

Chapter 2

Experimental Psychiatric Illness and Drug Abuse Models: From Human to Animal, an Overview

Scott Edwards and George F. Koob

Abstract

Preclinical animal models have supported much of the recent rapid expansion of neuroscience research and have facilitated critical discoveries that undoubtedly benefit patients suffering from psychiatric disorders. This overview serves as an introduction for the following chapters describing both in vivo and in vitro preclinical models of psychiatric disease components and briefly describes models related to drug dependence and affective disorders. Although there are no perfect animal models of any psychiatric disorder, models do exist for many elements of each disease state or stage. In many cases, the development of certain models is essentially restricted to the human clinical laboratory domain for the purpose of maximizing validity, whereas the use of in vitro models may best represent an adjunctive, well-controlled means to model specific signaling mechanisms associated with psychiatric disease states. The data generated by preclinical models are only as valid as the model itself, and the development and refinement of animal models for human psychiatric disorders continues to be an important challenge. Collaborative relationships between basic neuroscience and clinical modeling could greatly benefit the development of new and better models, in addition to facilitating medications development.

Key words: Animal model, Anxiety, Depression, Drug addiction, Preclinical model, Psychiatric disorders, Stress

1. Introduction

The past two decades have seen monumental growth in the neurosciences, with a particular focus on the investigation of cellular and molecular correlates of psychiatric disorders. From these studies, various neuroadaptations in cortical and subcortical circuitry have been proposed to mediate the transition to specific disease states (e.g., refs. 1, 2). These advances in basic neuroscience research have relied heavily on the development and refinement of animal

models, and a critical review of past and current methods will support future endeavors toward the ultimate goal of providing better therapeutics for the clinical setting. This overview serves as an introduction for the following chapters describing both in vivo and in vitro preclinical models of psychiatric disorders. The appropriate modeling of such disease states is particularly challenging given that, despite significant progress, our fundamental understanding of the brain is still rudimentary compared with other systems. One upside to this dilemma is that investigators are able to concomitantly discover new mechanisms of brain function while striving to serve the unmet health needs of society. An important point to always remember is that the refinement of animal models for psychiatric diseases is a never-ending process for neuroscientists. Particularly when working with animals, the generation of positive data should never by itself justify the accuracy or validity of the methods employed. Although models are not necessarily intended to be perfect, the robustness and utility of data generated by them can only be as good as the model itself. Thus, models are almost never described as ultimate or complete, and investigators should continuously question and refine existing models for the benefit of future scientists and patients. In a similar vein, investigators should never be unduly anchored to existing models if a more valid model can be conceptualized.

2. Conceptual Framework for Animal Models of Psychiatric Disorders

Animal models for a complete syndrome of a psychiatric disorder are highly unlikely to be attainable either conceptually or practically. Thus, although there are no perfect animal models of any psychiatric disorder, models do exist for many elements of each disease state or stage. As such, an animal model can be viewed as an experimental preparation or set of reproducible methods developed to study a given phenomenon found in humans. Certain areas of the human condition are obviously a challenge to replicate in animal studies (e.g., comorbidity, polysubstance dependence, child abuse). Moreover, from a practical standpoint, psychiatric disorders are necessarily based on a nosology that is both complex and continually evolving and most certainly involves multiple subtypes, diverse etiology, and constellations of many different disorders. Thus, an approach to the development of animal models that has gained widespread acceptance is that animal models are most likely to have construct validity when the model mimics only the specific signs or symptoms associated with the psychopathological condition (3).

It is, therefore, essential to understand exactly what conditions the animal model is intending to describe. Yet, the confounding

influences of psychiatric comorbidity have at their basis overlapping and interconnected neuroanatomical and neurochemical systems. This is no better illustrated than when the treatment (or modeling) of one symptom produces an undesirable (yet familiar) side effect. This dilemma often forces us to choose between strictly limiting the number of variables under investigation (good science) and accurately modeling what may be a constellation of symptoms (good modeling). Moreover, many psychiatric diseases can be conceptualized as progressive timelines consisting of transitory stages of disease, each with unique criteria, necessitating multiple levels of depth and breadth to describe the composite phenomenon. For example, drug dependence can be described as a transition from recreational to excessive drug use, with accompanying psychiatric symptoms that may in turn exacerbate dependence. In addition to perturbing brain reward chemistry directly, repeated illicit drug exposure results in the recruitment of conditioning factors that may also support dependence with reexposure to these same environmental factors during protracted abstinence. Moreover, factors related to age (4–7), gender (8,9), and even circadian rhythms (10,11) are critical components of practically all psychiatric disease states. Nonetheless, the focus of animal models on a given component or stage of the disordered process eliminates a fundamental problem associated with basic models of human psychopathology, namely, the frustration of attempting to completely validate the entire syndrome. When definitive data related to a specific domain of the disorder can be generated, the confidence of cross-species validity is increased substantially. This framework also leads to a more pragmatic and reproducible approach to the study of the neurobiological mechanisms of the behavior in question.

3. Construct and Other Validities Associated with Animal Models

The most relevant conceptualization of validity for animal models of psychiatric disorders is the concept of *construct validity* (12). Construct validity refers to the interpretability, “meaningfulness,” or explanatory power of each animal model and thereby incorporates most other measures of validity in which multiple measures or dimensions are associated with conditions known to affect the construct (13). A procedure has construct validity if there are statistical or deterministic propositions that relate constructs to observables derived from the procedure (14). An alternative conceptualization of construct validity is the requirement that models meet the concept of functional equivalence, defined as “assessing how controlling variables influence outcome in the model and the target disorder” (15). The most straightforward process for testing

functional equivalence has been argued to be through common experimental manipulations, which should have similar effects in both the animal model and the target disorder (15). This process is very similar to the broad use of the term *predictive validity* (see below). By comparison, *face validity* often represents the starting point in the development of animal models in which animal syndromes are produced that resemble those found in humans (16). *Reliability* refers to the stability and consistency with which the variable of interest can be measured both within and between laboratories. Reliability is achieved when, following objective repeated measurement of the variable, small within- and between-subject variability is observed, and the phenomenon is readily reproduced under reasonably similar environmental circumstances (for review, see ref. 17). *Predictive validity* in the more narrow sense refers to the model's ability to accurately predict the human phenomenon based on the response of the model system. Predictive validity is used most often in animal models of psychiatric disorders to refer to the ability of the model to identify pharmacological agents with potential therapeutic benefits in humans (16, 18). Alternatively, others have argued that this type of predictive validity can be considered more explicitly as "pharmacological isomorphism," which is the use of clinically relevant standards as positive controls to validate a model or set of procedures (19, 20). However, when predictive validity is more broadly expanded to explore the underlying physiological mechanisms of action related to psychiatric disorders, others have argued that it can incorporate other types of validity (e.g., etiological, convergent or concurrent, discriminant) considered to be important for animal models and thereby recenters around the concept of construct validity (21).

However, it is critical to note that the particular behavior being used for an animal model may not even necessarily be symptomatic of the disorder, but nonetheless must be defined objectively and observed reliably. Indeed, the behavioral output being observed may be seen in both pathological and nonpathological states but still have predictive validity. A good example of such a case would be the widespread use of positive reinforcement or reward as an animal model of addiction. Drug reinforcement does not necessarily lead to addiction (e.g., the widespread social drinking of alcohol), but the self-administration of alcohol has major predictive validity for the binge/intoxication stage of addiction (described in the next section and in ref. 22), and it would appear impossible to model addiction without an initial positive reinforcement stage.

The next sections of this chapter provide an overview of current models of drug dependence and affective disorders. Hopefully, it becomes clear that many of these models overlap to a great extent in terms of utility because many psychiatric disorders share common neural substrates.

4. Current Models of Psychiatric Illness and Drug Addiction

4.1. Overview of Current Models of Drug Addiction

Drug addiction has been conceptualized as a disorder that progresses from impulsivity to compulsivity in a connected cycle comprising three stages that correspond to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (23, 24): first, preoccupation/anticipation, second, binge/intoxication, and third, withdrawal/negative affect. In the past, much of the focus of animal and in vitro studies have been on the anatomical and synaptic sites and signaling mechanisms in the central nervous system on which drugs of abuse act initially to produce their positive reinforcing effects. More recently, new animal models of the motivational effects of dependence with more face validity for the human condition have been developed and employed. Motivation can be defined here as a “rough label for the relatively persisting states that make an animal initiate and maintain actions leading to particular outcomes or goals” (25).

Thus, animal models of addiction on specific drugs, such as psychostimulants, opioids, alcohol, nicotine, and Δ^9 -tetrahydrocannabinol (THC), can be categorized by models relevant to different stages of the addiction cycle (26). Animal models for the binge/intoxication stage of addiction can be considered as measuring acute drug reward/reinforcement, in which reward can be defined as a positive reinforcer with some added emotional value, such as pleasure, and positive reinforcement is represented by any event that increases the probability of an operant response. Animal models of positive reward and reinforcement are extensive and well-validated and include intravenous or intracranial drug self-administration, place conditioning, and states of reduced brain reward thresholds (27). Brain reward thresholds are measured by intracranial self-stimulation (ICSS) methodology, in which animals press a lever to obtain electrical stimulation of the medial forebrain bundle (28). Animal models of the withdrawal/negative affect stage include conditioned place aversion (vs. preference) to either spontaneous or precipitated withdrawal from chronic drug exposure, states of increased brain reward thresholds, and dependence-induced increases in drug-seeking behavior during withdrawal. Rodents increase intravenous or oral self-administration with extended access to the drugs and during withdrawal from the dependent state, measured both by an increased amount of drug administration and working exponentially harder to obtain the drug. Such increased self-administration in dependent animals has been observed with cocaine, methamphetamine, nicotine, heroin, and alcohol (29–34). Finally, the use of yoked-administration models allows for the investigation of neuroadaptations associated with reinforcement-related vs. noncontingent drug exposure. For example, Edwards et al. found that individual preferred levels of cocaine self-administration in rats positively correlated

with the phosphorylation state of cyclic adenosine monophosphate response element binding protein (CREB) in the central nucleus of the amygdala, although this relationship was not observed in rats receiving passive cocaine infusions at identical levels and patterns as their self-administering partners (35).

Animal models of craving, representing the preoccupation/anticipation stage of drug addiction, involve reinstatement of drug seeking behavior following extinction training and subsequent exposure to the drugs themselves, cues/contexts paired with drug self-administration, or stressors (36, 37). The latency to reinstate responding, or the amount of responding on the previously extinguished lever, is hypothesized to reflect the motivation for drug-seeking behavior. Stress-induced reinstatement most often involves the application of acute stressors that reinstate drug seeking, such as footshock, although more natural stressors should be utilized (38). In rats with a history of dependence, protracted abstinence can be defined as a period after acute physical withdrawal has disappeared and is often accompanied by elevations in drug intake or drug-seeking behavior (e.g., 2–8 weeks post withdrawal from chronic drug self-administration). Protracted abstinence has also been linked to increased brain reward thresholds and increases in sensitivity to anxiety-like behavior that have been shown to persist after acute withdrawal symptoms have subsided in animals with a history of dependence. Stress-induced reinstatement of drug-seeking and stress-induced reinstatement of anxiety-like states during protracted abstinence represent models of the persistent preoccupation/anticipation (craving) stage of the addiction cycle.

Most of the animal models discussed above have predictive validity for some components of the addiction cycle (compulsive use, withdrawal, or craving) and are highly reliable. Consistency and stability of the measures, small within-subject and between-subject variability, and reproducibility of the phenomenon are characteristic of most of the measures employed in animal models of dependence, with the possible exception of conditioned place preference (39). For the positive reinforcing effects of drugs, drug self-administration, ICSS, and conditioned place preference have been shown to have predictive validity. Animal models of withdrawal can be focused on either motivational constructs of withdrawal or physical or somatic signs. Animal models of conditioned drug effects (e.g., reinstatement, place preference) are successful in predicting the potential for conditioned drug effects in humans. Predictive validity is more problematic for such concepts as craving largely because of the inadequate formulation of the concept of craving in humans (see below, and refs. 13, 40, 41). Clearly, much remains to be explored about the face validity and predictive validity of unconditioned positive and negative motivational states and particularly the conditioned positive and negative motivational states associated with drug use and withdrawal. However, the responsibility

for filling the gaps in knowledge may lie more in the human clinical laboratory setting than in the animal models domain.

4.2. Overview of Animal Models of Depression

Depression is a major health care problem and a significant economic burden to society. Animal models of depression with good predictive validity but limited construct validity include the forced swim test, tail suspension test, learned helplessness models, olfactory bulbectomy, and maternal deprivation (42). However, there has been a shift away from traditional animal models of depression to more focused “endophenotype-like” approaches. This section describes animal models that have face validity and in some cases a degree of reliability and construct validity for a key element underlying depression: reward deficits.

Reward and motivational deficits represent critical underlying elements of a number of psychiatric disorders, ranging from drug addiction to major depression. Animal models of such deficits are well-established and include brain stimulation reward thresholds and preference for a sweet solution, among many others. The use of these two models is described here in the context of a depression model, and evidence for face and construct validity is described. Similar to drug dependence, major depressive disorder (or unipolar depression) is a chronic relapsing disorder that has a significant genetic component (43, 44) and a long-hypothesized contribution from stress-related mechanisms (45). Major depressive periods show a kindling-like effect, in which episodes worsen over time if left untreated. Suicide is a very possible outcome of affective disorders, with 60–70% of severely depressed individuals having suicidal ideations and 10–15% ultimately attempting suicide (46). The present section describes animal models that have face validity and, in certain cases, a degree of reliability and construct validity for measuring reward deficits associated with depression. For brevity, we only review the chronic mild stress model as an animal model of depression induction.

Chronic and unpredictable mild stress produces a number of behavioral and physiological abnormalities that have face validity for the various symptoms of depression (47). These include decreased sexual and exploratory behavior, sleep abnormalities, immune and hypothalamic-pituitary-adrenal axis dysregulation, and hedonic deficits measured by sucrose consumption, place conditioning, and brain stimulation reward. In the chronic unpredictable stress model, rats are exposed to various moderate environmental perturbations consisting of several weeks of random stressors, such as food and water deprivation, circadian cycle disruption, cage tilt, soiled cage, temperature fluctuations, stroboscopic lighting, exposure to an empty bottle following water deprivation, and the presence of foreign objects in the cage (48, 49). However, it is essential to employ a regimen of stressors that limits the potential confound of stress-induced weight loss (50). The chronicity

and unpredictability of these challenges can produce depression-like motivational symptoms measured by the techniques described below.

4.2.1. Brain Stimulation Reward

As mentioned earlier in the chapter, brain stimulation reward (or intracranial self-stimulation) is a procedure in which animals perform an operant response to receive highly rewarding electrical stimulation to their medial forebrain bundle. Brain stimulation reward has many advantages over assaying the pursuit and consumption of natural rewards. It directly activates brain reward systems, bypassing much of the periphery of hedonic circuitry. Additionally, no confounding motivational deficit states (e.g., food or water deprivation) are required for the procedure. Responses are also controllable, with very small increments in reward value systematically changing behavior. Using brain stimulation reward, Jean-Luc Moreau and colleagues have consistently shown reliable hedonic deficits following chronic mild stress by measuring threshold changes in the ventral tegmental area that are reversed by chronic administration of a number of antidepressant treatments (47).

4.2.2. Sucrose Consumption

Sucrose is a highly rewarding sweet substance in rodents, and rats show a concentration-dependent increase in both consumption and preference for sucrose that forms an inverted U-shaped function (51). Reduced preference for a sucrose solution in rats has been hypothesized to reflect a decreased sensitivity to reward homologous with anhedonia (52). Sucrose consumption/preference is normally monitored by tracking, over repeated test sessions, the decrease in the consumption of or preference for a palatable, low-concentration (1–2%) sucrose solution in the home cage. Chronic sequential exposure to mild unpredictable stress has been found to decrease the consumption of palatable sweet solutions, and in some cases preference for palatable sweet solutions (48, 49, 53, 54). Additionally, the chronic mild stress-induced decrease in palatable solution intake has been replicated with social stress (55), novelty stress (56), and forced swim stress (49). Perhaps more impressive have been numerous studies showing that the decrease in consumption or preference was reversible by chronic but not acute antidepressant treatment (48, 49, 53, 57–60).

Clearly, there are significant differences among the sensitivity, reliability, and construct validity of these two measures of motivational deficits. Brain stimulation reward provides a reliable and sensitive measure of a reward deficit in drug withdrawal, consistent with reward deficiencies described in the human condition. It also has been validated via manipulation of both reward and performance variables (61). Neuropharmacological validation of brain stimulation reward has shown that agents that decrease thresholds increase reward in humans (e.g., drugs of abuse), and agents that increase thresholds generally produce dysphoric responses in

humans, lending some predictive, and thus construct validity to this measure. However, sucrose consumption or preference measures, although used extensively, may have much less reliability and validity. The majority of data in fact largely indicate that humans with major depressive episodes report a magnified “craving” for sweets, in contrast to rats exposed to chronic mild stress. However, sucrose consumption following chronic mild stress does have predictive validity for antidepressant treatment, which is presumably one rationale for its popularity. The use of sucrose consumption/preference has increased asymptotically in the literature. Another reason for the extensive use of sucrose consumption/preference presumably corresponds to the ease of measurement compared with brain stimulation reward, which requires specialized operant equipment.

4.3. Overview of Current Models of Anxiety Disorders

Anxiety is a common emotion and represents an integrated response to the trials and tribulations of life. Anxiety is adaptive when mild, but may be incapacitating when present at extreme and intractable levels. Anxiety appears in several clinically recognizable forms. Patients who suffer from persistent, diffuse psychological feelings of dread, unremitting nervousness, tension, and worry accompanied by motor tension, vigilance, and autonomic hyperactivity in the absence of obvious external stressors are distinguished from patients who are relatively symptom-free until an acute precipitated anxiety or panic attack occurs. Panic attacks are accompanied by subjective feelings of terror, apprehension, and fear of dying. Somatic symptoms occur across multiple physiological systems and include dyspnea, sweating, faintness, and trembling. The signs and symptoms of panic disorder are similar to those occurring during a life-threatening situation or during intense physical exercise. Further diagnostic distinctions are made among patients with anxiety caused by post-traumatic stress disorder (PTSD), obsessive-compulsive disorder, and phobic disorders (23). Anxiety can also be a prominent component of other psychiatric disorders, including schizophrenia, affective illness, and substance abuse. Clinical anxiety research has rapidly evolved with the delineation of several subtypes of specific anxiety states. Unknown is whether these subtypes and their distinctive signs and symptoms reflect a unitary phenomenon or are independent syndromes with separate neurobiological substrates. Several animal models of general anxiety disorder have been developed, including the operant conflict test (62) and social interaction test (63). Unfortunately, there are few, if any, animal models sufficiently validated to discriminate among the various subtypes of anxiety disorders. A future challenge will be to develop animal models that reflect specific aspects of these clinical anxiety syndromes. What follows is a description of two examples of currently used animal models of anxiety-like states: the elevated plus maze and defensive burying task.

4.3.1. *Elevated Plus Maze*

This ethologically based exploratory model of anxiety measures how animals, typically rats and mice, respond to a novel approach-avoidance situation by measuring the relative investigation of two distinct environments: a lit, exposed runway and a dark, walled runway intersected to form a plus sign. Both runways are elevated high off the floor. No motivational constraints are necessary, and the animal is free to remain in the darkened arm or venture onto the open and exposed arms. This type of approach-avoidance situation is a classic animal model of “emotionality” (64) and is very sensitive to treatments that produce disinhibition (such as sedative/hypnotic drugs) and stress (65). Moreover, the simplicity of the elevated plus maze provides a high degree of utility in measuring emotional reactivity to experimental treatments. Accordingly, the elevated plus maze has been the subject of several hundred studies of rodent emotionality since the description and validation of the modern testing protocol in 1984 and 1985 (66, 67). Experimental treatments, such as γ -aminobutyric acid (GABA) inverse agonists, which reduce open-arm exploration, are identified as anxiogenic-like in the elevated plus maze, whereas drugs, such as GABA agonists (which increase open-arm exploration), are anxiolytic-like (68). Other dedicated reviews (69, 70) offer critical examination of the validity of the elevated plus maze as a model of anxiety. A similar test, termed defensive withdrawal, consists of an illuminated open field with a small, enclosed, and darkened chamber situated near one corner of the field and shows comparable validity to the elevated plus maze (71).

4.3.2. *Defensive Burying*

Rodents have a natural defense reaction (sometimes termed “active coping”) to unfamiliar and potentially dangerous objects by spraying material over the object, leading to total coverage of the threatening object. The best-known procedure to measure this behavior employs a metal prod protruding into the animal’s cage from which, at first contact, a mild electric shock is delivered (72). The total time spent burying the prod with bedding material, the total number of burying acts, and the height of bedding material deposited over the prod serve as validated measures of emotionality in this test (73, 74). In an environment without bedding material, in which the active burying option is not possible, subjects adopt a passive strategy by exhibiting immobility in locations away from the probe (75). The anxiety disorder most effectively modeled in terms of face validity by the defensive burying task may be a specific phobia (formerly, simple phobia), the essential feature of which is a marked and persistent fear of clearly discernible, circumscribed objects or situations (23). The lack of extinction of burying behaviors with repeated exposure to the inducing stimuli has provided some comparison of this animal model of anxiety to obsessive-compulsive disorders, the symptomatology for which includes repetitive acts aimed at preventing or reducing distress (76).

Regarding face and construct validity, the majority of animal models of anxiety have been developed to identify anxiolytic drugs and to reject nonanxiolytic drugs. The two standard models reviewed here appear to have good predictive validity for drugs that are effective in the treatment of generalized anxiety disorder. Each of the models has its strengths and weaknesses that need to be recognized, and use of multiple models always provides a convergent validation of research findings. One major difference between animal models of anxiety and the clinical disorders is that most patients requiring drug treatment for anxiety are presenting with high trait anxiety, whereas all animal tests are based on conditions that presumably reflect transient changes in state anxiety. The tests described above measure adaptive responses to a test situation, not a pathological state. However, as long as these tests are predictive of various aspects of the pathological state, then they have some validity as animal models (17). Clearly, the use of specific genetic strains and molecular genetic manipulations allows for the exploration of state vs. trait similarities or differences. Finally, individual differences most likely play a very large role in terms of vulnerability versus resilience to anxiety-related disorders (77, 78) and therefore represents a much-needed dimension of animal modeling.

5. The Case for Preclinical Human Models

In many cases, the development of certain models is practically restricted to the human clinical laboratory domain for maximizing both internal and external validity, and a good case of this is the phenomenon of substance craving. Craving can be conceptualized as an intersection of exteroceptive or interoceptive cues with an individual's motivational impetus to seek and take a substance. Human laboratory models are well-suited for studying craving mechanisms, given the immediacy of effects obtained under well-controlled conditions (79) as well as allowing for subjective reporting. This approach also represents a cost-effective and efficient way to facilitate the understanding of psychological mechanisms underlying human behavior and to identify and develop new treatment options (80). By comparison, animal models, particularly the extinction-reinstatement model, may be best suited to study the neuronal mechanisms involved in drug-seeking behavior (37). Although subjective reports of craving are impossible to obtain in animals, the reinstatement model has appreciable face validity for relapse behavior (15). Unfortunately, as others have pointed out (81), whether the model has predictive validity is currently unknown because few clinical or preclinical human trials have tested effective drugs or conditions employed in the model. Thus, collaborative relationships between basic neuroscience and preclinical human

modeling could be useful in facilitating medications development, as well as in developing new and better models. This conceptualization has been termed the “Rosetta Stone” approach and represents a bridge between predictive and construct validity toward the mutual benefit of both approaches (82). For example, numerous animal studies show that stress both increases previously extinguished drug-seeking behavior in response to alcohol-associated cues and potentiates the effects of other environmental cues previously associated with alcohol self-administration (32). Although comparative studies in humans appear inconsistent, they also allow for the separation of specific negative emotional stimuli into those that precipitate craving from those that do not. Marlatt and Gordon (83) suggest that negative experiences with which the subject has an intimate history (such as social pressures or job loss) are more likely to be associated with relapse to drinking. Mason et al. (84) found that aversive but personally irrelevant cues (such as images of a threatening snake) were ineffective at inducing craving, whereas preferred beverage-related cues were very effective. As discussed above, such distinctions can be engineered back into preclinical models as refinements, in which more relevant and specific social stressors (e.g., maternal separation) can be incorporated into animal models of stress-induced reinstatement and neuroplasticity (38).

6. In Vitro Methods to Facilitate the Modeling and Treatment of Psychiatric Disorders

While lacking in some obvious aspects of validity versus whole-animal models, in vitro models may best represent an adjunctive, well-controlled means to model specific neuroplastic mechanisms associated with psychiatric disorders. One example of this utility is represented by studies of the transcription factor Δ FosB by Eric Nestler and colleagues. Δ FosB accumulates in a brain region-specific manner in response to several types of chronic neural stimulation, a phenomenon hypothesized to be attributable to the unusual stability of the protein. The persistence of Δ FosB engenders the transcription factor with long-lasting effects on gene expression well after the termination of the original stimulus (e.g., chronic stress or illicit drug exposure), particularly considering the broad amplifying effects of transcriptional regulation on physiological systems. Thus, the unique characteristics of Δ FosB have established this protein as a candidate “molecular switch” mediating the transition to drug dependence (85). After a series of studies examining the precise nature of Δ FosB’s stability in vitro (e.g., (86–88)), the group was able to engineer viral constructs that overexpressed phosphorylation site-specific mutant forms of Δ FosB in whole animals and demonstrated a disruption of the effects of chronic cocaine in rats given this intervention (89). These studies benefited from a

strategic combination of *in vitro* (including both implemental reagent development and investigatory aims) and *in vivo* methodologies.

Another pioneering use of *in vitro* work in the field of drug abuse has been the continuing search for ethanol's binding sites, which has produced a list of defined criteria for ethanol targets and several leading receptor candidates (90). Identification of this site would not only satisfy a long-standing pharmacological curiosity, but may also provide a direct therapeutic target for alcoholism. A related problem is how endogenous opioids and exogenous opiates/opioids differentially regulate desensitization, endocytosis, and subsequent resensitization of the μ -opioid receptor (a long-established target). Jennifer Whistler and colleagues determined that the endogenous ligands at μ receptors mediate a normal recycling of receptors *in vitro*, presumably corresponding to a dynamic and adaptive signal for the organism's benefit. In contrast, morphine stimulation of μ receptors leads to either a protracted desensitization of receptors or other intracellular changes that most likely contribute to the pronounced *in vivo* tolerance and dependence that occur with chronic morphine exposure (91). These results could have a dramatic impact on drug development strategies, which have mostly relied on adjusting ligand efficacy or the duration of action in an attempt to design better analgesic agents with reduced potential for dependence. As an alternative, Berger and Whistler (92) proposed that drug candidates (or even drug cocktails) can be screened for their ability to regulate endocytosis, similar to endogenous ligands. In terms of medications development, a more comprehensive elucidation of the mechanisms of action of current treatments for dependence should facilitate the discovery of therapeutic drugs with even greater efficacy. For example, the anticraving drug acamprosate is commonly hypothesized to function by dampening hyperglutamatergic states associated with alcohol dependence and withdrawal. However, given the vast array of neuronal targets for modulating excitatory neurotransmission (e.g., AMPA/NMDA receptor channels vs. metabotropic glutamate receptors vs. presynaptic vesicle release mechanisms), a better understanding of acamprosate targets is needed (93). Such investigations, aided by *in vitro* methodology, could also shed light on the neurobiological mechanisms underlying relapse to other drugs of abuse. A comprehensive understanding of drug tolerance and dependence (as well as therapeutic drug mechanisms) will most likely require the coalescence of multiple knowledge bases at various levels of analysis, from molecular to behavioral.

Finally, the complexity and validity of cell culture studies can be enhanced by the use of primary culture systems (94). When maintained under tightly regulated culture conditions, neurons extend axons and dendrites and form physiologically and functionally active synaptic contacts (95). Cocultures composed of neurons from two distinct neuronal populations can further aid in the

characterization of simple circuits. Gao and Wolf (96) used cocultures containing rat ventral tegmental area (VTA, dopamine-synthesizing neurons) and excitatory prefrontal cortex (PFC) neurons to investigate mechanisms of dopamine–glutamate interactions within this isolated PFC–VTA circuit, a neuronal pathway hypothesized to drive synaptic transmission relevant to stress sensitization and drug-seeking behavior in vivo (97–99).

7. Conclusion

The refinement of basic models of psychiatric illness will continue to be a challenging endeavor and will be greatly facilitated by both horizontal (across disease models) and vertical (in vitro, in vivo, and human model) integration. Specific techniques associated with preclinical models and the investigation of changes in the central nervous system that are associated with these models are subjects described in the chapters that follow. Altogether, these tools will undoubtedly continue to provide valuable insights into the etiology of psychopathologies associated with drug addiction and other psychiatric disorders.

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