Published in final edited form as:

Methods Mol Biol. 2012 ; 829: 3–30. doi:10.1007/978-1-61779-458-2_1.

New Frontiers in Animal Research of Psychiatric Illness

Arie Kaffman and John H. Krystal

Abstract

Alterations in neurodevelopment are thought to modify risk of numerous psychiatric disorders, including schizophrenia, autism, ADHD, mood and anxiety disorders, and substance abuse. However, little is known about the cellular and molecular changes that guide these neurodevelopmental changes and how they contribute to mental illness. In this review, we suggest that elucidating this process in humans requires the use of model organisms. Furthermore, we advocate that such translational work should focus on the role that genes and/or environmental factors play in the development of circuits that regulate specific physiological and behavioral outcomes in adulthood. This emphasis on *circuit development*, as a fundamental unit for understanding behavior, is distinct from current approaches of modeling psychiatric illnesses in animals in two important ways. First, it proposes to replace the *diagnostic and statistical manual of mental disorders* (DSM) diagnostic system with measurable endophenotypes as the basis for modeling human psychopathology in animals. We argue that a major difficulty in establishing valid animal models lies in their reliance on the DSM/*International Classification of Diseases* conceptual framework, and suggest that the Research Domain Criteria project, recently proposed by the NIMH, provides a more suitable system to model human psychopathology in animals. Second, this proposal emphasizes the developmental origin of many (though clearly not all) psychiatric illnesses, an issue that is often glossed over in current animal models of mental illness. We suggest that animal models are essential to elucidate the mechanisms by which neurodevelopmental changes program complex behavior in adulthood. A better understanding of this issue, in animals, is the key for defining human psychopathology, and the development of earlier and more effective interventions for mental illness.

Keywords

Animal models; Mental illness; Neurodevelopment; Endophenotype; RDoC

1. Introduction

Many previous reviews have discussed the difficulties involved in constructing animal models of mental illnesses, such as schizophrenia, depression, and autism (1–4). Most reviews have used a hierarchical list of criteria introduced by Paul Willner (1) to assess validity of animal models of depression, including face validity, predictive validity, and construct validity. Face validity reflects the ability of the model to recapitulate specific anatomical, biochemical, or behavioral features of the human condition (e.g., large ventricles in an animal model of schizophrenia (5)). Predictive validity describes the model's ability to mimic clinical response in humans (e.g., response to chronic but not acute course of antidepressant medication (6)) while construct validity reflects the ability of the model to recapitulate at least some aspects of the underlying human pathophysiology (e.g., transgenic mice models of Rett syndrome (7) or experimental autoimmune encephalitis (EES) as a model of multiple sclerosis (8)).

One issue that received surprisingly little attention in this discussion is the notion that animal models should primarily be used to improve clinical outcomes in humans. In other

words, construct validity is a key feature in modeling because it provides new insights about the pathophysiology, which in turn is necessary to develop new treatment options in humans. This clinical utility criterion requires that construct validity recapitulates at least one aspect, but not necessarily all aspects, of the human condition. For example, EES is an imperfect model of multiple sclerosis, yet it was used to elucidate the mechanism by which interleukin-17T helper cells gain entry into the brain and induce encephalopathy in mice. Blocking this step in mice prevents the induction of EES, providing a novel therapeutic target for multiple sclerosis (8). The absence of reliable genetic markers or pathognomonic pathological lesions has placed psychiatry in a unique category among all other branches of medicine, and has presented an enormous challenge for establishing animal models with construct and predictive validities (4). This assertion is supported by the observation that, to the best of our knowledge, not a single example exists in which work in animals led to the development of novel and effective interventions in mental illness, though some rare exceptions to this observation may occur in the near future (9–11).

In the first section, we summarize some of the main challenges for establishing animal models with construct and clinical validity of mental illness. We suggest that two related factors are mainly responsible for the slow progress in the development of such animal models. These include: (1) the reliance on the *diagnostic and statistical manual of mental disorders* (DSM)/ *International Classification of Diseases* (ICD) diagnosis system as a conceptual framework for establishing current models and (2) the almost exclusive focus on adult psychopathology while ignoring important neurodevelopmental changes that are responsible for these changes. In the second section, we examine important conceptual and technological advances that are likely to establish animal models with improved construct and predictive validities. These include the development of alternative strategies for diagnosing mental illness, identification of large effect-size genes implicated in mental illness, genomic and proteomic approaches, vehicles for region-specific manipulation of gene expression in animal models, and availability of inducible pluripotent cells from humans carrying mutations in large effect-size genes. In the final section, we provide two examples that demonstrate how these new technologies could be used to define underlying neurodevelopmental changes that are responsible for the behavioral deficits in adulthood. We suggest that this type of neurodevelopmental approach is necessary to establish animal models with better construct and predictive validity.

1.1. The Five Challenges

Here, we describe five main reasons that account for the relative slow progress in our ability to develop animal models with good construct and predictive validities for psychiatric conditions. First, the absence of known pathognomonic lesions in mental illness prevented the use of traditional pathological investigations available in all other branches of medicine, including common neurological conditions. Second, only recently, reliable genetic markers of psychiatric illnesses have become available (12, 13). The absence of such genetic markers, for many years, created an enormous obstacle in the construction of valid models of psychiatric illness. Third, some outcomes may be specific to humans and difficult to model in animals even if good genetic models are available. For example, mutation in the *FoxP2* gene causes significant language impairment in humans (14, 15) that is challenging to model in mice (for an interesting attempt to address this issue, see ref. 16). Fourth, the complexity of the human brain and its relative inaccessibility have allowed for only rudimentary understanding of how the brain generates emotions, constructs perception, and focuses attention. See more on this issue in the Chapter 36 in this book. The lack of clarity on how normal mentation is generated in humans makes it difficult to explain how these processes are impaired in mental illness. Fifth, the current diagnosis system of mental illness

provides an inadequate framework for establishing animal models with good construct and predictive validities.

1.2. The Development of the DSM/ICD System

Despite a long-standing appreciation of the difficulty involved in using the current DSM system to establish animal models of mental illness, no viable alternatives are currently available, and relatively little attention has been paid to its role in hindering the development of valid animal models. We start to examine this issue by providing an important historical perspective on how the DSM system was originally created and why it provides an inadequate system for animal work.

In the absence of psychopathological understanding of mental illness, the field has created diagnostic criteria for mental illness that were primarily aimed at achieving good inter-rater reliability (for an excellent review on this issue, see ref. 17). The rationale for this effort was first to establish a common language by which different clinicians could agree on the diagnosis. Each diagnostic category should share a particular set of symptoms, similar natural history, and response to interventions (17–20). The underlying assumption was that such effort would group together individuals with similar pathology allowing for further characterization of the underlying pathology and therefore improving clinical outcome. An important stipulation of this heuristic attempt was the notion that it serves as a “work in progress proposal” that needs to be continuously examined, particularly in terms of its clinical utility (20).

This approach for defining and categorizing mental disorders is shared by two of the most commonly used classification systems for mental disorders: DSM published by the American Psychiatric Association and its international counterpart the ICD produced by the World Health Organization. Despite the absence of biomarkers to aid diagnosis, these manuals yield good inter-rater reliability levels that are similar to other branches of medicine (18, 19, 21).

1.3. The Absence of a Developmental Perspective

Perhaps, the most significant conceptual drawback of the DSM/ICD system is its failure to recognize that alterations in neurodevelopment play an important role in programming adult psychopathology (22, 23). Several lines of evidence support this assertion. First, retrospective and prospective studies have consistently shown that maltreatment early in life is a major risk factor for the development of adult psychopathology. Recent analysis from the *national comorbidity replication survey* suggested that maltreatment accounts for roughly a third of all adult psychopathology and almost half of all childhood psychopathology in the general population (24), and far greater prevalence in individuals with chronic mental illness (25, 26). Several randomized clinical trials demonstrated that interventions that improve quality of parental care in high-risk children led to robust and sustained improvement in several behavioral and cognitive outcomes (27–29), consistent with the notion that parental care plays an important role in neurodevelopment and the presence of behavioral abnormalities later in life. Work in rodents and nonhuman primates identified neurodevelopmental changes in circuits that regulate fear, stress reactivity, cognition, and reward sensitivity in offspring exposed to low levels of parental care (see Table 1), providing important insights into the mechanisms by which parental care influences neurodevelopment and adult behavior (30, 31).

Second, many of the recently identified genes implicated in mental illness are expressed in high levels during development and regulate neurodevelopmental processes, such as neural stem cell proliferation, migration, differentiation, and synaptogenesis (32–37).

Third, recent neuroimaging studies have identified brain changes in asymptomatic high-risk adolescents that resemble those seen in full-blown adult psychopathology (38, 39). For example, work from the Personal Assessment and Crisis Evaluation (PACE) clinic in Melbourne, Australia, developed a strategy to identify asymptomatic adolescents that are at high risk for developing schizophrenia (40). The rate of conversion from this presymptomatic stage to schizophrenia spectrum disorder (SSD) is roughly 40% per year, allowing for the identification of neurodevelopmental biomarkers that predict conversion to SSD in this population (41). This work identified changes in prefrontal white matter in individuals that later develop psychotic symptoms (42). Similar changes were reported in both first-episode (43) and established patients with schizophrenia (44) demonstrating that developmental abnormalities in prefrontal white matter are present prior to the onset of full-blown SSD. Similarly, fMRI studies with high-risk, asymptomatic, adolescent daughters of mothers with recurrent depression showed abnormal processing of reward/punishment that is also seen in depressed individuals (39), consistent with the notion that abnormal development of reward circuitry is present prior to the development of depressive episodes.

Fourth, almost all forms of adult psychopathology are preceded by childhood psychopathology, suggesting that neurodevelopmental changes in childhood play an important role in modifying the risk for adult psychopathology (22, 45–49). For example, a longitudinal assessment of psychopathology in a large birth cohort ($n = 1,037$) from ages 11 until 26 showed that 75% of adulthood psychopathology was diagnosed before age 18 with roughly half diagnosed prior to age 15 (50). In some cases, childhood symptoms preceded a similar pattern in adulthood (i.e., childhood anxiety preceded adult anxiety), but in many cases childhood diagnosis evolved from one DSM category in childhood into a different one in adulthood. For instance, 40% of the individuals diagnosed with SSD in adulthood were diagnosed with anxiety disorder between the ages of 11 and 15 (50). The view that adult psychopathology is an extension of childhood psychopathology is consistent with a growing body of research showing that obesity early in life is a major risk factor for developing obesity in adulthood (51–54). Indeed, roughly two-thirds of children with the highest body mass index quartile continue to be in this category in adulthood, and over half of the individuals with adult obesity were obese in childhood (52, 54).

The lack of developmental perspective in the DSM/ICD system creates an artificial chasm between childhood and adult psychiatry. This myopic view of psychopathology focuses attention on adult interventions at the expense of early interventions that are likely to be more efficacious. The comparison to treatment of adult obesity is again illuminating with its recent emphasis on early prevention strategies (55).

Since animal models of psychiatric illness have traditionally followed the DSM/ICD framework, a similar trend in preclinical work has focused mainly on adult behavior with little attention paid to important neurodevelopmental processes that guide these behaviors. For example, elegant work in mice has shown that some adult mice are more susceptible to the long-term consequences of repeated social defeat compared to others (56). Most of the work so far has focused on the mechanisms by which exposure to chronic stress causes long-lasting behavioral changes in adult animals with little attention paid to the underlying developmental processes that program this differential response to stress. Similarly, work in rats has shown that high impulsivity plays an important role in mediating compulsive drug-seeking behavior in adult animals (57). Again, no effort has been made so far to assess the developmental processes that establish impulsivity in adult rodents. Even animal models that study the effects of early-life stress (ELS) on adult behavior have mainly focused on characterizing adult behavior while neglecting to track the developmental processes responsible for these changes.

Animal models of psychiatric care are as good as the questions we ask. The absence of a developmental perspective in the way we conceptualize mental illness hinders the development of animal models with improved construct and predictive validity.

1.4. The Heterogeneity and Subjectivity Pitfalls

A more common criticism of the DSM/ICD approach is that its good inter-rater reliability was achieved by using a set of polythetic (i.e., a system that allows one to choose a subset of options from a larger menu of options) and arbitrarily chosen criteria that placed individuals with very different presentations and psychopathologies in the same category (17). For example, clinicians can diagnose major depression by selecting five from a pool of nine possible criteria, many of which can deviate from baseline in either direction (e.g., decrease or increase sleep). In theory, this approach can be used to diagnose depression in two individuals that share no common symptoms. This heterogeneity within a given diagnosis probably contributed to the slow progress of identifying robust genetic biomarkers associated with mental illness (13, 17). This suggestion is supported by a growing body of work showing that genes with robust association to mental illness do not align themselves along specific DSM/ICD diagnoses (12, 13).

From an animal modeling perspective, such a system is problematic for several reasons. First, it relies exclusively on subjective reports with no objective measurements to support the diagnosis (4, 22). Second, the arbitrary and polythetic nature of the definition is impractical to model in animals, leading different researchers to focus on different aspects of the diagnosis (e.g., sucrose preference as a measure of anhedonia or immobility in the forced swim test (4)). This in turn created an ambiguity as to what constitutes an appropriate animal model of depression or schizophrenia, especially given that core features, such as anhedonia, are not specific for depression and can be seen in other conditions, such as negative symptoms of schizophrenia, schizoid personality disorders, chronic pain, or long-standing substance abuse (58).

1.5. The Comorbidity Issue

The DSM/ICD system is predicated on the assumption that these categories represent distinct and nonoverlying psychopathologies. There is now a growing body of evidence to suggest that such assumption is unlikely to be correct (17). For example, over 80% of individuals with major depression have additional DSM diagnoses and roughly 90% of individuals with generalized anxiety report lifetime comorbidity with other DSM diagnoses (59). High rates of comorbidities were described for many other DSM diagnoses (60–63), indicating that comorbidity among some psychiatric conditions is far more common than the pure single diagnosis. The reason for this high rate of comorbidity is currently unclear but it may reflect the ability of a single pathology to present itself in different ways—in the same way that retinopathy, renal failure, and peripheral neuropathy are all manifestations of poorly controlled diabetes. This is consistent with the observations that exposure to early-life adversity increases the risk for a broad spectrum of psychopathologies (24, 64, 65), and that genes that increase the risk for schizophrenia also increase the risk for other psychopathologies (13). We suggest that the paucity of animal work studying the issue of comorbidity is its absence from the DSM/ICD conceptual framework.

2. The Opportunity

The availability of novel technologies, coupled with a new effort led by the NIMH to develop a novel conceptual framework for defining psychopathology (described below), is likely to transform psychiatric care in the upcoming decades. The next section summarizes

some of these key advances and their potential to improve construct and predictive validities of animal models of mental illness.

2.1. The Research Domain Criteria as a Novel Conceptual Framework for Diagnosing Mental Illness

The inherent heterogeneity within each DSM/ICD category, its inability to account for the high rates of comorbidities, and the lack of developmental framework have raised serious doubts about the utility of the DSM/ICD approach to uncover underlying pathology and guide treatment. These concerns have led the NIMH to embark on an ambitious effort to develop an alternative approach for diagnosing and treating mental illness, known as the NIMH Research Domain Criteria (RDoC) (17, 66). The RDoC differs from the DSM/ICD approach in several important ways. First, it relies on advances in neuroscience as the guiding principles for defining psychopathology rather than clinical expertise. Second, it places brain circuit rather than a group 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 (67, 68). Other examples include the role of the striatum and orbital prefrontal cortex in reward prediction (39, 69–72) or the role that the hippocampus plays in spatial and episodic memory (73, 74). Third, pathology is defined as boundaries placed on a quantifiable and objective measurement related to a particular circuit output (e.g., abnormally delayed response in the Go NoGo paradigm (75), a failure to extinct in a fear conditioning paradigm (67), or abnormal p300 event-related recordings (76)). This definition emphasizes the need to anchor pathology to measurable objective information rather than the exclusive reliance on gathering subjective reports in the DSM/ICD system (22, 66). It also assumes that pathology is best defined by placing a boundary on a continuous spectrum in a manner analogous to the definition of hypertension or obesity and not as a discrete category, such as bacterial pneumonia and most DSM/ICD diagnoses (17, 22). It is important to recognize that pathology is not equivalent to functional impairment (i.e., morbidity) but can also be defined as a risk factor for impairment. This important concept is again consistent with the clinical diagnosis of hypertension and diabetes that are risk factors for stroke and cardiovascular disease but are not associated with any measurable impairment by themselves. Pathology in these conditions is defined at the boundary by which interventions seem to reduce morbidity and mortality (17, 22). It is, therefore, possible that some measurements of stress reactivity may justify interventions even in asymptomatic individuals as a way to prevent the onset of depression. Finally, abnormal output of a particular circuit may modify the risk across different DSM/ICD categories providing a possible explanation for the high rate of morbidity. For example, poor impulse control may predispose for increased risk for suicidality, substance abuse, and/or emotional liability/aggression.

These measurable endophenotypes are then analyzed in two directions. Upward analysis investigates the relationship between a particular response to clinically relevant impairment (i.e., morbidity) and response to interventions. Downward analysis studies the molecular and cellular details that modify output of this circuit (66). The RDoC approach is a more compatible framework for animal work because of its reliance on an objective measurable phenotype that reflects an output from a specific brain circuit. The RDoC initiative is at its infancy and its implementation is likely to be both challenging and imperfect. For example, most of the focus has been on using this tool as an alternative diagnostic system for human pathology with little attention paid to how to integrate animal work into this effort. Moreover, it is currently unclear how neurodevelopmental perspective, on circuit assembly, is incorporated into this new effort. Nevertheless, the development of an alternative approach to the DSM/ICD system represents an important and necessary step for the development of animal models with improved validities.

2.2. Microarrays

Recent advances in high-throughput sequencing technologies provided complete genetic maps for the human genome, and a growing list of genomes from other model organisms (for details, see <http://www.ncbi.nlm.nih.gov/genomes/leuks.cgi>). This genomic revolution allowed for the development of unbiased methods to screen for changes in RNA and protein levels associated with human psychopathology in a manner that was not available before. Despite many limitations due to sample heterogeneity, this approach was able to reproducibly identify “cellular markers” that distinguish affected and control groups (77–80). One of the most robust findings has been the identification of abnormal expression of genes implicated in myelination in the prefrontal cortex of individuals with schizophrenia (80–83). These findings are consistent with neuroimaging studies demonstrating white matter abnormalities in the prefrontal cortex of asymptomatic high-risk individuals (42), first-onset unmediated individuals (43), and in individuals with chronic schizophrenia (44). However, white matter abnormalities are unlikely to be a specific marker for schizophrenia (84–90) and therefore additional work is needed to determine how these changes modify brain function and risk for psychopathology.

Genomic tools have been instrumental in bridging the gap between gene expression and behavior in animal models that led to new insights about parallel processes in humans. A good example is the discovery that low BDNF levels in the nucleus accumbens (NA) appear to mediate vulnerability to chronic stress in mice and in humans (56). Microarrays provide also a powerful tool to probe for important differences in the neurobiology between humans and other model organisms. Such information is sorely needed to evaluate and address important limitations in our ability to model unique human traits in animals. For instance, the transcription factor FoxP2 plays an important role in the development of language in humans (14, 91). The amino acid composition of FoxP2 is highly conserved among rodents, nonhuman primates, and humans suggesting that it serves an important common function in mammals. Interestingly, there are only three amino acid changes between the mouse and the human protein, two of which are unique to humans and are not found in other apes (92). This striking finding suggests that the FOXP2 gene underwent important changes in recent history that may have modified its transcriptional activity, allowing for the development of language in humans. This hypothesis was recently tested using a genomic approach comparing the transcriptional activity of the human and the chimpanzee’s FOXP2 genes (93). Interestingly, transgenic mice expressing the human FOXP2 gene show altered vocalization demonstrating the complexity of studying unique human behaviors in rodents (16). In summary, microarrays provide a powerful tool to refine the construct and predictive validities of animal models of psychiatric illnesses.

2.3. Novel Genetic Pathways Implicated in Mental Illness

Recent studies documented significant variability in genomic content among individuals that was not previously recognized (94, 95). This genomic variability, termed copy number variation (CNV), is common but in most cases appears to be inconsequential. However, in rare instances, it interferes with expression of genes that mediate risk for the development of several psychiatric illnesses. For a good review on this issue, see ref. 13. Increased CNV burden has been found in individuals with schizophrenia compared to matched controls (96–99). CNVs that are associated with increased risk for schizophrenia appear to target genes implicated in synaptic development (12, 97). The observation that the rate of CNV in noncoding DNA was similar in schizophrenics and controls (97) suggests that the increased burden of CNV is not due to a general increase in genomic instability. A small portion of these CNVs is located in specific hotspots in the genome that confer high risk for schizophrenia (e.g., 1q21.1, 15q11.2, 15q13.3, and 22q11.2). The genomic instability in these regions is most likely due to flanking tandem repeats driving nonallelic homologous

recombination in these regions. These more common CNVs account for only 2–4% of cases of schizophrenia (13). In fact, most individuals with schizophrenia have a single-unique hit that is not shared with other individuals with schizophrenia (13, 97). Interestingly, a particular CNV is not specific for schizophrenia and may also increase the risk for other mental illnesses, such as autism, mental retardation, or mood disorders (12, 13, 100). Together, these findings suggested that genomic instability is responsible for rare hits in many different developmental genes that substantially modify the risk of mental illness in a manner that does not follow a specific DSM/ICD category.

DNA microarray provides a powerful and inexpensive technology to identify CNVs associated with high risk for mental illness (101). However, this technology is now being replaced by new advances that are able to sequence the entire coding sequence of the human genome (i.e., exomes) at a cost of \$4,000 or less (101, 102). In fact, the cost associated with sequencing the entire human genome is likely to reach the NIH goal of \$1,000 within the next decade (102). This technology provides single-base resolution of individual genomes and has already been used successfully for gene discovery and to guide novel interventions (103–107).

The explosive nature of these advances undoubtedly generates a long list of genes and pathways implicated in mental illness (see step 1 in Fig. 1). Such a list provides a tremendous opportunity for progress, but this progress requires the development of clinical and preclinical tools to bridge the gap between genes and complex behavior. From a clinical point of view, there is a need to map intermediate phenotypes (i.e., endophenotypes, and see step 2 in Fig. 1) that more closely track these genetic variations (for two good examples of such endophenotypes, see refs. 76, 108). Animal models, especially in mice where genetic manipulations allow for deletion and overexpression of genes, are critical for elucidating the mechanism by which genes implicated in mental illness modify neurodevelopment and adult behavior (see steps 6–9 in Fig. 1). In Subheading 3, we provide more details on how this approach will likely improve construct and predictive validity of animal models of psychiatric illness.

2.4. Viral Gene Delivery and Pluripotent Stem Cells

Advancements in molecular and cellular biology have provided several new tools to bridge the gap between gene expression and human psychopathology. Here, we briefly describe two such examples. The first example is the development of synthetic viruses that can be used to manipulate gene expression within a specific brain region or a cell type. This technology takes advantage of the fact that viral particles are made by assembling a protein capsid shell around a DNA (or RNA) sequence. The capsid shell allows the virus to attach itself and gain entry into specific cells. Once inside the cell, the particle disassembles delivering the DNA into the infected cell providing the necessary information to assemble new particles that can then go on to infect other cells. By modifying the genetic sequence packaged in these particles, one can deliver genetic instructions that modify expression of specific genes in neurons or glial cells without expressing other viral genes that harm these cells (109, 110). In animals, this method provides a powerful tool to determine how changes in gene expression, within a particular brain region, modify brain function and behavior. This approach was used to show that high levels of brain-derived neurotrophic factor (BDNF) produced in the ventral tegmental area (VTA) are necessary to mediate susceptibility to social defeat in mice (56). Viruses can also be used to modify gene expression in other model organisms, such as nonhuman primates, where transgenic animals are not yet available. Finally, viral gene delivery in humans is likely to provide a promising novel strategy for interventions in the future (111, 112).

The second example is the development of novel stem cell technology that can reprogram differentiated somatic cells (i.e., fibroblasts) into pluripotent stem cells by expressing a defined combination of transcription factors. The inducible pluripotent cells (iPS) can then be differentiated in vitro to give rise to different cell types, including neurons and glial cells (113). In practical terms, this approach allows us to harvest fibroblasts from individuals carrying a mutation of interest and their normal siblings, transform them into neurons, and examine their structural, biochemical, and electrophysiological properties in the dish. A recent report has demonstrated significant improvements in the methodology and the establishment of an iPS cell line from fibroblasts obtained from an 8-year-old girl with Rett syndrome (114). This method may help circumvent the current inaccessibility of fresh brain tissue for pathological examination, providing an important tool for validating findings in animal models as well as guiding preclinical work.

3. Section 3: Lessons from developmental work in rodents

In this last section, we provide two examples to demonstrate how developmental work in rodents can provide important insights into the pathophysiology of mental illness in humans. We suggest that this kind of developmental work is necessary to improve construct and predictive validity of current animal models of psychiatric illness.

3.1. Lessons from DISC1

In 1970, Patricia Jacobs and her colleagues reported an aberrant translocation in an 18-year-old boy that had severe conduct disorder but was otherwise healthy (115). The balanced translocation occurred between chromosomes 1 and 11, t(1:11) (q43,q21), and was detected across three generations in many members of his extended family (carriers $n = 34$, noncarriers $n = 43$) (76, 116). No phenotypic abnormalities were noted during the initial evaluation, but a follow-up study, conducted 20 years later, found unusually high incidence of psychiatric hospitalizations in this family (116). Carriers of this translocation showed high incidence of schizophrenia ($n = 7$), bipolar disorder ($n = 1$), unipolar depression ($n = 10$), adolescent conduct disorder ($n = 2$), and minor depression ($n = 1$) (116). All the individuals with severe mental illness in this family carried the translocation with a maximum LOD score of 7.1 for linkage between this translocation and severe mental illness. Interestingly, only 60% of the carriers developed severe psychopathology suggesting that the presence of the translocation alone is not sufficient to induce severe mental illness (76, 100).

Initial characterization of the translocation suggested that it disrupted at least two genes (117, 118), named Disrupted in Schizophrenia 1 and 2 (i.e., DISC1 and DISC2). DISC2 appears to be a noncoding RNA that is transcribed in the opposite direction of DISC1 and is believed to regulate DISC1 expression. A large number of studies have now confirmed an association between mutations in DISC1 and increased risk for a host of psychopathologies (reviewed in refs. 100, 119), providing the field with one of its first reliable genetic biomarkers for human psychopathology.

3.2. The Search for an Endophenotype

One important lesson from the DISC1 discovery is the need to better characterize the clinical presentation associated with this genetic biomarker. For instance, despite its name, abnormal expression of DISC1 is not a specific genetic marker for schizophrenia and a substantial portion of individuals that carry the t(1:11) translocation develop depression or show no evidence of mental illness (76, 116). This nonspecific relationship has been described now for many other large-effect genes, underscoring the importance of defining an intermediate phenotype (i.e., an endophenotype) that better segregates with the mutation and

also links it to clinical impairment (12, 13, 108). An interesting example of such an endophenotype is the finding that carriers of the t(1:11) translocation showed a decrease in amplitude of the P300 event-related potential not seen in noncarrier family members (76). Importantly, this test was able to distinguish between carriers and noncarriers regardless of their clinical diagnosis (depression vs. schizophrenia) or clinical impairment (mental illness vs. no mental illness). The observation that decreased P300 amplitude was seen in carriers with no evidence of mental illness is not necessarily problematic as long as this biomarker is a good predictor of the development of mental illness, an assertion that is supported by some reports (120–122). This is analogous to the observation that many individuals with hypertension do not develop stroke (i.e., clinical morbidity), yet we prevent stroke by treating hypertension because hypertension is a good predictor for future morbidity and it is amenable to treatment. Moreover, hypertension is a risk factor for many forms of morbidities, including heart disease and kidney disease, demonstrating that a single-risk factor can have different clinical presentations. In summary, a good endophenotype should provide an objective measurement that distinguishes between carriers and noncarriers and relay information regarding risk for clinical impairment (see step 2 in Fig. 1).

The DSM/ICD system may be useful for describing clinical impairment but not for uncovering psychopathology or guiding treatment. The RDoC initiative should facilitate the identification of endophenotypes that are likely to be more informative regarding the pathophysiology and intervention strategies. Such endophenotypes would also improve construct validity of animal models in psychiatric research by providing important postmarks to guide this work (step 7, Fig. 1). Similarly, work with transgenic animals carrying mutations in these genes can provide important insights for testing specific endophenotypes in humans (step 7, Fig. 1). For example, reports from several groups demonstrated a decrease in prepulse inhibition (PPI) (i.e., a measurement of sensorimotor gating) in transgenic animals carrying mutations in the DISC1 gene (5, 123, 124). These deficits appear to be due to inappropriate maturation of dopamine innervations in the prefrontal cortex of these mice (124). Defects in PPI have been documented in individuals with schizophrenia and other mental illnesses, but to the best of our knowledge have not been used to distinguish between carrier and noncarrier family members with a mutated DISC1 gene. This kind of reciprocal translational work demonstrates how work in animals can guide the identification of an endophenotype in humans.

4. Animal Models Play a Key Role in Elucidating the Mechanism by Which Large Effect-Size Genes Modify Behavior

Animal work has played an instrumental role in elucidating the mechanisms by which mutations in DISC1 modify behavior. For example, biochemical and cellular studies have shown that the DISC1 protein regulates at least two major cellular pathways that are necessary for neural stem cell (NSC) proliferation, migration, and synaptogenesis (119). One pathway involves a direct inhibition of GSK3 β allowing for stabilization of β -catenin, which in turn is necessary for cell-cycle entry and NSC proliferation (34). The second pathway involves recruiting microtubule-assembly proteins, such as LIS1 and NUDEL, into a dynein-mediated motor complex that transports the complex to the centrosome. Disruption of this latter pathway interferes with centrosome-mediated cellular functions, such as cell division, cell migration, and neurite outgrowth (125, 126).

Several lines of transgenic animals with dysregulated DISC1 activity have been characterized and much work has been done to demonstrate behavioral and anatomical alterations in adulthood (5, 123, 127). Recent work has focused on trying to understand how changes in DISC1 levels modify behavior in animals. Mao et al. (2009) showed that DISC1 knockdown is associated with decreased proliferation of NSC in the hippocampus of adult

mice, a decrease that was associated with increased helpless behavior in the forced swim test. Abnormalities in NSC proliferation and helpless behavior were eliminated after administering GSK3 β inhibitors (34). In other words, DISC1 inhibition of GSK3 β is necessary for NSC proliferation and therefore GSK3 β inhibitors can compensate for DISC1 loss of function. Availability of iPS cell lines from family members with and without a mutation in DISC1 can assess the validity of these findings in humans. These examples provide a vivid demonstration of how a molecular understanding of the underlying biology in rodents can potentially lead to novel interventional strategies in humans (steps 8 and 9 in Fig. 1).

Several observations suggest that many of the behavioral abnormalities seen in animals with mutated DISC1 are neurodevelopmental in nature. First, DISC1 modifies processes, such as NSC proliferation, migration, and synaptogenesis, that are essential for normal neurodevelopment. Second, expression of DISC1 peaks during embryogenesis and the juvenile (i.e., adolescence-like) period (32). Despite this well-accepted notion that DISC1 is likely to play an important role in neurodevelopment, most effort so far has been on characterizing abnormal behavior in adulthood with little effort made to link these behavioral abnormalities to specific neurodevelopmental changes. A unique exception to this general trend has been a recent report by Niwa et al. (2010). These authors first developed a method that allowed them to transiently knock down DISC1 mainly in pyramidal prefrontal cortex cells (124). This transient decrease in DISC1 (from embryonic day 14 to roughly postnatal day 10) caused abnormal dendritic development in these neurons that persisted into adulthood despite the restoration of normal levels of DISC1 in these cells at 2 weeks after birth. Next, the authors wanted to know whether defects in dendritic arborization impaired the ability of these cells to receive dopaminergic innervations that normally mature during young adulthood (128). Indeed, defects in dendritic arborization were associated with impaired dopaminergic input and deficits in several prefrontal mediated tasks that were apparent in late (i.e., postnatal day 56) but not early adolescence (i.e., postnatal day 28). This work demonstrates how early developmental abnormalities affect later developmental processes accounting for the emergence of abnormal behavior in early adulthood. We suggest that this kind of developmental approach is likely to improve construct and predictive validity of animal models of psychiatric illness by defining the underlying pathology and facing the challenges of treating developmental psychopathology in adulthood (see steps 8 and 9 in Fig. 1).

In conclusion, understanding the mechanisms by which DISC1 modifies the risk for psychopathology in humans requires the identification of reliable endophenotypes that link this gene with objective measurements that predict the risk for clinical impairment. Animal models provide an instrumental tool to understand how alterations in this gene modify adult behavior and this work can aid in identifying endophenotypes in humans. Current animal work has focused on characterizing adult behavior in animals with abnormal DISC1 protein activity with little attention paid to how neurodevelopmental changes contribute to these underlying behavioral abnormalities. A better understanding of the neurodevelopmental processes by which disruption of DISC1 modifies adult behavior represents an important and promising area of research for future animal work.

4.1. Parental Care Programs Neurodevelopment

Childhood maltreatment represents one of the most significant risk factors for the development of mental illness in adulthood. The importance of this issue as a major public health concern was recently acknowledged by several influential sources, including the World Health Organization and the Institute of Medicine (129, 130). Roughly, 1.5 million children are abused or neglected each year in the USA and these alarming statistics have been documented now for the past 30 years (30). In the absence of effective interventions,

maltreated children go on to develop a host of behavioral, emotional, cognitive, and medical sequelae that are chronic and in most cases refractory to psychiatric treatment (24, 130–133). The relationship between ELS and mental illness has now been demonstrated using both retrospective and prospective studies (reviewed in ref. 130), and several reports have consistently found that more than half (!) of the individuals with chronic mental illness have been physically, verbally, or sexually abused early in life (25, 26). Finally, in a recent report from the Institute of Medicine, the total cost related to ELS was estimated at \$247 billion annually (129), placing it at equal footing with the estimated costs for all cancers combined.

The observations that many of the symptoms associated with exposure to ELS are present early in life and persist into adulthood suggest that exposure to ELS is somehow able to modify brain development in a manner that influences the risk for mental illness in adulthood. The molecular and cellular mechanisms by which ELS influences such diverse and severe clinical outcomes are still poorly understood in humans, but similar outcomes in rodents and nonhuman primates suggest that at least some aspects of this process could be further studied in animal models (30, 131). Here, we briefly summarize key findings from Dr. Michael Meaney's laboratory on the developmental sequelae of maternal neglect model in the rat. These are presented to illustrate how developmental work on early adversity in animals can inform us about parallel processes in humans.

Maternal behavior during the first postnatal week is normally distributed in rats such that some dams lick and groom (LG) their pups almost three times as much compared to others (134). High- and low-LG dams were defined as those that are 1 SD above and below the mean, respectively, creating two nonoverlapping extremes of maternal care—reviewed in ref. 135. Longitudinal follow-up studies demonstrated a host of behavioral differences between adult offspring of high- and low-LG dams (see Table 1). Levels of LG peak during the first 2 days after birth in both high- and low-LG dams followed by a gradual decline to similar low levels at around postnatal 9 (134). These observations suggest that differences in frequency of LG during the first 9 days after birth are somehow able to alter many behavioral outcomes in adulthood. Cross-fostering studies showed that most behavioral outcomes are dictated by levels of postnatal maternal care provided by the adopting dam rather than the biological dam, and that tactile stimulation provided by the dam during a critical period of development is responsible for these changes in behavior, reviewed in ref. 31.

Exposure to high levels of LG during the first week of life is necessary to induce a cascade of developmental changes that ultimately lead to the removal of DNA methylation from a promoter that controls glucocorticoid receptor (GR) expression in the hippocampus (for a detailed review, see ref. 136). Demethylation of this regulatory element allows the transcription factor NGFI-A to bind this promoter, resulting in higher levels of GR in the hippocampus of offspring raised by high-LG dams (137, 138). Low levels of LG are not sufficient to trigger demethylation of this promoter resulting in low expression levels of GR in offspring raised by low-LG dams. Once established, the DNA methylation at this promoter persists into adulthood accounting for the higher levels of GR in the hippocampus of adult offspring raised by high-LG compared to those of low-LG dams. High levels of GR in the hippocampus allow for more efficient termination of the release of corticosterone from the adrenal gland explaining why offspring of high-LG dams have a more blunted hypothalamic-pituitary-adrenal (HPA) response to stress compared to offspring of low-LG dams (137–139).

This work has demonstrated how early-life events cause stable alterations in gene expression that modify HPA reactivity in adulthood, providing an important paradigm to explain how early adversity could modify vulnerability to mental illness in adulthood. The relevance of

these findings to human psychopathology was examined in a recent postmortem study showing higher levels of DNA methylation and lower GR in the hippocampus of individuals exposed to early maltreatment compared to matched controls (140). This work provides a plausible molecular model to explain previous data documenting increased stress reactivity in both humans and nonhuman primates exposed to ELS (131), and demonstrates the potential of using animal models to elucidate some of the molecular mechanisms by which exposure to stress early in life modifies vulnerability to stress in adult humans (steps 7–9, Fig. 1).

Changes in DNA methylation is likely to be only one of many molecular mechanisms by which events early in life modify the risk for psychopathology in adulthood and there is a need to use unbiased genomic strategies to further characterize other developmental pathways modified by ELS. Some examples include neural stem proliferation/survival/differentiation, synaptogenesis, and synaptic pruning. Moreover, current work has mainly focused on demonstrating behavioral or physiological changes in adult animals exposed to ELS, with little attention paid to the underlying developmental changes that are responsible for these changes. Finally, additional effort is needed to explain how ELS is able to modify so many different behavioral outcomes in adulthood (see Table 1). Such work is likely to shed new light on the underlying developmental processes that are responsible for the high rate of comorbidity seen in some forms of mental illness (59, 60, 62, 141).

In summary, exposure to maltreatment early in life represents a major risk factor for a host of psychopathologies in humans. Animal models of ELS are likely to provide valuable molecular insights into the underlying pathology that is likely to improve our ability to diagnose and treat this common form of psychopathology.

5. Conclusions

In the absence of viable alternatives, attempts to model human psychopathology in animals have relied almost exclusively on the DSM/ICD conceptual framework. Here, we suggest that this conceptual framework is inadequate for studying human psychopathology and therefore inappropriate to guide this issue in animals. We propose that animal work should focus instead on the role that genes and/or environmental factors play in the development of circuits that regulate specific physiological and behavioral outcomes in adulthood. Such an approach is consistent with the notion that most (though clearly not all) adult psychopathology is programmed earlier in development and is necessary to elucidate the underlying biology of a growing list of developmental genes implicated in human psychopathology. A better understanding of these processes in animals improves construct and predictive validity of animal models of mental illness and facilitates the development of earlier diagnostic and interventional strategies that are likely to improve clinical outcomes.

Acknowledgments

This work was supported by NIMH 1KO8MH074856, DANA foundation Program in Brain and Immuno-imagine 2011, the Clinical Neuroscience Division of the VA National Center for PTSD, the NIAAA Center for the Translational Neuroscience of Alcoholism (P50-AA012870-09), and CTSA Grant Number UL1 RR024139 from the National Center for Research Resources.

References

1. Willner P. The validity of animal models of depression. *Psychopharmacology (Berl)*. 1984; 83:1–16. [PubMed: 6429692]
2. Kellendonk C, Simpson EH, Kandel ER. Modeling cognitive endophenotypes of schizophrenia in mice. *Trends Neurosci*. 2009; 32:347–358. [PubMed: 19409625]

3. Chadman KK, Yang M, Crawley JN. Criteria for validating mouse models of psychiatric diseases. *Am J Med Genet B Neuropsychiatr Genet.* 2009; 150B:1–11. [PubMed: 18484083]
4. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. *Nat Neurosci.* 13:1161–1169. [PubMed: 20877280]
5. Hikida T, Jaaro-Peled H, Seshadri S, Oishi K, Hookway C, Kong S, Wu D, Xue R, Andrade M, Tankou S, Mori S, Gallagher M, Ishizuka K, Pletnikov M, Kida S, Sawa A. Dominant-negative DISC1 transgenic mice display schizophrenia-associated phenotypes detected by measures translatable to humans. *Proc Natl Acad Sci USA.* 2007; 104:14501–14506. [PubMed: 17675407]
6. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science.* 2003; 301:805–809. [PubMed: 12907793]
7. Shahbazian M, Young J, Yuva-Paylor L, Spencer C, Antalffy B, Noebels J, Armstrong D, Paylor R, Zoghbi H. Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. *Neuron.* 2002; 35:243–254. [PubMed: 12160743]
8. Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, Uccelli A, Lanzavecchia A, Engelhardt B, Sallusto F. C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. *Nat Immunol.* 2009; 10:514–523. [PubMed: 19305396]
9. Berry-Kravis E, Hessler D, Coffey S, Hervey C, Schneider A, Yuhas J, Hutchison J, Snape M, Tranfaglia M, Nguyen DV, Hagerman R. A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *J Med Genet.* 2009; 46:266–271. [PubMed: 19126569]
10. Sanacora G, Zarate CA, Krystal JH, Manji HK. Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. *Nat Rev Drug Discov.* 2008; 7:426–437. [PubMed: 18425072]
11. Grillon C. D-cycloserine facilitation of fear extinction and exposure-based therapy might rely on lower-level, automatic mechanisms. *Biol Psychiatry.* 2009; 66:636–641. [PubMed: 19520359]
12. State MW. Another piece of the autism puzzle. *Nat Genet.* 2010; 42:478–479. [PubMed: 20502490]
13. Sebat J, Levy DL, McCarthy SE. Rare structural variants in schizophrenia: one disorder, multiple mutations; one mutation, multiple disorders. *Trends Genet.* 2009; 25:528–535. [PubMed: 19883952]
14. Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP. A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature.* 2001; 413:519–523. [PubMed: 11586359]
15. Hurst JA, Baraitser M, Auger E, Graham F, Norell S. An extended family with a dominantly inherited speech disorder. *Dev Med Child Neurol.* 1990; 32:352–355. [PubMed: 2332125]
16. Enard W, Gehre S, Hammerschmidt K, Holter SM, Blass T, Somel M, Bruckner MK, Schreiwies C, Winter C, Sohr R, Becker L, Wiebe V, Nickel B, Giger T, Muller U, Groszer M, Adler T, Aguilar A, Bolle I, Calzada-Wack J, Dalke C, Ehrhardt N, Favor J, Fuchs H, Gailus-Durner V, Hans W, Holzlwimmer G, Javaheri A, Kalaydjiev S, Kallnik M, Kling E, Kunder S, Mossbrugger I, Naton B, Racz I, Rathkolb B, Rozman J, Schrewe A, Busch DH, Graw J, Ivandic B, Klingenspor M, Klopstock T, Ollert M, Quintanilla-Martinez L, Schulz H, Wolf E, Wurst W, Zimmer A, Fisher SE, Morgenstern R, Arendt T, de Angelis MH, Fischer J, Schwarz J, Paabo S. A humanized version of Foxp2 affects cortico-basal ganglia circuits in mice. *Cell.* 2009; 137:961–971. [PubMed: 19490899]
17. Hyman SE. The diagnosis of mental disorders: the problem of reification. *Annu Rev Clin Psychol.* 6:155–179. [PubMed: 17716032]
18. Helzer JE, Clayton PJ, Pambakian R, Reich T, Woodruff RA Jr, Reveley MA. Reliability of psychiatric diagnosis. II. The test/retest reliability of diagnostic classification. *Arch Gen Psychiatry.* 1977; 34:136–141. [PubMed: 843173]
19. Helzer JE, Robins LN, Taibleson M, Woodruff RA Jr, Reich T, Wish ED. Reliability of psychiatric diagnosis. I. A methodological review. *Arch Gen Psychiatry.* 1977; 34:129–133. [PubMed: 320954]
20. Feighner JP, Robins E, Guze SB, Woodruff RA Jr, Winokur G, Munoz R. Diagnostic criteria for use in psychiatric research. *Arch Gen Psychiatry.* 1972; 26:57–63. [PubMed: 5009428]

21. Pies R. How “objective” are psychiatric diagnoses?: (guess again). *Psychiatry* (Edmont). 2007; 4:18–22. [PubMed: 20428307]
22. Widiger TA, Clark LA. Toward DSM-V and the classification of psychopathology. *Psychol Bull.* 2000; 126:946–963. [PubMed: 11107884]
23. Thompson BL, Levitt P. The clinical-basic interface in defining pathogenesis in disorders of neurodevelopmental origin. *Neuron.* 2010; 67:702–712. [PubMed: 20826303]
24. Green JG, McLaughlin KA, Berglund PA, Gruber MJ, Sampson NA, Zaslavsky AM, Kessler RC. Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: associations with first onset of DSM-IV disorders. *Arch Gen Psychiatry.* 67:113–123. [PubMed: 20124111]
25. Muenzenmaier K, Meyer I, Struening E, Ferber J. Childhood abuse and neglect among women outpatients with chronic mental illness. *Hosp Community Psychiatry.* 1993; 44:666–670. [PubMed: 8192738]
26. Bryer JB, Nelson BA, Miller JB, Krol PA. Childhood sexual and physical abuse as factors in adult psychiatric illness. *Am J Psychiatry.* 1987; 144:1426–1430. [PubMed: 3674223]
27. Olds D, Henderson CR Jr, Cole R, Eckenrode J, Kitzman H, Luckey D, Pettitt L, Sidora K, Morris P, Powers J. Long-term effects of nurse home visitation on children’s criminal and antisocial behavior: 15-year follow-up of a randomized controlled trial. *Jama.* 1998; 280:1238–1244. [PubMed: 9786373]
28. Olds DL, Kitzman H, Cole R, Robinson J, Sidora K, Luckey DW, Henderson CR Jr, Hanks C, Bondy J, Holmberg J. Effects of nurse home-visiting on maternal life course and child development: age 6 follow-up results of a randomized trial. *Pediatrics.* 2004; 114:1550–1559. [PubMed: 15574614]
29. Olds DL, Robinson J, Pettitt L, Luckey DW, Holmberg J, Ng RK, Isacks K, Sheff K, Henderson CR Jr. Effects of home visits by paraprofessionals and by nurses: age 4 follow-up results of a randomized trial. *Pediatrics.* 2004; 114:1560–1568. [PubMed: 15574615]
30. Kaffman A. The silent epidemic of neurodevelopmental injuries. *Biol Psychiatry.* 2009; 66:624–626. [PubMed: 19747590]
31. Kaffman A, Meaney MJ. Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights. *J Child Psychol Psychiatry.* 2007; 48:224–244. [PubMed: 17355397]
32. Schurov IL, Handford EJ, Brandon NJ, Whiting PJ. Expression of disrupted in schizophrenia 1 (DISC1) protein in the adult and developing mouse brain indicates its role in neurodevelopment. *Mol Psychiatry.* 2004; 9:1100–1110. [PubMed: 15381924]
33. Duan X, Chang JH, Ge S, Faulkner RL, Kim JY, Kitabatake Y, Liu XB, Yang CH, Jordan JD, Ma DK, Liu CY, Ganesan S, Cheng HJ, Ming GL, Lu B, Song H. Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain. *Cell.* 2007; 130:1146–1158. [PubMed: 17825401]
34. Mao Y, Ge X, Frank CL, Madison JM, Koehler AN, Doud MK, Tassa C, Berry EM, Soda T, Singh KK, Biechele T, Petryshen TL, Moon RT, Haggarty SJ, Tsai LH. Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3 β /beta-catenin signaling. *Cell.* 2009; 136:1017–1031. [PubMed: 19303846]
35. Berkel S, Marshall CR, Weiss B, Howe J, Roeth R, Moog U, Endris V, Roberts W, Szatmari P, Pinto D, Bonin M, Riess A, Engels H, Sprengel R, Scherer SW, Rappold GA. Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. *Nat Genet.* 42:489–491. [PubMed: 20473310]
36. Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C, Bourgeron T. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat Genet.* 2003; 34:27–29. [PubMed: 12669065]
37. Laumonnier F, Bonnet-Brilhault F, Gomot M, Blanc R, David A, Moizard MP, Raynaud M, Ronce N, Lemonnier E, Calvas P, Laudier B, Chelly J, Fryns JP, Ropers HH, Hamel BC, Andres C, Barthelemy C, Moraine C, Briault S. X-linked mental retardation and autism are associated with a

- mutation in the NLGN4 gene, a member of the neuroligin family. *Am J Hum Genet.* 2004; 74:552–557. [PubMed: 14963808]
38. Correll CU, Hauser M, Auther AM, Cornblatt BA. Research in people with psychosis risk syndrome: a review of the current evidence and future directions. *J Child Psychol Psychiatry.* 2010; 51:390–431. [PubMed: 20214698]
 39. Gotlib IH, Hamilton JP, Cooney RE, Singh MK, Henry ML, Joormann J. Neural processing of reward and loss in girls at risk for major depression. *Arch Gen Psychiatry.* 67:380–387. [PubMed: 20368513]
 40. Pantelis C, Velakoulis D, Wood SJ, Yucel M, Yung AR, Phillips LJ, Sun DQ, McGorry PD. Neuroimaging and emerging psychotic disorders: the Melbourne ultra-high risk studies. *Int Rev Psychiatry.* 2007; 19:371–381. [PubMed: 17671870]
 41. Yung AR, Phillips LJ, McGorry PD, McFarlane CA, Francey S, Harrigan S, Patton GC, Jackson HJ. Prediction of psychosis. A step towards indicated prevention of schizophrenia. *Br J Psychiatry Suppl.* 1998; 172:14–20. [PubMed: 9764121]
 42. Walterfang M, Yung A, Wood AG, Reutens DC, Phillips L, Wood SJ, Chen J, Velakoulis D, McGorry PD, Pantelis C. Corpus callosum shape alterations in individuals prior to the onset of psychosis. *Schizophr Res.* 2008; 103:1–10. [PubMed: 18562178]
 43. Walterfang M, McGuire PK, Yung AR, Phillips LJ, Velakoulis D, Wood SJ, Suckling J, Bullmore ET, Brewer W, Soulsby B, Desmond P, McGorry PD, Pantelis C. White matter volume changes in people who develop psychosis. *Br J Psychiatry.* 2008; 193:210–215. [PubMed: 18757979]
 44. Woodruff PW, McManus IC, David AS. Meta-analysis of corpus callosum size in schizophrenia. *J Neurol Neurosurg Psychiatry.* 1995; 58:457–461. [PubMed: 7738554]
 45. Ferdinand RF, Verhulst FC. Psychopathology from adolescence into young adulthood: an 8-year follow-up study. *Am J Psychiatry.* 1995; 152:1586–1594. [PubMed: 7485620]
 46. Ferdinand RF, Verhulst FC, Witznitzer M. Continuity and change of self-reported problem behaviors from adolescence into young adulthood. *J Am Acad Child Adolesc Psychiatry.* 1995; 34:680–690. [PubMed: 7775363]
 47. Hofstra MB, Van der Ende J, Verhulst FC. Pathways of self-reported problem behaviors from adolescence into adulthood. *Am J Psychiatry.* 2002; 159:401–407. [PubMed: 11870003]
 48. Hofstra MB, van der Ende J, Verhulst FC. Child and adolescent problems predict DSM-IV disorders in adulthood: a 14-year follow-up of a Dutch epidemiological sample. *J Am Acad Child Adolesc Psychiatry.* 2002; 41:182–189. [PubMed: 11837408]
 49. Hofstra MB, Van der Ende J, Verhulst FC. Continuity and change of psychopathology from childhood into adulthood: a 14-year follow-up study. *J Am Acad Child Adolesc Psychiatry.* 2000; 39:850–858. [PubMed: 10892226]
 50. Kim-Cohen J, Caspi A, Moffitt TE, Harrington H, Milne BJ, Poulton R. Prior juvenile diagnoses in adults with mental disorder: developmental follow-back of a prospective-longitudinal cohort. *Arch Gen Psychiatry.* 2003; 60:709–717. [PubMed: 12860775]
 51. Biro FM, Wien M. Childhood obesity and adult morbidities. *Am J Clin Nutr.* 91:1499S–1505S. [PubMed: 20335542]
 52. Deshmukh-Taskar P, Nicklas TA, Morales M, Yang SJ, Zakeri I, Berenson GS. Tracking of overweight status from childhood to young adulthood: the Bogalusa Heart Study. *Eur J Clin Nutr.* 2006; 60:48–57. [PubMed: 16132057]
 53. Guo SS, Chumlea WC. Tracking of body mass index in children in relation to overweight in adulthood. *Am J Clin Nutr.* 1999; 70:145S–148S.
 54. Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med.* 1997; 337:869–873. [PubMed: 9302300]
 55. Reilly JJ, Armstrong J, Dorosty AR, Emmett PM, Ness A, Rogers I, Steer C, Sherriff A. Early life risk factors for obesity in childhood: cohort study. *BMJ.* 2005; 330:1357. [PubMed: 15908441]
 56. Krishnan V, Han MH, Graham DL, Berton O, Renthall W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell.* 2007; 131:391–404. [PubMed: 17956738]

57. Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ. High impulsivity predicts the switch to compulsive cocaine-taking. *Science*. 2008; 320:1352–1355. [PubMed: 18535246]
58. Gorwood P. Neurobiological mechanisms of anhedonia. *Dialogues Clin Neurosci*. 2008; 10:291–299. [PubMed: 18979942]
59. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, Wittchen HU, Kendler KS. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry*. 1994; 51:8–19. [PubMed: 8279933]
60. Kessler RC, DuPont RL, Berglund P, Wittchen HU. Impairment in pure and comorbid generalized anxiety disorder and major depression at 12 months in two national surveys. *Am J Psychiatry*. 1999; 156:1915–1923. [PubMed: 10588405]
61. Moffitt TE, Caspi A, Harrington H, Milne BJ, Melchior M, Goldberg D, Poulton R. Generalized anxiety disorder and depression: childhood risk factors in a birth cohort followed to age 32. *Psychol Med*. 2007; 37:441–452. [PubMed: 17201999]
62. Ormel J, VonKorff M, Ustun TB, Pini S, Korten A, Oldehinkel T. Common mental disorders and disability across cultures. Results from the WHO Collaborative Study on Psychological Problems in General Health Care. *Jama*. 1994; 272:1741–1748. [PubMed: 7966922]
63. Olfson M, Fireman B, Weissman MM, Leon AC, Sheehan DV, Kathol RG, Hoven C, Farber L. Mental disorders and disability among patients in a primary care group practice. *Am J Psychiatry*. 1997; 154:1734–1740. [PubMed: 9396954]
64. Mullen PE, Martin JL, Anderson JC, Romans SE, Herbison GP. The long-term impact of the physical, emotional, and sexual abuse of children: a community study. *Child Abuse Negl*. 1996; 20:7–21. [PubMed: 8640429]
65. Bebbington PE, Bhugra D, Brugha T, Singleton N, Farrell M, Jenkins R, Lewis G, Meltzer H. Psychosis, victimisation and childhood disadvantage: evidence from the second British National Survey of Psychiatric Morbidity. *Br J Psychiatry*. 2004; 185:220–226. [PubMed: 15339826]
66. Insel T, Cuthbert B, Garvey M, Heinssen R, Pine DS, Quinn K, Sanislow C, Wang P. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am J Psychiatry*. 2010; 167:748–751. [PubMed: 20595427]
67. Delgado MR, Nearing KI, Ledoux JE, Phelps EA. Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. *Neuron*. 2008; 59:829–838. [PubMed: 18786365]
68. LeDoux JE. Emotion circuits in the brain. *Annu Rev Neurosci*. 2000; 23:155–184. [PubMed: 10845062]
69. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev*. 1998; 28:309–369. [PubMed: 9858756]
70. O'Doherty J, Kringelbach ML, Rolls ET, Hornak J, Andrews C. Abstract reward and punishment representations in the human orbitofrontal cortex. *Nat Neurosci*. 2001; 4:95–102. [PubMed: 11135651]
71. Roesch MR, Olson CR. Neuronal activity related to reward value and motivation in primate frontal cortex. *Science*. 2004; 304:307–310. [PubMed: 15073380]
72. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron*. 2002; 34:13–25. [PubMed: 11931738]
73. Squire LR. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev*. 1992; 99:195–231. [PubMed: 1594723]
74. Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron*. 65:7–19. [PubMed: 20152109]
75. Strauss GP, Frank MJ, Waltz JA, Kasanova Z, Herbener ES, Gold JM. Deficits in Positive Reinforcement Learning and Uncertainty-Driven Exploration Are Associated with Distinct Aspects of Negative Symptoms in Schizophrenia. *Biol Psychiatry*. 2011
76. Blackwood DH, Fordyce A, Walker MT, St Clair DM, Porteous DJ, Muir WJ. Schizophrenia and affective disorders – cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. *Am J Hum Genet*. 2001; 69:428–433. [PubMed: 11443544]

77. Bray NJ. Gene expression in the etiology of schizophrenia. *Schizophr Bull.* 2008; 34:412–418. [PubMed: 18334509]
78. Karssen AM, Li JZ, Her S, Patel PD, Meng F, Evans SJ, Vawter MP, Tomita H, Choudary PV, Bunney WE Jr, Jones EG, Watson SJ, Akil H, Myers RM, Schatzberg AF, Lyons DM. Application of microarray technology in primate behavioral neuroscience research. *Methods.* 2006; 38:227–234. [PubMed: 16469505]
79. Newton SS, Bennett A, Duman RS. Production of custom microarrays for neuroscience research. *Methods.* 2005; 37:238–246. [PubMed: 16308153]
80. Uranova NA, Vostrikov VM, Vikhreva OV, Zimina IS, Kolomeets NS, Orlovskaya DD. The role of oligodendrocyte pathology in schizophrenia. *Int J Neuropsychopharmacol.* 2007; 10:537–545. [PubMed: 17313698]
81. Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, Haroutunian V, Fienberg AA. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proc Natl Acad Sci USA.* 2001; 98:4746–4751. [PubMed: 11296301]
82. Sugai T, Kawamura M, Iritani S, Araki K, Makifuchi T, Imai C, Nakamura R, Kakita A, Takahashi H, Nawa H. Prefrontal abnormality of schizophrenia revealed by DNA microarray: impact on glial and neurotrophic gene expression. *Ann N Y Acad Sci.* 2004; 1025:84–91. [PubMed: 15542704]
83. Tkachev D, Mimmack ML, Ryan MM, Wayland M, Freeman T, Jones PB, Starkey M, Webster MJ, Yolken RH, Bahn S. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet.* 2003; 362:798–805. [PubMed: 13678875]
84. Coplan JD, Abdallah CG, Tang CY, Mathew SJ, Martinez J, Hof PR, Smith EL, Dwork AJ, Perera TD, Pantol G, Carpenter D, Rosenblum LA, Shungu DC, Gelernter J, Kaffman A, Jackowski A, Kaufman J, Gorman JM. The role of early life stress in development of the anterior limb of the internal capsule in non-human primates. *Neurosci Lett.* 2010; 480:93–96. [PubMed: 20541590]
85. Cotter D, Mackay D, Chana G, Beasley C, Landau S, Everall IP. Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. *Cereb Cortex.* 2002; 12:386–394. [PubMed: 11884354]
86. De Bellis MD, Keshavan MS, Beers SR, Hall J, Frustaci K, Masalehdan A, Noll J, Boring AM. Sex differences in brain maturation during childhood and adolescence. *Cereb Cortex.* 2001; 11:552–557. [PubMed: 11375916]
87. De Bellis MD, Keshavan MS, Shifflett H, Iyengar S, Beers SR, Hall J, Moritz G. Brain structures in pediatric maltreatment-related posttraumatic stress disorder: a sociodemographically matched study. *Biol Psychiatry.* 2002; 52:1066–1078. [PubMed: 12460690]
88. Vataja R, Pohjasvaara T, Leppavuori A, Mantyla R, Aronen HJ, Salonen O, Kaste M, Erkinjuntti T. Magnetic resonance imaging correlates of depression after ischemic stroke. *Arch Gen Psychiatry.* 2001; 58:925–931. [PubMed: 11576030]
89. Walterfang M, Yucel M, Barton S, Reutens DC, Wood AG, Chen J, Lorenzetti V, Velakoulis D, Pantelis C, Allen NB. Corpus callosum size and shape in individuals with current and past depression. *J Affect Disord.* 2009; 115:411–420. [PubMed: 19019454]
90. Aston C, Jiang L, Sokolov BP. Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. *Mol Psychiatry.* 2005; 10:309–322. [PubMed: 15303102]
91. Fisher SE, Lai CS, Monaco AP. Deciphering the genetic basis of speech and language disorders. *Annu Rev Neurosci.* 2003; 26:57–80. [PubMed: 12524432]
92. Enard W, Przeworski M, Fisher SE, Lai CS, Wiebe V, Kitano T, Monaco AP, Paabo S. Molecular evolution of FOXP2, a gene involved in speech and language. *Nature.* 2002; 418:869–872. [PubMed: 12192408]
93. Konopka G, Bomar JM, Winden K, Coppola G, Jonsson ZO, Gao F, Peng S, Preuss TM, Wohlschlegel JA, Geschwind DH. Human-specific transcriptional regulation of CNS development genes by FOXP2. *Nature.* 2009; 462:213–217. [PubMed: 19907493]
94. Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, Maner S, Massa H, Walker M, Chi M, Navin N, Lucito R, Healy J, Hicks J, Ye K, Reiner A, Gilliam TC, Trask B, Patterson N, Zetterberg A, Wigler M. Large-scale copy number polymorphism in the human genome. *Science.* 2004; 305:525–528. [PubMed: 15273396]

95. Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C. Detection of large-scale variation in the human genome. *Nat Genet.* 2004; 36:949–951. [PubMed: 15286789]
96. Schizophrenia Consortium. Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature.* 2008; 455:237–241. [PubMed: 18668038]
97. Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, Nord AS, Kusenda M, Malhotra D, Bhandari A, Stray SM, Rippey CF, Roccanova P, Makarov V, Lakshmi B, Findling RL, Sikich L, Stromberg T, Merriman B, Gogtay N, Butler P, Eckstrand K, Noory L, Gochman P, Long R, Chen Z, Davis S, Baker C, Eichler EE, Meltzer PS, Nelson SF, Singleton AB, Lee MK, Rapoport JL, King MC, Sebat J. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science.* 2008; 320:539–543. [PubMed: 18369103]
98. Rujescu D, Ingason A, Cichon S, Pietilainen OP, Barnes MR, Touloupoulou T, Picchioni M, Vassos E, Ettinger U, Bramon E, Murray R, Ruggeri M, Tosato S, Bonetto C, Steinberg S, Sigurdsson E, Sigmundsson T, Petursson H, Gylfason A, Olason PI, Hardarsson G, Jonsdottir GA, Gustafsson O, Fossdal R, Giegling I, Moller HJ, Hartmann AM, Hoffmann P, Crombie C, Fraser G, Walker N, Lonnqvist J, Suvisaari J, Tuulio-Henriksson A, Djurovic S, Melle I, Andreassen OA, Hansen T, Werge T, Kiemenev LA, Franke B, Veltman J, Buizer-Voskamp JE, Sabatti C, Ophoff RA, Rietschel M, Nothen MM, Stefansson K, Peltonen L, St Clair D, Stefansson H, Collier DA. Disruption of the neurexin 1 gene is associated with schizophrenia. *Hum Mol Genet.* 2009; 18:988–996. [PubMed: 18945720]
99. Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, Steinberg S, Fossdal R, Sigurdsson E, Sigmundsson T, Buizer-Voskamp JE, Hansen T, Jakobsen KD, Muglia P, Francks C, Matthews PM, Gylfason A, Halldorsson BV, Gudbjartsson D, Thorgeirsson TE, Sigurdsson A, Jonasdottir A, Bjornsson A, Mattiasdottir S, Blondal T, Haraldsson M, Magnusdottir BB, Giegling I, Moller HJ, Hartmann A, Shianna KV, Ge D, Need AC, Crombie C, Fraser G, Walker N, Lonnqvist J, Suvisaari J, Tuulio-Henriksson A, Paunio T, Touloupoulou T, Bramon E, Di Forti M, Murray R, Ruggeri M, Vassos E, Tosato S, Walshe M, Li T, Vasilescu C, Muhleisen TW, Wang AG, Ullum H, Djurovic S, Melle I, Olesen J, Kiemenev LA, Franke B, Sabatti C, Freimer NB, Gulcher JR, Thorsteinsdottir U, Kong A, Andreassen OA, Ophoff RA, Georgi A, Rietschel M, Werge T, Petursson H, Goldstein DB, Nothen MM, Peltonen L, Collier DA, St Clair D, Stefansson K. Large recurrent microdeletions associated with schizophrenia. *Nature.* 2008; 455:232–236. [PubMed: 18668039]
100. Muir WJ, Pickard BS, Blackwood DH. Disrupted-in-Schizophrenia-1. *Curr Psychiatry Rep.* 2008; 10:140–147. [PubMed: 18474207]
101. Lifton RP. Individual genomes on the horizon. *N Engl J Med.* 2010; 362:1235–1236. [PubMed: 20220178]
102. Kircher M, Kelso J. High-throughput DNA sequencing – concepts and limitations. *Bioessays.* 32:524–536. [PubMed: 20486139]
103. Bilguvar K, Ozturk AK, Louvi A, Kwan KY, Choi M, Tatli B, Yalnizoglu D, Tuysuz B, Caglayan AO, Gokben S, Kaymakcalan H, Barak T, Bakircioglu M, Yasuno K, Ho W, Sanders S, Zhu Y, Yilmaz S, Dincer A, Johnson MH, Bronen RA, Kocer N, Per H, Mane S, Pamir MN, Yalcinkaya C, Kumandas S, Topcu M, Ozmen M, Sestan N, Lifton RP, State MW, Gunel M. Whole-exome sequencing identifies recessive WDR62 mutations in severe brain malformations. *Nature.* 2010; 467:207–210. [PubMed: 20729831]
104. Choi M, Scholl UI, Ji W, Liu T, Tikhonova IR, Zumbo P, Nayir A, Bakkaloglu A, Ozen S, Sanjad S, Nelson-Williams C, Farhi A, Mane S, Lifton RP. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. *Proc Natl Acad Sci USA.* 2009; 106:19096–19101. [PubMed: 19861545]
105. Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, Beck AE, Tabor HK, Cooper GM, Mefford HC, Lee C, Turner EH, Smith JD, Rieder MJ, Yoshiura K, Matsumoto N, Ohta T, Niikawa N, Nickerson DA, Bamshad MJ, Shendure J. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nat Genet.* 42:790–793. [PubMed: 20711175]

106. Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, Shendure J, Bamshad MJ. Exome sequencing identifies the cause of a mendelian disorder. *Nat Genet.* 42:30–35. [PubMed: 19915526]
107. Ng SB, Turner EH, Robertson PD, Flygare SD, Bigham AW, Lee C, Shaffer T, Wong M, Bhattacharjee A, Eichler EE, Bamshad M, Nickerson DA, Shendure J. Targeted capture and massively parallel sequencing of 12 human exomes. *Nature.* 2009; 461:272–276. [PubMed: 19684571]
108. Brunetti-Pierri N, Berg JS, Scaglia F, Belmont J, Bacino CA, Sahoo T, Lalani SR, Graham B, Lee B, Shinawi M, Shen J, Kang SH, Pursley A, Lotze T, Kennedy G, Lansky-Shafer S, Weaver C, Roeder ER, Grebe TA, Arnold GL, Hutchison T, Reimschisel T, Amato S, Geraghty MT, Innis JW, Obersztyn E, Nowakowska B, Rosengren SS, Bader PI, Grange DK, Naqvi S, Garnica AD, Bernes SM, Fong CT, Summers A, Walters WD, Lupski JR, Stankiewicz P, Cheung SW, Patel A. Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities. *Nat Genet.* 2008; 40:1466–1471. [PubMed: 19029900]
109. Kwon I, Schaffer DV. Designer gene delivery vectors: molecular engineering and evolution of adeno-associated viral vectors for enhanced gene transfer. *Pharm Res.* 2008; 25:489–499. [PubMed: 17763830]
110. Hommel JD, Sears RM, Georgescu D, Simmons DL, DiLeone RJ. Local gene knockdown in the brain using viral-mediated RNA interference. *Nat Med.* 2003; 9:1539–1544. [PubMed: 14634645]
111. McCown TJ. The future of epilepsy treatment: focus on adeno-associated virus vector gene therapy. *Drug News Perspect.* 2010; 23:281–286. [PubMed: 20603651]
112. Chen G, Twyman R, Manji HK. p11 and gene therapy for severe psychiatric disorders: a practical goal? *Sci Transl Med.* 2010; 2:54ps51.
113. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007; 131:861–872. [PubMed: 18035408]
114. Hotta A, Cheung AY, Farra N, Vijayaragavan K, Seguin CA, Draper JS, Pasceri P, Maksakova IA, Mager DL, Rossant J, Bhatia M, Ellis J. Isolation of human iPS cells using EOS lentiviral vectors to select for pluripotency. *Nat Methods.* 2009; 6:370–376. [PubMed: 19404254]
115. Jacobs PA, Aitken J, Frackiewicz A, Law P, Newton MS, Smith PG. The inheritance of translocations in man: data from families ascertained through a balanced heterozygote. *Ann Hum Genet.* 1970; 34:119–136. [PubMed: 5493841]
116. St Clair D, Blackwood D, Muir W, Carothers A, Walker M, Spowart G, Gosden C, Evans HJ. Association within a family of a balanced autosomal translocation with major mental illness. *Lancet.* 1990; 336:13–16. [PubMed: 1973210]
117. Millar JK, Christie S, Anderson S, Lawson D, Hsiao-Wei Loh D, Devon RS, Arveiler B, Muir WJ, Blackwood DH, Porteous DJ. Genomic structure and localisation within a linkage hotspot of Disrupted In Schizophrenia 1, a gene disrupted by a translocation segregating with schizophrenia. *Mol Psychiatry.* 2001; 6:173–178. [PubMed: 11317219]
118. Brandon NJ, Handford EJ, Schurov I, Rain JC, Pelling M, Duran-Jimeniz B, Camargo LM, Oliver KR, Behr D, Shearman MS, Whiting PJ. Disrupted in Schizophrenia 1 and Nudel form a neurodevelopmentally regulated protein complex: implications for schizophrenia and other major neurological disorders. *Mol Cell Neurosci.* 2004; 25:42–55. [PubMed: 14962739]
119. Brandon NJ, Millar JK, Korth C, Sive H, Singh KK, Sawa A. Understanding the role of DISC1 in psychiatric disease and during normal development. *J Neurosci.* 2009; 29:12768–12775. [PubMed: 19828788]
120. Blackwood DH, Glabus MF, Dunan J, O'Carroll RE, Muir WJ, Ebmeier KP. Altered cerebral perfusion measured by SPECT in relatives of patients with schizophrenia. Correlations with memory and P300. *Br J Psychiatry.* 1999; 175:357–366. [PubMed: 10789304]
121. O'Donnell BF, McCarley RW, Potts GF, Salisbury DF, Nestor PG, Hirayasu Y, Niznikiewicz MA, Barnard J, Shen ZJ, Weinstein DM, Bookstein FL, Shenton ME. Identification of neural circuits underlying P300 abnormalities in schizophrenia. *Psychophysiology.* 1999; 36:388–398. [PubMed: 10352563]

122. Turetsky BI, Cannon TD, Gur RE. P300 subcomponent abnormalities in schizophrenia: III. Deficits in unaffected siblings of schizophrenic probands. *Biol Psychiatry*. 2000; 47:380–390. [PubMed: 10704950]
123. Clapcote SJ, Lipina TV, Millar JK, Mackie S, Christie S, Ogawa F, Lerch JP, Trimble K, Uchiyama M, Sakuraba Y, Kaneda H, Shiroishi T, Houslay MD, Henkelman RM, Sled JG, Gondo Y, Porteous DJ, Roder JC. Behavioral phenotypes of Disc1 missense mutations in mice. *Neuron*. 2007; 54:387–402. [PubMed: 17481393]
124. Niwa M, Kamiya A, Murai R, Kubo K, Gruber AJ, Tomita K, Lu L, Tomisato S, Jaaro-Peled H, Seshadri S, Hiyama H, Huang B, Kohda K, Noda Y, O'Donnell P, Nakajima K, Sawa A, Nabeshima T. Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits. *Neuron*. 2010; 65:480–489. [PubMed: 20188653]
125. Kamiya A, Kubo K, Tomoda T, Takaki M, Youn R, Ozeki Y, Sawamura N, Park U, Kudo C, Okawa M, Ross CA, Hatten ME, Nakajima K, Sawa A. A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. *Nat Cell Biol*. 2005; 7:1167–1178. [PubMed: 16299498]
126. Morris JA, Kandpal G, Ma L, Austin CP. DISC1 (Disrupted-In-Schizophrenia 1) is a centrosome-associated protein that interacts with MAP1A, MIPT3, ATF4/5 and NUDEL: regulation and loss of interaction with mutation. *Hum Mol Genet*. 2003; 12:1591–1608. [PubMed: 12812986]
127. Koike H, Arguello PA, Kvajo M, Karayiorgou M, Gogos JA. Disc1 is mutated in the 129 S6/SvEv strain and modulates working memory in mice. *Proc Natl Acad Sci USA*. 2006; 103:3693–3697. [PubMed: 16484369]
128. Benes FM, Taylor JB, Cunningham MC. Convergence and plasticity of monoaminergic systems in the medial pre-frontal cortex during the postnatal period: implications for the development of psychopathology. *Cereb Cortex*. 2000; 10:1014–1027. [PubMed: 11007552]
129. National Research Council (US) and Institute of Medicine (US) Committee on the Prevention of Mental Disorders and Substance Abuse Among Children, Y., and Young Adults: Research Advances and Promising Interventions. Preventing mental, emotional and behavioral disorders among young people: progress and possibilities. National Academies Press; US, Washington (DC): 2009.
130. Gilbert R, Widom CS, Browne K, Fergusson D, Webb E, Janson S. Burden and consequences of child maltreatment in high-income countries. *Lancet*. 2009; 373:68–81. [PubMed: 19056114]
131. Kaffman A, Meaney M. Neurodevelopmental Sequelae of Postnatal Maternal Care in Rodents: Clinical and Research Implications of Molecular Insights. *J Child Psychol Psychiatry*. 2007; 48:224–244. [PubMed: 17355397]
132. Nemeroff CB, Heim CM, Thase ME, Klein DN, Rush AJ, Schatzberg AF, Ninan PT, McCullough JP Jr, Weiss PM, Dunner DL, Rothbaum BO, Kornstein S, Keitner G, Keller MB. Differential responses to psychotherapy versus pharmacotherapy in patients with chronic forms of major depression and childhood trauma. *Proc Natl Acad Sci U S A*. 2003; 100:14293–14296. [PubMed: 14615578]
133. Zeanah CH, Egger HL, Smyke AT, Nelson CA, Fox NA, Marshall PJ, Guthrie D. Institutional rearing and psychiatric disorders in Romanian preschool children. *Am J Psychiatry*. 2009; 166:777–785. [PubMed: 19487394]
134. Champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiol Behav*. 2003; 79:359–371. [PubMed: 12954431]
135. Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annu Rev Neurosci*. 2001; 24:1161–1192. [PubMed: 11520931]
136. Szyf M, McGowan P, Meaney MJ. The social environment and the epigenome. *Environ Mol Mutagen*. 2008; 49:46–60. [PubMed: 18095330]
137. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci*. 2004; 7:847–854. [PubMed: 15220929]

138. Weaver IC, D'Alessio AC, Brown SE, Hellstrom IC, Dymov S, Sharma S, Szyf M, Meaney MJ. The transcription factor nerve growth factor-inducible protein a mediates epigenetic programming: altering epigenetic marks by immediate-early genes. *J Neurosci.* 2007; 27:1756–1768. [PubMed: 17301183]
139. Weaver IC, Champagne FA, Brown SE, Dymov S, Sharma S, Meaney MJ, Szyf M. Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. *J Neurosci.* 2005; 25:11045–11054. [PubMed: 16306417]
140. McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, Turecki G, Meaney MJ. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci.* 2009; 12:342–348. [PubMed: 19234457]
141. Moffitt TE, Harrington H, Caspi A, Kim-Cohen J, Goldberg D, Gregory AM, Poulton R. Depression and generalized anxiety disorder: cumulative and sequential comorbidity in a birth cohort followed prospectively to age 32 years. *Arch Gen Psychiatry.* 2007; 64:651–660. [PubMed: 17548747]
142. Caldji C, Diorio J, Meaney MJ. Variations in maternal care alter GABA(A) receptor subunit expression in brain regions associated with fear. *Neuropsychopharmacology.* 2003; 28:1950–1959. [PubMed: 12888776]
143. Caldji C, Francis D, Sharma S, Plotsky PM, Meaney MJ. The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. *Neuropsychopharmacology.* 2000; 22:219–229. [PubMed: 10693149]
144. Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc Natl Acad Sci USA.* 1998; 95:5335–5340. [PubMed: 9560276]
145. Francis DD, Caldji C, Champagne F, Plotsky PM, Meaney MJ. The role of corticotropin-releasing factor – nor-epinephrine systems in mediating the effects of early experience on the development of behavioral and endocrine responses to stress. *Biol Psychiatry.* 1999; 46:1153–1166. [PubMed: 10560022]
146. Francis DD, Meaney MJ. Maternal care and the development of stress responses. *Curr Opin Neurobiol.* 1999; 9:128–134. [PubMed: 10072372]
147. Meaney MJ, Bhatnagar S, Larocque S, McCormick C, Shanks N, Sharma S, Smythe J, Viau V, Plotsky PM. Individual differences in the hypothalamic-pituitary-adrenal stress response and the hypothalamic CRF system. *Ann N Y Acad Sci.* 1993; 697:70–85. [PubMed: 8257024]
148. Anisman H, Zaharia MD, Meaney MJ, Merali Z. Do early-life events permanently alter behavioral and hormonal responses to stressors? *Int J Dev Neurosci.* 1998; 16:149–164. [PubMed: 9785112]
149. Champagne F, Diorio J, Sharma S, Meaney MJ. Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proc Natl Acad Sci USA.* 2001; 98:12736–12741. [PubMed: 11606726]
150. Champagne FA, Chretien P, Stevenson CW, Zhang TY, Gratton A, Meaney MJ. Variations in nucleus accumbens dopamine associated with individual differences in maternal behavior in the rat. *J Neurosci.* 2004; 24:4113–4123. [PubMed: 15115806]
151. Champagne FA, Weaver IC, Diorio J, Dymov S, Szyf M, Meaney MJ. Maternal Care Associated with Methylation of the Estrogen Receptor- α 1b Promoter and Estrogen Receptor- α Expression in the Medial Preoptic Area of Female Offspring. *Endocrinology.* 2006; 147:2909–2915. [PubMed: 16513834]
152. Champagne FA, Weaver IC, Diorio J, Sharma S, Meaney MJ. Natural variations in maternal care are associated with estrogen receptor α expression and estrogen sensitivity in the medial preoptic area. *Endocrinology.* 2003; 144:4720–4724. [PubMed: 12959970]
153. Parent CI, Meaney MJ. The influence of natural variations in maternal care on play fighting in the rat. *Dev Psychobiol.* 2008; 50:767–776. [PubMed: 18846499]
154. Bredy TW, Grant RJ, Champagne DL, Meaney MJ. Maternal care influences neuronal survival in the hippocampus of the rat. *Eur J Neurosci.* 2003; 18:2903–2909. [PubMed: 14656341]

155. Bredy TW, Humpartzoomian RA, Cain DP, Meaney MJ. Partial reversal of the effect of maternal care on cognitive function through environmental enrichment. *Neuroscience*. 2003; 118:571–576. [PubMed: 12699791]
156. Bredy TW, Zhang TY, Grant RJ, Diorio J, Meaney MJ. Peripubertal environmental enrichment reverses the effects of maternal care on hippocampal development and glutamate receptor subunit expression. *Eur J Neurosci*. 2004; 20:1355–1362. [PubMed: 15341607]
157. Champagne DL, Bagot RC, van Hasselt F, Ramakers G, Meaney MJ, de Kloet ER, Joels M, Krugers H. Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. *J Neurosci*. 2008; 28:6037–6045. [PubMed: 18524909]
158. Liu D, Diorio J, Day JC, Francis DD, Meaney MJ. Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nat Neurosci*. 2000; 3:799–806. [PubMed: 10903573]
159. Caldji C, Diorio J, Meaney MJ. Variations in maternal care in infancy regulate the development of stress reactivity. *Biol Psychiatry*. 2000; 48:1164–1174. [PubMed: 11137058]
160. Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science*. 1999; 286:1155–1158. [PubMed: 10550053]
161. Laplante P, Diorio J, Meaney MJ. Serotonin regulates hippocampal glucocorticoid receptor expression via a 5-HT7 receptor. *Brain Res Dev Brain Res*. 2002; 139:199–203.
162. Liu D, Caldji C, Sharma S, Plotsky PM, Meaney MJ. Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinephrine release in the hypothalamic paraventricular nucleus. *J Neuroendocrinol*. 2000; 12:5–12. [PubMed: 10692138]
163. Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*. 1997; 277:1659–1662. [PubMed: 9287218]
164. Meaney MJ, Aitken DH. The effects of early postnatal handling on hippocampal glucocorticoid receptor concentrations: temporal parameters. *Brain Res*. 1985; 354:301–304. [PubMed: 4052820]
165. Weaver IC, La Plante P, Weaver S, Parent A, Sharma S, Diorio J, Chapman KE, Seckl JR, Szyf M, Meaney MJ. Early environmental regulation of hippocampal glucocorticoid receptor gene expression: characterization of intracellular mediators and potential genomic target sites. *Mol Cell Endocrinol*. 2001; 185:205–218. [PubMed: 11738810]
166. Zhang TY, Chretien P, Meaney MJ, Gratton A. Influence of naturally occurring variations in maternal care on prepulse inhibition of acoustic startle and the medial prefrontal cortical dopamine response to stress in adult rats. *J Neurosci*. 2005; 25:1493–1502. [PubMed: 15703403]
167. Zhang TY, Hellstrom IC, Bagot RC, Wen X, Diorio J, Meaney MJ. Maternal care and DNA methylation of a glutamic acid decarboxylase 1 promoter in rat hippocampus. *J Neurosci*. 2008; 28:13130–13137. [PubMed: 20881131]
168. Huot RL, Thiruvikraman KV, Meaney MJ, Plotsky PM. Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. *Psychopharmacology (Berl)*. 2001; 158:366–373. [PubMed: 11797057]
169. Meaney MJ, Brake W, Gratton A. Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? *Psychoneuroendocrinology*. 2002; 27:127–138. [PubMed: 11750774]
170. Francis DD, Kuhar MJ. Frequency of maternal licking and grooming correlates negatively with vulnerability to cocaine and alcohol use in rats. *Pharmacol Biochem Behav*. 2008; 90:497–500. [PubMed: 18508115]
171. Brake WG, Zhang TY, Diorio J, Meaney MJ, Gratton A. Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *Eur J Neurosci*. 2004; 19:1863–1874. [PubMed: 15078560]

Fig. 1.

Animal work should play a central role in the development of more effective interventions for mental illness. Advances in sequencing technologies provide a growing list of candidate genes implicated in mental illness (step 1). Genes implicated in mental illness are used to define endophenotypes (step 2) that segregate with the genetic biomarker. Endophenotypes should also inform about pathophysiology (step 3) and help guide interventions (step 4) with improved clinical outcome (step 5). Work in animals plays a central role in the transition along steps 1–5. This includes characterization of neurodevelopmental pathways by which genes identified in step 2 affect brain function and adult behavior. Such work identifies additional genes implicated in these behavioral changes (step 6), helps define possible endophenotypes (step 7), informs about pathological changes (step 8), and helps in the development of novel diagnostic and interventional strategies (step 9).

Table 1

Alterations in levels of postnatal maternal care are associated with a broad range of behavioral changes in adulthood

Behavior	Circuit examined	Genes involved	References
Anxiety-like behavior	Amygdala-brain stem NE systems, such as NTS/LC	GABA-A $\gamma 2$ receptor, adrenoreceptor $\alpha 2$, CRF receptor	(142–147)
Maternal care	MPA–VTA–NAc	Oxytocin/oxytocin receptor and estrogen receptor	(148–152)
Play behavior	Unknown	Unknown	(153)
Hippocampal-dependent memory	Hippocampus	NMDA receptor subunits NR1, NR2A, NR2B, synaptophysin, acetylcholine esterase, BDNF	(154–158)
HPA reactivity	Hippocampus–PVN _h –pituitary	GR, MR, CRF	(137–139, 147, 148, 159–165)
Prepulse inhibition	mPFX	DA, COMT	(166, 167)
Substance abuse/reward	NAc	DAT, DA	(168–171)

Abbreviations: *BDNF* brain-derived neurotrophic factor, *CRF* corticotropin-releasing factor, *COMT* catechol-*O*-methyltransferase, *DA* dopamine, *DAT* dopamine transporter, *GABA* gamma-aminobutyric acid, *GR* glucocorticoid receptor, *MPA* medial preoptic area, *MR* mineralocorticoid receptor, *Nac* nucleus accumbens, *NE* norepinephrine, *NMDA* *N*-methyl-D-aspartate, *LC* locus ceruleus, *mPFX* medial prefrontal cortex, *NTS* nucleus tractus solitarius, *PVN_h* paraventricular nucleus of the hypothalamus, *VTA* ventral tegmental area