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Pathophysiological Toolkit

Genes to Circuits

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Abstract

Understanding the etiology and pathophysiology of neuropsychiatric disease requires the development of new tools (ranging from evolving diagnostic strategies to biomarkers) that can address the unique challenges of neuropsychiatric disease, including the current lack of tractable interfaces between what we can learn in the clinic and the tools available using model systems in the laboratory. This chapter outlines some of these tools, addressing pitfalls and opportunities, while acknowledging the iterative nature of bridging the gaps between different levels of inquiry.

How Do We Diagnose Human Brain Diseases?

The diagnosis of human brain diseases has historically relied on relating anatomical pathology to clinical presentation. In cases where gross anatomical changes are not apparent (e.g., psychiatric disorders), diagnosis is made exclusively based on signs and symptoms. Mismatches between these measures, as well as the more recent appreciation of extensive pleiotropy across brain disorders, underscore concerns about the lack of the etiological and pathophysiological validity of current diagnostic classification systems. Not surprisingly, then, pharmaceutical companies have recently withdrawn *en masse* from drug development for brain disease, citing the lack of good targets with genetic and pathophysiological validation that can be related to the symptom-based diagnostic categories and the heterogeneity of patients within a given disease category. If we are to proceed successfully with research on the etiology and

pathogenesis of brain diseases, improve individual patient diagnosis, and develop novel mechanism-based therapeutics, we need new approaches to disease classification that take these concerns into account. In recognition of this need, two wide-scale projects have recently been initiated: the Research Domain Criteria (RDoC) project, based in the United States, and the Human Brain Project (HBP), based in Europe. Here, we will briefly outline the basic approaches of these projects and highlight advantages and limitations of each.

The Research Domain Criteria Project

In early 2009, the National Institutes of Mental Health (NIMH) initiated the RDoC project[1] with the intent of creating a research system to support studies that will ultimately improve diagnosis and treatment. Mental disorders are increasingly being viewed as neurodevelopmental disorders that become manifest within and among neural circuits as a result of a fundamental biological risk and its interaction with various environmental contexts across development. RDoC reflects this perspective and is built around neural systems posited within five major domains of function:

1. negative valence (i.e., systems which respond to threats and other aversive situations),
2. positive valence (related to various aspects of reward and appetitive behavior),
3. cognitive systems,
4. social processes, and
5. arousal/regulatory systems.

These functional systems have been termed "constructs" to denote their status as noncomputable concepts, the nature and perceived function of which change over time with advancing research. Because the constructs are inherently dimensional, RDoC research designs emphasize using a wide range of subjects for research studies, to include both those with levels of psychopathology of comparable severity to disorders defined by current nosology (i.e., the Diagnostic and Statistical Manual of Psychiatric Disorders, 5th edition, DSM-5) as well as subjects representative of a broad range along the distribution of function within the population. Investigators are expected to specify their sampling frame clearly and to measure each construct with an array of measures. This might include genetics, molecular/cellular processes, neural circuit measures, other physiological measures, behavior, and self- or interviewer-reports, which, at the extreme nonfunctional end of a distribution, would comprise symptoms.

[1] http://www.nimh.nih.gov/research-priorities/rdoc/index.shtml (accessed March 2, 2015)

The withdrawal of pharmaceutical companies from psychiatric drug development occurred coincidentally at about the same time as the RDoC project was initiated. As a result, the RDoC framework has somewhat unexpectedly become an important component of the new experimental medicine approach to early clinical trials at NIMH. This paradigm emphasizes an RDoC-like specification of the clinical sample and a strong hypothesis-based approach to a particular CNS mechanism. Under this rubric, an investigator must:

1. demonstrate adequate engagement of the drug under investigation with the hypothesized molecular target, e.g., with a positron emission tomography (PET) ligand (Wietek et al. 2014), but with other CNS measures when PET ligands are not available;
2. determine appropriate dosing;
3. ideally demonstrate that adequate levels of target engagement result in a relevant change in a brain or behavioral measure (e.g., change in fMRI signal, event-related potential, or behavioral task); and
4. relate these measures to a preliminary clinical efficacy signal.

A "fail" at any stage of this "fast-fail" paradigm results in the discontinuation of the study series, thus saving time and funds for another target. More importantly, this allows the hypothesis concerning the proposed modification (e.g., agonist, antagonist) of the chosen target to be tested. Too often, in the recent history of CNS pharmacology, there has been no convincing test of such hypotheses (e.g., due to lack of evidence of target engagement or pathway modification), and this has led companies to return repeatedly to the same target, even in the face of multiple negative studies. Critically, emphasis is on targeting specified neural mechanisms and their behavioral outputs, rather than heterogeneous DSM disorders. This feature is highly consonant with the RDoC framework. A critical aspect of the early trials is to enroll subjects on the basis of their measurable deficits or impairment in the systems being tested, not simply because they are members of a particular diagnostic category. Clinical outcome is measured by assessing the mechanism of interest in addition to clinical symptom reduction or improvements in functioning. In this manner, NIMH hopes to provide more homogeneous patient groups to improve the chances of detecting a successful outcome during the early stages of treatment development.

An important caveat to this approach is that the RDoC is a research framework, not a completely specified system. Thus, the constructs and measurement units should be considered as a guide to research, rather than a replacement for extant diagnostic paradigms. Although the RDoC project is still at an early stage, the number of funded grants using RDoC constructs, instead of DSM categories, has increased steadily and is expected to accelerate in the future.

The Human Brain Project

Launched in 2013, and funded by the European Union, the HBP[2] is an initiative to build a working computer model of the human brain by 2023. Drawing on technical concepts pioneered in other areas of science and technology (e.g., simulation modeling, distributed query engines, real-time data visualization, statistics, and data mining algorithms), this approach aims to federate and integrate existing clinical and basic neuroscience data, with the proximate goal of redefining disease diagnosis—moving away from a symptom-based classification to one based on patterns of biological abnormality. Ultimately, the HBP aims to use information about how the brain is structured and organized to facilitate design and production of neuromorphic computing systems.

One initial focus of the HBP is to analyze and cluster available patient data to develop diagnostics and personalized treatments by identifying unique biological signatures of disease. Such signatures, it is hoped, will permit the "cleanup" of populations recruited for clinical trials. This "big-data" approach may also help in redefining nosology, by pointing out symptom clusters that have not been considered in the current framework. A recent study by Denny et al. (2013) has demonstrated the feasibility of a large-scale application of the phenome-wide association study paradigm within electronic medical records. Especially when linked to computational approaches, such as those implemented in the HBP, and incorporating neuroimaging methods, this could highlight new mechanisms and syndromal clusters that are relevant for treatment.

Future iterations of the HBP approach will attempt to use simulations based on such disease signatures to enable development of new drug discovery paradigms. The HBP will make its tools and data freely available to participating scientists, clinical researchers, and industries, who are prepared to share their data within a dedicated network through six technology platforms that provide a new model for very large-scale international scientific collaboration in the life and health sciences. Despite enthusiasm for this approach, some have voiced concern that available data sets, particularly for neuropsychiatry, are information-poor, and that the results of this model-free analysis will be insufficient to provide a mechanistic understanding of human brain disorders.

Other Considerations

Current diagnoses are based on the assumption that a criterion set is being matched to the clinical picture observed in an interview with the patient. However, there is good evidence that the clinical picture is highly variable over time and that there may be much interesting information, not so much in the cross-sectional picture at any given point in time, but rather in the variability of psycho(patho)logy in relationship to the ongoing environmental experience.

[2] https://www.humanbrainproject.eu (accessed March 2, 2015)

Advances in environmental assessment technology, including wearable computing devices, intelligent textiles, and the technology referred to as the "internet of things," should be harnessed to give clinicians a richer understanding of what their patients actually experience in the real world, and how their behavior changes in response to treatment.

Conclusions

The long-term goal of diagnosis is treatment, prognosis, and continuing the dialogue between clinical neuropsychiatry and basic neuroscience. Historical divisions between psychiatry and neurology should eventually be abandoned. Data collection should be informed by recent advances in genetics and neurobiology and prioritized over re-sorting extant data. Techniques that incorporate the molecular basis of disease into traditional diagnostic tools should continue to be developed.

How Do We Identify and Utilize Biomarkers?

Broadly defined, a biomarker is a measured characteristic that serves as an indicator of disease, a pathophysiologic process, or response to therapy. Biomarkers can be used in many different settings which may not be mutually exclusive. For example, a biomarker may report the status of the underlying pathophysiology, serve to delineate subcategories of disease, act as a reporter of a therapeutic intervention, or, in some cases, even act as a surrogate endpoint. This last category will receive special consideration here, since the definition of endpoints for clinical trials has been a major limitation for development of new therapies for both neurodegenerative and neurodevelopmental disorders.

Selective biomarkers may provide surrogate endpoints, but this requires that the biomarker meets specific criteria. To qualify as a surrogate, the biomarker must reflect the underlying pathophysiologic process of the disease. In addition, the tested intervention must affect the biomarker, and the intervention must affect the biomarker without altering the underlying intervention. The degree of the effect of the biomarker must be sufficient to measure clinical outcome and not just reach statistical significance. The timing of the effect on the biomarker must be appropriate for the length of the clinical trial. Finally, a surrogate must reflect toxicity of an intervention. This last criterion frequently prevents a biomarker from acting as a surrogate endpoint for a clinical study. Nevertheless, even if this criterion is not met, a biomarker could provide an important measure of target engagement or efficacy of the intervention.

In comparison with psychiatric diseases, neurological diseases have been more amenable to biomarker identification and utilization, although few are used in routine clinical practice. To provide a roadmap and to highlight pitfalls

for discovery, a number of examples from the neurology literature are examined below to illustrate the problem and possible solutions.

Parkinson Disease

Molecular imaging of the nigrostriatal pathway has used several tracers, including FDOPA, DAT (dopamine transporter) radioligands, or VMAT2 (vesicular monoamine transporter type 2) radioligands to measure presynaptic terminals of nigrostriatal neurons in the dorsal striatum. These have been used in clinical trials to provide insights into the possible efficacy of drugs that may reduce progression of Parkinson disease (PD). However, almost all of these clinical trials found discordant results between changes in the striatal uptake of these radiotracers and a clinical endpoint, as measured by a change in a PD motor rating scale. Several problems may have contributed to this. Of course, the rating scales are far from perfect. In addition, a convincing validation of any of these radiotracers, which would demonstrate that they were sensitive to changes in numbers of nigrostriatal neurons, has been lacking. In fact, this has only been completed recently in a nonhuman primate model of nigrostriatal neuronal loss induced by variable amounts of the selective dopaminergic neuronal toxin MPTP (Karimi et al. 2013). These studies demonstrated that the PET measures of the striatal uptake of each of these tracers correlates well with striatal dopamine as measured by high-performance liquid chromatography, with *in vitro* quantitative autoradiography of DAT or VMAT2 sites in striatum and with each other. However, all of these striatal terminal field measures reached near zero when the number of nigral cell bodies decreased by 50%. Most importantly, the Parkinsonian behavior of these animals correlated fully with the nigral cell counts, whereas the striatal PET measures hit the flooring effect with relatively mild Parkinsonism. This was surprising; however, Kordower et al. (2013) ascertained similar findings in postmortem brains of people with PD who died at various stages of disease severity. Once Parkinsonism was mild to moderate, they found that striatal measures of dopaminergic neurons were down to zero. Fortunately, aiming the PET at the midbrain provides measures of uptake of either the DAT or VMAT2 radioligand, which correlates well with nigral cell bodies and with Parkinsonism motor severity (Brown et al. 2013). The key point of these studies is that biomarkers used to measure disease severity must be adequately validated prior to the implementation of clinical trials and that animal model systems can play an important part in this validation.

Alzheimer Disease

Although pathological and neurodegenerative biomarkers can be identified before symptom onset, they do not share the same time course. For example, pathologic amyloid in the brain can be detected 15 years before symptom onset,

at which time the accumulation of this biomarker has peaked. Increases in tau protein can be detected five years before symptom onset, and tau continues to accumulate as symptoms progress. Although each of these biomarkers can independently predict future outcomes in symptomatic patients, in presymptomatic patients they are only predictive in combination. Currently, it is unknown whether these biomarkers will predict treatment outcome, which may depend on the mechanism of therapy.

Multiple Sclerosis

Gadolinium-enhanced lesions, reflecting inflammatory pathologic processes in brain, occur more frequently than individual clinical relapses of symptoms in patients with multiple sclerosis. The appearance and number of these lesions have thus been used as a biomarker of disease activity in therapeutic trials in remitting-relapsing multiple sclerosis. Biomarker use has the potential to reduce the length of time needed for clinical trials, if this meets the criteria for a surrogate endpoint (as discussed below). Nevertheless, European regulatory agencies still require a clinical endpoint for therapeutic trials. A critical limitation of this approach is that once a patient with multiple sclerosis develops a chronic progressive course, magnetic resonance-based measures may not adequately reflect the progressive neurodegenerative process.

From these examples we can infer that although basic mechanisms are informative, useful biomarkers can be developed even in the absence of a complete understanding of the pathogenesis of disease. Furthermore, even when the biomarker does not act as an endpoint measurement, it can serve to inform an understanding of pathogenesis. For example, PET measures of striatal uptake of radioligands may provide valuable information about the pathophysiology of Parkinsonian conditions (Criswell et al. 2011). Similarly, PET measures of the amyloid-β radioligand PiB may reveal coexisting abnormal deposition of amyloid-β in people with PD and cognitive impairment that is different from those with cognitive impairment due to Alzheimer disease (Campbell et al. 2013).

Future Directions: Psychiatric Disease

Neuropsychiatric diseases are susceptible to placebo effects, since the desired outcome is almost invariably a change in the patients' subjective experience of the world. For this reason, development of surrogate endpoints and biomarkers is of critical importance to translational research in this area. Previous attempts to identify biomarkers using cerebral spinal fluid and blood markers, microRNA changes, or REM latency measures have failed. These failures may reflect the etiologic complexity and phenotypic heterogeneity across cell types and indicate that a better understanding of pathogenic mechanisms will be required for biomarker development.

Despite these hurdles, studies with resting state glucose metabolism, measured with ^{18}F-fluorodeoxyglucose PET, reveal changes in limbic frontal circuits that reflect responses to pharmacotherapy or cognitive therapy in people with major depression (Seminowicz et al. 2004). Similarly, MRI-based measures of BOLD (blood oxygenation level dependent) signal activation in prefrontal or anterior cingulate cortex can predict efficacy for both antidepressant (Frodl et al. 2011) and cognitive behavioral therapies (Klumpp et al. 2013). Resting state network measures with BOLD MRI show great promise in identifying new biomarkers that reflect the various domains affected in neuropsychiatric disorders (Greicius 2008). The number of studies in this area has exploded over the last several years. However, careful attention to technical details of the imaging and detailed characterization of patient symptoms is necessary to determine how helpful these potential biomarkers will be for clinical trials.

Electrophysiological measures have also been explored as potential biomarkers in psychiatric disorders. Event-related potentials (ERPs), mismatched negativity (MMN), gamma oscillations, and electroretinograms are promising candidates. Electrophysiological methods provide a tool that reflects functional brain dynamic changes within milliseconds and may also be used as an ensemble of biomarkers. Furthermore, these methods have the advantage of being low-cost, fast, and well-tolerated by patients.

ERPs are characterized by a positive voltage deflection that occurs approximately 50 ms after the stimulus (P50), a negative voltage deflection at 100 ms after the stimulus (N100), and two more positive voltage deflections at approximately 200 ms (P200) and 300 ms (P300) poststimulus, respectively. In the paired-click paradigm, component amplitudes elicited by the first stimulus are normally greater than equivalent potentials elicited by a second stimulus. Schizophrenic individuals exhibit similar response amplitudes to both stimuli, yielding a lower ratio between them (Adler et al. 1982; Boutros et al. 1991). This gating deficit may result from a decreased response to the first stimulus and/or a failure to inhibit the second stimulus. ERP components are affected by certain pharmacological treatments and stimulus manipulations (Maxwell et al. 2004). Deficits in ERP components are reliable and robust findings in schizophrenia, indicating that ERP may be a potential biomarker for this disease.

MMN is a component of ERP and a promising biomarker candidate for psychotic disorders such as schizophrenia (Michie 2001; Michie et al. 2000; Näätänen and Kähkönen 2009; Rissling et al. 2010; Turetsky et al. 2007a; Umbricht and Krljes 2005). MMN, an exaggerated negative voltage deflection following the N100, is elicited when the qualitative features of a novel, or deviant, tone fail to match the pattern of a previous series of repetitive stimuli (Javitt et al. 2000; Light and Braff 2005). Reduced MMN amplitude is one of the most robust findings in schizophrenia (Lawrie et al. 2011), and the mean effect size is approximately 0.99 (Umbricht and Krljes 2005). Furthermore, MMN is regarded as a reliable, sensitive index of central auditory system plasticity, with important relationships to cognition and psychosocial functioning

(Rissling et al. 2013). For example, it has been reported that MMN amplitude is associated with social function (Light and Braff 2005), social cognition (Turetsky et al. 2007b; Wynn et al. 2010), and executive function (Toyomaki et al. 2008), whereas phonetic MMN amplitude is associated with verbal memory (Kawakubo et al. 2006) and social skills acquisition (Kawakubo and Kasai 2006; Kawakubo et al. 2007). Previous studies have shown that antipsychotic medication has little effect on MMN. However a recent report (Zhou et al. 2013) showed that antipsychotics such as aripiprazole improve MMN amplitude reduction in schizophrenia. MMN might offer promise for contributing to the continued development of pharmacologic and nonpharmacologic therapeutics (Light and Näätänen 2013; Näätänen 2008; Nagai et al. 2013).

Frequency oscillations have recently emerged as an important measure of brain activity in the study of schizophrenia. Oscillations represent the coordinated firing of clusters of neurons and are thought to help synchronize activity within and between brain regions. The theta and gamma bands have both been shown to be abnormal in schizophrenia (Ozerdema et al. 2013), and these deficits have been linked to the disease via heritability studies (Hall et al. 2011; Hong et al. 2008). Theta power is reduced in patients with schizophrenia and is associated with memory deficits seen in the illness (Brockhaus-Dumke et al. 2008; Schmiedt et al. 2005). Interestingly, symptoms of schizophrenia have been reported to correlate with increased synchronization of gamma oscillation, although mean gamma synchronicity is lower in patients with schizophrenia than in control subjects (Lee et al. 2003; Uhlhaas and Singer 2006). As oscillation is a biologically and clinically significant index of schizophrenia; it has the potential to serve as a biomarker for the disease. However, it remains to be seen whether this measure covaries with treatment.

Electroretinography (ERG) measures the electrical responses of various cells types of the retina to visual stimuli, including photoreceptors as well as bipolar, amacrine and ganglion cells. Excitatory synapses onto these cells, like most other neurons, contain postsynaptic density proteins, anchoring proteins such as Shank and Homer, and cell adhesion molecules such as neuroligin (Hoon et al. 2011; Stella et al. 2012), many of which have been implicated in autism (Dölen and Bear 2009; Grabrucker et al. 2011). Owing to its location outside of the skull, the unique access afforded by the retina may allow measurement of synaptic phenotypes with ERG recording in both model organisms and human patients. Furthermore, because ERGs are already in clinical use for the diagnosis of various retinal diseases, phenotypes captured with this technique might readily serve as biomarkers. Indeed, this opportunity is currently being explored by the Innovative Medicines Initiative through the European autism interventions project.

Neurodevelopmental diseases present unique challenges to the design of outcome measures. For example, improvements in cognition can be reflected in the "products" of learning (e.g., number of words a patient can utter) or in the "processes" that underlie learning (e.g., an accelerated rate of learning

new words) (Berry-Kravis et al. 2013). This distinction is critical because it seems likely that an intervention, which completely reverses impairments in learning capacity assayed by process measures, might require years of learning under conditions of restored capacity to accumulate improvements assayed by product measures, such as intelligence quotient. The evolving KiTAP test (Test of Attentional Performance for Children; Zimmermann et al. 2004) attempts to overcome this challenge by utilizing computer-based cognitive games designed to assay learning across multiple cognitive domains over time. Recently, KiTAP has been validated in fragile X patients across a wide range of cognitive ability, thus raising the hope that this computer-based cognitive assay will become a practicable behavioral outcome measure in future clinical trials (Knox et al. 2012).

Conclusions

The types of imaging, electrophysiological, and behavioral measures described above may provide biomarkers to help stratify or classify patients for clinical studies or symptomatic treatment. Additional work is required to determine whether these biomarkers can be useful for determining efficacy of a therapeutic intervention or target engagement, either as categorical or continuous variables. It will also be important to implement protocols for standardization and harmonization. In the next section we outline opportunities for developing novel biomarkers by focusing on tractable interfaces between basic science and clinical investigations.

How Do We Design "Tractable Interfaces" between Studies in Animal Model Systems and Patients?

Significant advances in understanding the pathogenesis of neuropsychiatric diseases have been aided by new key discoveries in genetics, which is beginning to provide important molecular entry points for the modeling of the respective diseases in genetically modified animals. However, translation of insights into pathogenic mechanisms from such model organisms back to the clinic has had only limited success. One main reason for this, hopefully transient, failure is that the broad spectrum of methods and readouts that can be employed in animal studies cannot usually be recapitulated or emulated in patients, where noninvasive methods are required. What is needed are "tractable interfaces": methods and readouts that can be employed in model organisms and patients alike, or readouts that can at least be emulated or approximated in the respective other realm. Furthermore, a deeper understanding of the human nervous system—leveraging experimental neuroscience approaches—promises to enable more targeted, controlled interventions.

As outlined above, ERPs, MMN, network oscillations measured by EEG, and ERGs belong to the category of tractable interfaces. They can reflect alterations in the function of defined circuits and neuronal networks that underlie the pathophysiology of major neuropsychiatric disorders. In addition, brain stimulation techniques combined with various functional readouts hold particular promise as tractable interfaces, because they can be applied in animal models and patients alike.

Brain stimulation techniques offer a fascinating opportunity to modulate specific neural networks, identify neural substrates, affect behavior, and address symptoms of disease. At the same time, brain stimulation represents a controllable input that can be clearly characterized so as to gain novel insights into the integrity and dynamics of neural networks. It has the potential to help patients while advancing scientific insights as it can bridge the gap between human and model systems. In addition, noninvasive brain stimulation approaches—compared to invasive ones—are safe, relatively easy to apply, and cost effective. Because they lack surgical invasiveness, application is possible in normal subjects as well as in patients across the entire age span.

To date, methods based in electromagnetic and electric stimulation, notably transcranial magnetic stimulation (TMS) and transcranial current stimulation (tCS), are the two most popular and best-studied noninvasive brain stimulation tools. There is, however, a fast-growing array of other noninvasive techniques to query and modulate brain activity, including methods that utilize ultrasound or light combined with transgene expression via viruses or other vectors. Noninvasive brain stimulation techniques can effectively modify, suppress, augment, or disrupt brain function, depending on stimulation, individual, context, and environmental characteristics. Applications include investigations into fundamental principles of brain function, efforts to translate insights from animal models to humans, research on brain-behavior relations, and novel therapeutic approaches for neurological and psychiatric illnesses.

Opportunities for Induction of Synaptic Plasticity-Like Changes in Humans

As mounting evidence from studies in model organisms suggest, an important pathogenic mechanism in neuropsychiatric disease is disruption of synaptic plasticity (Dölen and Bear 2009; Grabrucker et al. 2011; Lüscher and Malenka 2011; Paoletti et al. 2013). In animals, mechanistic studies of synaptic plasticity are typically carried out *ex vivo*, although whole cell recording techniques have more recently been adapted to enable *in vivo* synaptic plasticity studies (Chu et al. 2014). Synaptic plasticity has also been demonstrated in human hippocampal tissue excised from patients undergoing temporal lobe surgery for intractable epilepsy, and seems to share essential mechanisms described in animal models (Beck et al. 2000; Chen et al. 1996; Testa-Silva et al. 2010). Direct assays of *in vivo* synaptic plasticity are not currently feasible in humans;

however, measurements of circuit-level changes, which are thought to reflect these synaptic changes, can be obtained using several emerging noninvasive techniques (Bliss and Cooke 2011; Nitsche et al. 2012).

Noninvasive techniques used in human studies typically mimic electrical stimulation patterns used to induce long-term potentiation or depression (LTP and LTD, respectively), or spike timing-dependent plasticity (STDP), whereas other approaches attempt to modulate the mechanisms known to be required for plasticity. Below, we briefly review some of these including repetitive transcranial magnetic stimulation (rTMS), transcutaneous electrical nerve stimulation (TENS), sensory tetanization, interventional paired-associative stimulation (IPAS), direct current stimulation (DCS), and priming. (For further details, see Bliss and Cooke 2011; Nitsche et al. 2012.)

Repetitive Transcranial Magnetic Stimulation

rTMS delivers relatively small electrical currents generated by fluctuating magnetic fields administered over the skull using a magnetic coil. Single TMS pulses evoke ERPs, measured by EMG, EEG, or fMRI, and can be used to quantify cortical reactivity before and after a given intervention. Potentiation or depression of ERP amplitude can be induced by delivering either high- or low-frequency trains of rTMS pulses, mimicking stimulation patterns for LTP and LTD induction in animals (Goldsworthy et al. 2012; Rothkegel et al. 2010; Ziemann et al. 2004, 2008). Significantly, changes in excitability induced by rTMS last for minutes to hours (Rossi et al. 2009; Siebner et al. 2009; Thut and Pascual-Leone 2010; Ziemann et al. 2008). Currently, this technique is limited to excitation of neural circuitry in structures relatively close to the brain surface and cannot yet replace invasive techniques such as deep brain stimulation. Noninvasive stimulation of deep targets (e.g., the thalamus, basal ganglia, and brainstem nuclei) may soon be enabled by emerging "deep" rTMS technologies (Bersani et al. 2013) and neuro-navigated TMS protocols (Julkunen et al. 2009).

With rTMS, it is also possible to deliver two pulses—a test pulse and a conditioning pulse—applied in rapid succession to the same cortical region. Although this method has been termed "paired-pulse TMS," it should not be confused with paired pulses recorded with local field potential and whole cell electrodes. Changes in paired-pulse ratios after induction of synaptic plasticity have been used to localize the expression to the pre- or postsynapse (Granger and Nicoll 2014; Manabe et al. 1993); however this inference can only be made under the condition that what is being recorded is a monosynaptic response (Voronin 1994). In contrast, paired-pulse TMS is polysynaptic, thought to be mediated by GABAergic transmission, and is used to measure intracortical inhibition and facilitation. Three variants of this protocol exist: short-interval intracortical inhibition, long-interval intracortical inhibition, and intracortical facilitation. At this time, it is unclear whether ERP changes induced by these

protocols share mechanisms in common with synaptic plasticity. (For a more detailed discussion, see Ziemann et al. 2008.)

Transcutaneous Electrical Nerve Stimulation

Although interpreting synaptic changes that occur across multiple nodes of a circuit is complicated, previous studies have shown that high-frequency TENS delivered directly to peripheral nerves through the skin in the forearm induces plasticity of cortical ERPs (van den Broeke et al. 2010). Similarly, in animal models, removal of a digit induces nociceptive changes that are transmitted across at least three synapses, ultimately causing induction of LTP in the anterior cingulate cortex (Chen et al. 2014a; Wei and Zhuo 2001). These parallels raise the possibility that TENS-evoked changes in cortical ERP response properties may have the potential to serve as readout of polysynaptic plasticity in the human brain.

Sensory Tetanization

Studies of *in vivo* experience-dependent modification of sensory-evoked responses have demonstrated that plasticity can be induced in primary sensory cortices of humans without electrical stimulation. For example, the amplitude of the visually-evoked potential can be potentiated in the visual cortex using a photic tetanus that consists of flashing or phase-reversing visual stimulus presented on a computer screen (Ross et al. 2008; Teyler et al. 2005). Similarly, other studies have shown that tetanic auditory stimulation can evoke potentiation of the auditory-evoked potential (Clapp et al. 2005; Zaehle et al. 2007). Importantly, many of these circuit-level plastic changes have been shown to be stimulus specific, localized to a particular synapse, and to share induction and expression mechanisms with synaptic plasticity (Bliss and Cooke 2011). Although only a handful of studies have used similar measures to identify pathogenic changes in animal models of neuropsychiatric disease (Dölen et al. 2007; Tropea et al. 2009), the identification of sensory tetanization-induced plasticity in humans, in conjunction with the development of stimulus-specific response potentiation biomarker assays in mice, raises the very real hope that these measures could serve as a tractable interface between models and disease (Cooke and Bear 2012).

Interventional Paired-Associative Stimulation

IPAS is a refinement of the rTMS method, which combines sensory- and electrically-evoked stimulation. Induction protocols aim to mimic STDP by timing the delivery of electrical stimulation (rTMS) to either before (LTD-like) or after (LTP-like) the height of the sensory-evoked ERP (Sowman et al. 2014; Wolters et al. 2003, 2005). Although the parallels to STDP are necessarily

imperfect (since IPAS activates polysynaptic pathways), this approach offers the advantage of regional specificity (Bliss and Cooke 2011), likely due to the fact it requires lower frequency stimulation than rTMS alone and that the activated pathways represent the subset recruited by relevant sensory circuits.

Direct Current Stimulation

An older approach, DCS applies continuous weak current (rather than frequency modulated pulses, as in rTMS) using two electrodes fitted on the scalp surface, and has been shown to induce plastic changes in the ERP (Cheeran et al. 2008). Although the exact mechanisms of this plasticity are unclear (Bliss and Cooke 2011), low-frequency stimulation, which would ordinarily induce LTD, when combined with DCS, has been shown in rodents to instead induce LTP (Fritsch et al. 2010). Furthermore, DCS causes the release of brain-derived neurotrophic factor (BDNF) (Figurov et al. 1996), and BDNF knockout mice show impaired motor learning and absence of DCS plasticity (Fritsch et al. 2010). Interestingly, these phenotypes are recapitulated in patients who carry a polymorphism associated with reduced BDNF concentrations and show diminished motor cortical circuit plasticity induced by DCS/rTMS (Antal et al. 2010; Cheeran et al. 2008; Kleim et al. 2006).

Priming

Synaptic metaplasticity (i.e., the plasticity of plasticity; Hulme et al. 2013) has also been reproduced in humans by brief application of DCS or a train of low-frequency rTMS to the region of interest prior to the induction of LTP-like plasticity with rTMS or motor training (Bütefisch et al. 2004; Carey et al. 2006; Cosentino et al. 2012; Lang et al. 2004; Nitsche et al. 2007). Significantly, synaptic metaplasticity has been shown to be disrupted in a variety of animal models of neuropsychiatric disease (Hulme et al. 2013), suggesting that development of this assay as a biomarker would be of value to translational neuroscience.

As exciting as these parallels between noninvasive-evoked modifications in human brain and synaptic plasticity are, caution should be exercised in equating these phenomena. For example, *ex vivo* field potentials are recorded at the site of excitatory postsynaptic potential (EPSP) generation, and are only used when the anatomical arrangement of the brain area in question produces synchronous synaptic currents that can be attributed to a single input pathway. In contrast, ERPs in humans are recorded at a distance, and necessarily reflect the summation of polysynaptic EPSPs and action potentials; as a result, a change in the ERP amplitude may reflect some combination of synaptic LTP or LTD, increased or decreased inhibitory tone, as well as alterations of the intrinsic excitability of the underlying cell population (Bliss and Cooke 2011). This mismatch may have important consequences for extrapolating findings in

animal models to the clinical setting. For instance, exaggerated mGluR5 LTD has been observed in hippocampal field potential recordings in the knockout mouse model of fragile X syndrome. However, because this change is modest, region and induction protocol specific (Sidorov et al. 2013), and the hippocampus is relatively distal to the surface of the human brain, this phenotype would likely be obscured in an ERP recording. As a result, it is likely that testing the translatability of therapeutic targets identified in mouse models using these assays must await development of technologies to address these limitations.

Noninvasive Imaging

Noninvasive imaging provides another tractable interface between top-down studies on symptomatic and syndromic features of human disease and molecular, cellular, or circuit-based analyses of disease-related pathophysiological mechanisms in animal models. PET, fMRI, MRS, electrophysiological techniques, and, as discussed above, TMS can all be used in animals as well as in humans. However, given the differences in spatial and temporal scales, further technical developments are necessary and caveats regarding the interpretation of comparisons between model organisms and patients will have to be overcome. Specific disruptive techniques (e.g., TMS, deep brain stimulation, ultrasound and gamma rays) can also be used to lesion reversibly or irreversibly the brain with high spatial fidelity in humans and animals. These techniques can be combined and used for investigative purposes and treatment, separately or in combination. The applicability of PET depends on finding specific molecular or metabolic targets as well as on the question of whether pharmacokinetics and drug distributions or mechanisms are being explored. MRI in functional and structural modes is developing rapidly. The BOLD technique remains very useful in structural imaging, and the quantitative measurement of tissue characteristics through the use of a series of specific MR sequences provides an exciting opportunity for future work. Electrophysiological studies—both invasive, in the form of electrode arrays in patients or animal models, and noninvasive, in the form of EEG or event potential recordings—are further techniques with high temporal resolution that can, in principle, be used in humans and animals alike.

Optogenetics

As each of the new technologies described above is further developed in humans, an important iterative process is needed to confirm the observed mechanisms in model organisms. Here, optogenetic tools (Boyden et al. 2005) can help to identify relevant pathways in animal models and to understand in more detail which parts of the brain need to be electrically stimulated to achieve a desired effect. Contrary to electrical stimulation, where one cannot be certain that only a local area or a particular pathway is affected, optogenetic manipulations

allow for very precise stimulation. By stimulating or inhibiting axons while simultaneously measuring the impact on neural activity and on behavior, it is now possible to measure the impact of manipulating activity in a particular pathway very specifically. This will help identify disease-related projection pathways in the brain and determine where to place ideally a stimulating electrode for a particular purpose. Further, it can also help explain effects seen with electrical stimulation (e.g., by cell type-specific optogenetic stimulation) and reduce side effects of electrical stimulation by refining the target site.

Conclusions

An important appeal of each of the methods described above lies in their potential to help patients while gaining critical insights into fundamental questions of brain function and pathophysiology of nervous system diseases. It is likely that scientific and clinical interest in noninvasive brain stimulation, imaging, and optogenetics will continue to expand as the spectrum of their applications is nearly inexhaustible. The most transformative approach in this context would be to truly integrate technological, basic, and clinical neuroscience with therapeutic efforts. This could lead, for example, to an exploration of combined behavioral and brain stimulation interventions that are individually targeted, perhaps based on more sophisticated understanding of individual circuit dysfunctions as well as individual genetic and epigenetic factors.

How Do We Model Neuropsychiatric Disease?

Understanding the etiology and pathophysiology of complex human neuropsychiatric disorders will require modeling disease in systems ranging from humans to stem cells. While this will enable insights appropriate to each model system, the development of novel therapeutics ultimately requires that these models converge at tractable interfaces. In the previous discussion, we laid out a roadmap for developing technologies to achieve this convergence. Here we describe these model systems in greater detail, in an effort to highlight the appropriateness of each to answering a particular question. Rather than stratifying models into “top-down” and “bottom-up” heuristics, we highlight opportunities to interface between systems, as well as the iterative nature of this process.

Role of Human Genetics for Developing Models

Genetic technology has exploded over the last several years, and soon we will have the ability to obtain full genetic information for each person. Gene manipulation in model systems can provide a platform for investigations of the consequences of genetic mutations on neurochemistry, plasticity, circuits, and

behavior in a manner that cannot be done in humans. Advances in human genetics have facilitated tremendous progress toward identifying pathogenic or risk genes for several neuropsychiatric disorders, and this information has been applied to model systems. The genetic variations in autism spectrum disorders (ASD), for example, are quite diverse, encompassing rare and common alleles, sequence and structural variants, and *de novo* as well as transmitted mutations. Moreover, in addition to gene discovery in what is often referred to as "common idiopathic" forms of these disorders, there are also multiple examples of established "syndromes" caused by monogenic mutations, including fragile X and Rett syndromes, highly penetrant copy number variants, including 22q11 deletion syndrome (also known as velocardiofacial syndrome), and rare recessive syndromes. It is important to note that the clinical presentation of these genetic disorders can vary widely and that distinctions between syndromic and idiopathic categories are largely historical. It also remains to be determined whether the underlying pathophysiology of the relevant psychiatric manifestations differs as a result of divergent transmission modes.

The characterization of differences in penetrance, effect size, and likelihood that a given risk gene may be implicated in more than one disease state will inform both the selection of genes for modeling in nonhuman systems and the strategies that will need to be applied. For example, mutations carrying relatively large effects and mapping to coding segments of the genome may be suitable to be immediately modeled in an animal system, whereas noncoding single nucleotide polymorphisms found in association with a condition may require additional fine mapping and systems biological analysis before pursuing *in vivo* model experiments.

Given the finding of a very high level of locus heterogeneity for both schizophrenia and ASD, efforts are underway to use a variety of approaches to organize disparate genes into more biologically meaningful groups. These include protein-protein interaction analysis and gene ontology approaches that seek to identify molecular pathways of interest. In addition, several recent studies have leveraged genome-wide expression data from typically developing brain (in humans and other species) to gain traction on the question of when and where specific risk genes might show pathophysiological convergence. Studies in both autism and schizophrenia have implicated mid-fetal cortical development (Gulsuner et al. 2013; Parikshak et al. 2013; Willsey et al. 2013). One recent study mapped an ASD-associated co-expression network based on only high-confidence ASD genes to layer 5/6 projection neurons in mid-fetal development (Willsey et al. 2013). All of these "systems biological" approaches are aimed at prioritizing key parameters in model systems studies, including which cell types, signal pathways, and circuits may be of particular interest.

Etiological and symptom heterogeneity has led some to propose that it might be more useful to consider risk genes as distinct clinical entities (i.e., the existence of "autisms" rather than a single "autism"). Others have suggested an analogy to Alzheimer disease, which is considered a single clinical entity

based on pathology, despite the fact that only 10% of the cases have been linked to a specific genetic cause. The remaining 90% likely result from a set of changes that take place during aging which, presumably, compromises the ability of neurons to process proteins correctly. Thus, it is taken for granted that a single therapeutic, when it is finally found, will be effective on all patients with Alzheimer disease. This makes a huge difference in the search for therapeutics and explains why knowing the number of classes (in the treatment sense) of diseases is so essential. In the long run, the complexity of psychiatric disorders, both in terms of their waxing and waning symptoms and genetic etiology, suggests that a spectrum of phenotypic and etiologic factors will need to be considered when defining individual disease classes for the development of tractable models and treatment studies.

The distinction between defining a "disease," based on symptoms or pathology, or "diseases," based on genes, could influence selection of models, since these definitions may lead to different predictions about the level (i.e., biochemical, synaptic, circuit, behavior) of pathogenic convergence across etiologies. For example, at one extreme, it could be argued that because the "diseases" are primarily defined by the genetic etiology, the key property required of a model is construct validity (e.g., genetic homology) and the ability to therapeutically target convergent biochemical pathogenic mechanisms. Accordingly, behavioral readouts would be secondary, and a demonstration of face validity for human symptoms need not be prioritized. This view is particularly amenable to using iPS or IN cell-based models to subclassify complex disorders. At the other extreme, one could argue that because the "disease" is primarily defined by the symptoms and pathology, models which achieve the best face validity (e.g., behavioral changes that recapitulate human symptoms) will provide the greatest opportunity for understanding pathogenesis. Accordingly, therapeutic strategies would focus on correcting conserved circuit and behavioral abnormalities, independent of the heterogeneity of genetic disruptions that produced them. This view is particularly useful when the etiology of the disorder is unknown and may require development of model organisms (e.g., marmosets, discussed below) amenable to interrogation of more complex behaviors.

Between these two extremes lies the view that since some of the identified risk genes encode synaptic proteins (e.g., receptor, downstream signaling, and adhesion molecules) with known functions that are largely conserved across species, understanding pathogenesis at the level of synapses will yield the greatest short-term opportunity for therapeutic intervention. The term "synapsopathy" was first coined to describe this view as it applies to autism (Bear et al. 2008; Dölen and Bear 2009) and to distinguish this disorder from those brain diseases where the primary pathology is localized to a specific brain region (e.g., substantia nigra in PD). Despite enthusiasm for the idea, an inherent challenge is that the brain is remarkably heterogeneous in its cellular composition. The wide variety of cell types might thus be the basis for genetic "pathoclisis," a selective vulnerability of subsets of neurons to the effects of

mutation. Indeed, recent studies of Huntington disease and autism raise the possibility that genetic pathoclisis will be an important pathogenic mechanism, with profound implications for developing therapeutics (Dölen and Bear 2009; Paul et al. 2014). Moreover, synapsopathy and pathoclisis need not be mutually exclusive; together they might account for symptom heterogeneity despite overlapping clinical presentation. Understanding the relative contribution of each of these mechanisms to the pathogenesis of disease will likely inform decisions about the suitability of modeling synaptic disruptions at the cellular or circuit level, so as to guide future development of tractable interfaces. For example, synapsopathic features might be particularly amenable to ERPs, MMN, gamma oscillations, and ERG measurements (discussed above), whereas TMS, tCS, and brain imaging (see previous discussion) of specific circuits might be more appropriate for interrogating disease symptoms that result from pathoclisis. Of course, a risk of focusing solely on genes, the functions of which are currently somewhat familiar, is that many of the genome-wide significant loci associated with disorders, such as schizophrenia, are noncoding (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Thus we may end up ignoring important pathogenic mechanisms which, in the long run, need to be understood.

Model Systems: Mice

To date, the modeling of identified risk genes has primarily been conducted in mice using constitutive or conditional transgenic systems. This model organism offers the opportunity to interrogate pathogenesis at the level of biochemical, synaptic, circuit, and behavioral mechanisms. Moreover, the availability of a number of other molecular genetic tools—such as BAC-Cre-recombinase driver and BAC-EGFP reporter lines, viral-mediated gene transfer (Callaway 2008; Grimm et al. 2008; Luo et al. 2008; Marie and Malenka 2006; Neve et al. 2005; Salinas et al. 2010), RNA interference (Morris and Mattick 2014), optogenetics (Boyden et al. 2005; Lima and Miesenböck 2005; Lin et al. 2009), genetically encoded voltage (Cao et al. 2013; Dimitrov et al. 2007; Siegel and Isacoff 1997) and calcium sensors (Chen et al. 2014a; Wang et al. 2004) and pharmacosynthetics (Dong et al. 2010; Lee et al. 2013)—has made it possible to address how complex, goal-directed behaviors occur from organized networks of neurons. Many of the plasmids for these molecular manipulations are available from Addgene,[3] a nonprofit, public repository. An in-depth review of the molecular toolkit is beyond the scope of this discussion. Here we will present an overview, with a focus on resources, advantages, and limitations, followed by a handful of examples that have demonstrated the power of these approaches to help understand the pathophysiology of brain disease.

[3] https://www.addgene.org/ (accessed March 3, 2015)

The Gene Expression of the Nervous System Atlas project,[4] in collaboration with the Intramural Program of the National Institute of Mental Health, offers transgenic BAC-EGFP reporter and BAC-Cre recombinase driver lines which allow for cell-specific gene manipulations in the mouse CNS. The aim of this project is to provide the scientific community with reporter and Cre driver lines that will target selected neuronal or glial populations in the brain and spinal cord. An important caveat is that cell type specificity must be confirmed for each line, since BAC transgenics are not generated by targeted mutation; furthermore, cell type specificity in adult brain need not be reflective of expression patterns during development. In addition to this resource, a vast array of constitutive and conditional knockout and knockin mice are available through commercial repositories, such as Jackson Labs.[5] When transgenic mice, particularly conditional knockouts, for the gene of interest are not available, RNA interference is a viable alternative at most synapses. Because various forms of RNA interference are encoded by relatively short sequences, it is feasible, and indeed often necessary, to control for off-target effects using molecular replacement strategies (Alvarez et al. 2006; Jurado et al. 2014).

The introduction of exogenous DNA into neurons by viral transfection has become a standard technique in molecular neurobiology, particularly as our understanding of viral tropism, life cycle, transport, and toxicity has led to the development of increasingly sophisticated recombinant strategies. An illustrated comparison of the viruses commonly used for neuroscience (Table 10.1) highlights the relevant features of each (for in-depth reviewa, see Callaway 2008; Grimm et al. 2008; Luo et al. 2008; Marie and Malenka 2006; Neve et al. 2005; Salinas et al. 2010). This approach can be a powerful adjunct or alternative to the BAC driver lines, particularly when synapse-specific or developmentally restricted expression is required. Nevertheless, *in vivo* viral infection requires labor-intensive stereotaxic injection, except in cases where the vector can cross the blood-brain barrier (e.g., AAV-9; Foust et al. 2009). Finally, this approach is also a promising method under development for therapeutic gene delivery in human patients, with the caveat that viral immunogenicity is often species specific, so not all vectors used in mice are appropriate for humans (Mingozzi and High 2011).

The ability to optically stimulate molecularly specified neurons (termed "optogenetics") has transformed neuroscience (Boyden et al. 2005; Lima and Miesenböck 2005; Lin et al. 2009). Stimulation of neurons with metal or glass electrodes, while still a mainstay, can only resolve individual input pathways when these are arranged in such a way that they can be physically segregated (e.g., Schafer collateral versus perforant path inputs to the CA1 region of the hippocampus). However, this anatomical arrangement is exceptional: in the vast majority of brain regions, inputs are intermingled, and thus stimulation

4 http://www.gensat.org (accessed March 3, 2015)

5 http://jaxmice.jax.org/ (accessed March 3, 2015)

Table 10.1 Comparison of commonly used viruses in neuroscience.

Virus	Description	Reference
Recombinant Sindbis (SIN) virus	• Used when rapid, robust gene expression is required • Suffers from precipitous neuronal toxicity, limiting use to short-term expression studies	Chung et al. (2013) Marie and Malenka (2006)
Lentiviral virus (LV)	• Widely used when relatively sparse transfection is desired (e.g., to examine cell autonomous effects) • Appropriate for both acute and long-term expression since LV shows very low cellular toxicity • Because it is a retrovirus, insertion of exogenous DNA into the host genome may disrupt native transcription (positional effect) • Since LV is enveloped, it can be pseudotyped with rabies virus glycoproteins to enable retrograde transport in neurons • Utility of presynaptic uptake and retrograde transport has been exploited most readily with recombinant rabies virus	Klapoetke et al. (2014)
Rabies virus (RbV)	• Is rapidly and robustly expressed • Has relatively slower neurotoxicity compared to SIN and HSV • Because it is an RNA virus, expression cannot be driven by cell type-specific promoters, although alternative strategies have been developed	Callaway (2008) Wickersham et al. (2007)
Canine adeno virus type 2 (CAV-2)	• Is also taken up presynaptically and retrogradely transported • Is a promising alternative to RbV, since it has very low neurotoxicity and is a DNA virus with a relatively high cloning capacity of (36–38 kb)	Hnasko et al. (2006) Salinas et al. (2010)
Adeno-associated virus (AAV)	• Most widely used due to its rapid expression and lack of pathogenicity • New hybrid serotypes have been developed by merging desirable qualities of naturally occurring AAV capsids • AAV-DJ serotype has exceptional neuronal tropism and is particularly well suited for manipulations requiring rapid, robust expression • Limited by relatively restricted cloning capacity (5 kb)	Grimm et al. (2008) Xu et al. (2012)
Herpes simplex virus (HSV)	• Exceptionally high cloning capacity of 150 kb • Efficient transgene expression • Depending on the envelope proteins, potential for anterograde and retrograde transport • Advantages are counterbalanced by neuronal toxicity and immunogenicity	Neve et al. (2005)

of known inputs to a specific cell type is frequently impossible. Furthermore, it is increasingly apparent that many of the assumptions concerning input and output homogeneity, even in well-circumscribed pathways, do not hold (e.g., co-release of transmitters, novel parallel pathways) (Graves et al. 2012; Tritsch et al. 2012; Varga et al. 2009). In addition, optogenetics enables convergence of cellular and behavioral studies since molecularly isolated inputs can be stimulated to both record electrical responses in specific neurons as well as to interrogate the behavioral consequences of evoked responses. This is particularly important in brain regions where the "receptive fields" of the neurons in question are internal states and are not reliably evoked by direct manipulation of the sensory or motor environment. Despite the remarkable advances enabled by the implementation of optogenetics, its current use is restricted by the speed with which the optogenetic proteins can be delivered to membranes. It often takes many weeks or months for adequate expression of optogenetic proteins in axon terminals, thus limiting experiments to late stages in development. Future iterations will likely improve subcellular targeting (e.g., dendritic versus axonal membranes), channel properties (for better temporal fidelity at higher stimulation frequencies), opsin properties (e.g., for better resolution of distinct activation wavelengths), and toxicity due to overexpression.

Genetically encoded voltage (Cao et al. 2013; Dimitrov et al. 2007; Siegel and Isacoff 1997) and calcium (Chen et al. 2014a; Wang et al. 2004) sensors represent a parallel set of emerging technologies, which will do for the recording electrode what optogenetics has done for the stimulating electrode: allow sub-(action potential)-threshold recordings in molecularly specified neurons, both *ex vivo* and *in vivo*. Currently, voltage sensors are limited to *in vitro* approaches in mammalian systems, and the use of genetically encoded calcium indicators *in vivo* is restricted to microscopically accessible brain regions (e.g., the somatosensory cortex). However, ongoing development of brighter fluorophores and endoscopic techniques may likely overcome these hurdles in the near future (Deisseroth and Schnitzer 2013; St-Pierre et al. 2014).

Finally, the G protein-coupled receptor superfamily represents the canonical targets of more than 30% of clinically available pharmacotherapies (across all indications), largely because these molecules are readily druggable, act as modulators of nearly every known physiological process, and have been implicated in the etiology or pathogenesis of numerous disorders. Despite this profile, interrogating the neuronal function of these receptors is complicated by the fact that a single endogenous ligand typically binds multiple receptors, can modulate different signaling pathways through a single G protein-coupled receptor, and are expressed across mixed populations of cells within a given brain region. The advent of second-generation molecularly encoded, pharmacosynthetics, namely the designer receptors exclusively activated by designer drugs, has made deconstructing this functional complexity a tenable goal (Dong et al. 2010; Lee et al. 2013).

While many of these techniques can be used independently in other model organisms, the power of modeling in mice is the opportunity to use a combinatorial approach. For example, AAV (adeno-associated virus)-mediated expression of channelrhodopsin-2 in the striatum of BAC-transgenic Cre driver lines under control of regulatory elements for the dopamine D1 or D2 receptor has enabled direct activation of basal ganglia circuitry implicated in PD. These studies have validated the long-standing hypothesis that two parallel pathways exert bidirectional control over motor behavior and, furthermore, shown that in a mouse model of PD, direct pathway activation rescues movement phenotypes (Kravitz et al. 2010). Another approach has combined viral-mediated expression of pharmacosynthetics with Cre-mediated targeting of neurons which receive inputs from agouti-related protein-expressing neurons in the arcuate nucleus, to interrogate G protein-mediated feeding behaviors relevant to the pathogenesis of insatiability in Prader-Willi syndrome (Atasoy et al. 2012). Others have capitalized on the ability of rabies virus and AAV to infect selectively pre- and postsynaptic neurons, respectively, combining this technology with conditional knockout and knockin reporter mouse lines. Such approaches, for example, allowed the characterization of oxytocin and serotonin receptor-containing inputs to the nucleus accumbens and implicated a novel circuit in the pathogenesis of social deficits seen in autism (Dölen et al. 2013).

Despite the remarkable technological opportunities available in mice, determining the suitability of this organism for modeling disease requires the consideration of genetic similarities and differences between mouse and humans. Sequencing of the mouse and human genome has revealed that in protein-coding regions, there is 97% alignment between orthologs, and in 1:1 orthologs 85% DNA sequence identity. These genes are the most likely to have maintained ancestral function in both species and are therefore most appropriately targeted as disease models (Church et al. 2009). Despite this remarkable homology in protein-coding regions, only 33% of whole genomes align, with 60% DNA sequence identity in aligned regions. This discrepancy is due, in part, to structural variants and segmental duplications (i.e., evolutionarily young and rapidly evolving parts of the genome) in gene regulatory regions, as well as to transcribed microRNA and noncoding RNA sequences (Church et al. 2009). Modeling disease that affects these nonhomologous genomic regions will require alternate systems. For example, patient-derived induced pluripotent stem cells may be appropriate for modeling schizophrenia, since most identified schizophrenia risk variants are regulatory (e.g., cis trans transcription regulators) affecting the time, place, and rate of gene expression. Currently, the success of such an approach will depend on the degree to which the pathogenesis of disease is cell autonomous, although chimeric and organoid systems may be able to overcome this limitation in the future (Anderson and Vanderhaeghen 2014).

In addition to these genetic differences, anatomical differences (e.g., a small medial prefrontal cortex) as well as a limited repertoire of behavioral assays for interrogating complex cognitive function (e.g., episodic learning, language) limit the use of mice for modeling all features of complex neuropsychiatric disease. New genome-editing technologies, such as clustered, regularly interspaced, short palindromic repeats, and associated cas genes, have made it possible to make precise genetic manipulations in many other organisms, including primates (Ran et al. 2013). Considerations such as size, cost, generation time, and ability to breed in captivity will importantly influence the selection of organism for genetic modeling.

The common marmoset, *Callithrix jacchus*, a New World monkey, has significant advantages from a genetic perspective. It is small in size (~400 g), reaches sexual maturity at 12 months, and breeds rapidly in captivity, typically producing two pairs of fraternal twins per year. The neuroanatomy of common marmoset is well described. Like macaques, but unlike rodents, marmosets have a well-developed prefrontal cortex, a critical region for many cognitive functions that are impaired in human psychiatric disorders. Furthermore, marmosets are very social and communicative and can perform some higher cognitive tasks developed for macaque monkeys and humans. The marmoset genome has recently been sequenced, laying the necessary groundwork for genetic manipulations. Moreover, the optogenetic tools described above are also being developed for use in primates (Diester et al. 2011; Galvan et al. 2012).

A number of other organisms offer potential as model systems for understanding the pathogenesis of complex neuropsychiatric disease. For example, SAGE labs[6] has developed several rat models of PD, Alzheimer disease, schizophrenia, and autism, as well Cre lines for specific expression of floxed constructs in dopaminergic neurons. The larger size of rats makes them more amenable to *in vivo* recording as well as an extensive set of well-characterized behavioral assays. Other species of potential interest for future genetic model development are the Etruscan shrew (active touch; Brecht et al. 2011), the prairie vole (pair bonding; Barrett et al. 2013), and the scrub jay (episodic memory; Raby et al. 2007).

Modeling Genes and Environment

The importance of environmental risk factors for neurodevelopmental disorders is well established. Thus, in the long run, it will be essential to interrogate the role of environmental influences in model systems. A major complication, however, is that multiple environmental variables have been identified as potential triggering factors for brain disorders when associated with genetic susceptibility. In schizophrenia, for instance, obstetric/birth complications, loss of parents in early childhood, prenatal infection, physical or psychosocial abuse,

6 http://www.sageresearchlabs.com/research-models (accessed March 3, 2015)

cannabis use in adolescence, and urbanicity may represent up to 60% of the contributing etiological factors. Many of these factors are difficult to replicate in animals and each one (e.g., urbanicity) consists of many variables, any one of which may critically contribute to the pathogenesis of psychiatric disorders. Nevertheless, attempts have been made to model experimentally some of these factors in animals. For instance, rodent models of prenatal infection using poly I:C treatment (which induces a cytokine-mediated viral-like response) or maltreatment in early life through maternal separation have been found to recapitulate some components of symptomatology. However, to examine gene-environment interactions optimally in experimental animal models, these manipulations need to be combined with animals that express genes, the influences of which on brain function are most susceptible to environmental variables. The analyses of chromatin states and epigenetic alterations across the genome in patients may be one way to gain knowledge on the potential genetic loci affected by nongenomic mechanisms (i.e., DNA methylation, noncoding RNAs). However, this will be a challenging endeavor since epigenetic modifications are often cell type specific.

Conclusions

Interfacing between model systems is critical to approach the daunting complexity of human neuropsychiatric disorders. As our understanding of the etiology and pathogenesis of brain disorders grows, these approaches should be used in an iterative fashion, with the ultimate goal of designing novel therapeutic strategies for the myriad of diseases for which current treatments are of modest utility.