sleep are observed across patients with MDD;[2] nevertheless, circadian disruption remains a widely understudied domain for testing the face validity of various mouse models of MDD. Multiple standard methods exist for quantifying sleep architecture in mice, including methods that can be used to monitor changes across long intervals.[63,64] Mice housed on a 12-hour light–dark cycle typically sleep 60% of the light cycle and 40% of the dark cycle.[65] Since insomnia in MDD is typically marked by decreased sleep during normal periods of sleep, and hypersomnia in MDD is characterized by increased sleep during normal periods of wakefulness, we propose that mouse models of MDD should be evaluated using a similar standard.

Anxiety is widely utilized in mice as a behavioral correlate of MDD-like behavior. While anxiety is often comorbid with MDD in humans,[66,67] anxiety is not a central feature of the disorder.[2] Anxiety is typically modeled in mice using tasks such as the elevated plus maze and exploratory behavior in an open field to quantify the effect of anxiogenic stimuli (i.e., a well-lit area of a chamber, or the open arm of an elevated maze) on exploratory behavior.[42] However, a major confound arises when using these tasks to quantify anxiety-like behaviors in murine models of MDD. Given that MDD alters motivated behaviors (as noted previously), behavioral changes

that are interpreted as increased anxiety may simply reflect changes across nonfear-related brain systems that modulate exploratory behaviors in mice. For these reasons, we recommend using caution when interpreting anxiety-like behaviors as correlates of MDD-related actions. Interestingly, anxiety-like behaviors often precede the onset of depressive-like behaviors, particularly in response to compulsive drug taking, or exposure to intense stress.[68] In rodents, ultrasonic vocalizations are easily detected, quantifiable, and interpreted to reflect a range of emotional states, including anxiety-like behaviors that occur in response to distressful conditions like those just mentioned.[69,70]

Multiple biochemical and molecular alterations, including changes in cortical gene expression[71] and levels of CNS serotonin function,[72–74] have been described in patients with MDD. Recent studies have demonstrated that several putative mouse models of MDD exhibit these biochemical or molecular alterations as well,[8,71] suggesting that biochemical and molecular alterations observed in MDD can be used as a measure of face validity in various models. Along these same lines, functional alterations across cortical and limbic brain areas[75] and changes in functional connectivity across cortically dependent emotional and cognitive networks have been described in patients with MDD.[76] Given the recent development of techniques that allow brain activity to be acquired concurrently across entire brain networks in freely behaving mice,[77] it is likely that the neurophysiological alterations observed in MDD[78] can be used to define novel neurophysiological measures of face validity in mouse models of MDD.

Establishing criteria for murine models of MDD

The criteria for MDD define a collection of behavioral alterations that tend to co-occur in humans. While MDD is certainly heterogeneous in nature, the co-occurrence of these behavioral alterations across heterogeneous human populations suggests that discrete biological changes likely lie upstream of the collection of behavioral manifestations observed in the disorder. Based on this principle, we propose that MDD in mice should be modeled based on the upstream biological changes that confer risk for MDD or the manifestation of a distinct collection of behavioral alterations (rather than individual behavioral alterations alone).

Nine clear behavioral criteria have been established for MDD in humans; however, one-third of them (i.e., poor mood, guilt, and suicidal thinking) cannot be assessed in mice. Thus, we will define a novel behavioral syndrome in mice that reflects many of the criteria for MDD in humans (i.e., exhibits face validity with MDD). Here, we will coin this disorder mouse affective syndrome (MAS, see Fig. 3). MAS will be defined based on three domains. The first domain will consist of MDD criteria that

Domain	Criteria	Behavioral Measurement
Reward Related	Anhedonia	Intracranial self-stimulation (ICSS) Sucrose preference test (if no changes in food consumption)
	Decreased Concentration	Delayed 5 hole nose poke test (requires intact reward system)
Homeostatic Factors	Psychomotor Retardation or Agitation	Total dark-cycle locomotion (Increased or decreased)
	Insomnia or Hypersomnia	Increased sleep during dark cycle Decreased sleep during light cycle
	Appetite or weight change	Total food consumption change or weight change (+/- 5%)
Biomarkers	Biochemical and Molecular Markers	Changes in cortical gene expression (for example)
	Neurophysiological Markers	Enhanced cortico-limbic network synchrony (for example)

Figure 3. **Criteria for mouse affective syndrome.**

cannot be adequately quantified in mice in the absence of motivated behavior (i.e., anhedonia, working memory/attention). We have grouped these two criteria into the same domain since behavioral correlates of diminished working memory/attention in mouse models of MDD may actually reflect deficits in hedonic processing (as described previously). The second domain will consist of MDD criteria that can be quantified in mice in the absence of motivated behavior (sleep, appetite changes, and psychomotor retardation/activation). The third domain will consist of biological or neurophysiological biomarkers identified in humans subjects with MDD.[16,78] Several examples of putative biomarkers are listed in Figure 3; however, this list is certainly not exhaustive. Behavioral dysfunction across three areas within the first two domains (at least one in each domain), or two areas within the first two domains (one in each domain), and one area within the third domain, will be sufficient to meet the criteria for MAS. Thus, based on our proposal, MAS will be defined as dysfunction across three domains that exhibit face validity with MDD, and any given model may be described as having MAS irrespective of the construct validity of that model. Second, we propose that mouse models that exhibit subthreshold changes in MAS behaviors should be referred to as models of the behavioral domains they exhibit. For example, mice that exhibit anhedonia but do not meet the criteria for MAS should be described as a "mouse model of anhedonia." We raise this suggestion since anhedonia (for example) is not only observed in MDD, but across other neuropsychiatric disorders such as schizophrenia, and isolated anhedonia is not sufficient to meet the criteria for MDD in patients.

Mice engineered to express genetic mutations identified in human MDD populations (i.e., construct validity) should be described within the context of a "genetic mouse model of MDD risk." Notably, these models need not exhibit any behaviors consistent with MAS since they are models of disease risk and not MDD itself. If such mice also meet criteria for MAS, they should be described as a "genetic mouse model of MDD." Conversely, mice designed (genetically, pharmacologically, or otherwise) to model salient biological mechanisms thought to contribute to MDD in humans should be described as mouse models of that feature. For example, we propose that mice genetically modified to exhibit hyposerotonergia should be described as a "genetic mouse model of hyposerotonergia," unless they are more appropriately described using one of the previously mentioned categories.

Conclusion

Here, we propose several criteria for advancing the validity of mouse models of MDD. Overall, these criteria will allow for defining novel syndromes in mice that more effectively mirror components of MDD in humans. This syndrome is based on measured changes across behavioral, neurophysiological, and postmortem analyses of molecular and cellular domains that are correspondingly dysfunctional in MDD. Some behaviors, particularly those classically utilized to model MDD in mice (i.e., the TST, FST, and FSE tasks), are not included in the behavioral correlates proposed here; however, further neurobiological justification on the utility of these tasks for modeling MDD symptoms may warrant their inclusion as valid behavioral correlates of MAS in the future. In the end effect, the application of more stringent forms of clarification and *in vivo* assessments for components of depressive, or depressive-like, symptoms will facilitate the translation of experimental findings across basic science and clinical research boundaries, and allow for the development of better diagnostic and treatment strategies.

Conflicts of interest

The authors declare no conflicts of interest.

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