9

How Do We Define and Modulate Circuits in Animals That Are Relevant to Pathophysiology in Humans?

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Abstract

How do investigators define and modulate circuits in animals that are relevant to the pathophysiology of human neurodegenerative diseases? Animal models provide a variety of advantages for translational investigations related to neurodegenerative diseases, with the best models revealing new understanding of the mechanisms of disease and stimulating new therapeutic interventions. However, animal models have also led to many missteps with investigators following false paths. This review addresses the purpose of these animals models, describes how to identify relevant circuit abnormalities, provides examples of how such models relate to human neurodegenerative diseases, warns of critical limitations of such models, and finally suggests that better tools be developed for these translational investigations.

Introduction

How do investigators define and modulate circuits in animals that are relevant to the pathophysiology of human neurodegenerative diseases? To address the series of relevant issues about such animal models and circuits, it is helpful to begin by reviewing the purpose of animal models and what we mean by circuits. Different model systems may be optimal or totally inadequate to address selected research questions, and an investigator must understand the various intricacies that determine the appropriate selection of the right model system. Animal models provide a platform for translational studies that cannot be done safely or as efficiently in humans. Such models provide a variety of advantages for investigations related to neurodegenerative diseases, with

the best models revealing new understanding of the underlying pathophysiology of disease and stimulating development and testing of new therapeutic interventions. In the past, most clinical-anatomic correlation studies focused on specific regional deficits and the corresponding changes in behavior. Recent studies now focus on how such regional deficits affect brain circuits. In this context, a brain circuit could refer to any linked parts of the brain or perhaps, more appropriately, to a pathway with a start and finish that coincide. Of course, the designated "start" or "finish" can just be any arbitrary nodes along a pathway; they do not necessarily imply initiation or completion. However, the notion of circuits has extended to networks with recent development of techniques that directly identify or assess the integrity of networks. Thus, studies now commonly investigate changes in brain networks or circuits which underlie normal or abnormal behaviors without obvious isolated focal defects. Animal models have revealed insights into dysfunction of such circuits, but they have also led investigators astray. In this chapter, I review the purpose of defining these animals models and describe how relevant circuit abnormalities can be identified. Examples are provided on how such models relate to human neurodegenerative diseases. Critical limitations of such models are discussed and the suggestion is made to develop better tools to permit translational investigations.

Purposes of Animal Models

The selection of an animal model for an investigation requires matching the model system with the research goals. Early animal models focused on identifying biochemical or pharmacologic effects produced by known defects found in human neuropathology. For example, the loss of nigral neurons and subsequent striatal dopamine deficiency found in brain tissues from people with Parkinson disease (Hornykiewicz and Birkmayer 1961) eventually led to toxin-induced models of nigrostriatal dopaminergic neuron injury that mimicked that which occurred in Parkinson disease. Defects in nigrostriatal dopaminergic neurons can be replicated in animals through direct injection, into the relevant brain region, of a neurotoxin (e.g., 6-OHDA) or through direct intracerebral or systemic administration of a protoxin like MPTP or the complex I inhibitor rotenone. Such toxin-induced models have been used to investigate the function of the nigrostriatal pathway and effects of loss of nigral input on basal ganglia circuits, as revealed through changes in glucose metabolism (Wooten and Collins 1981). The changes in circuits found in such models eventually led to new models of the functional anatomy of basal ganglia circuits (Albin et al. 1989; Mink 1996).

Similarly, genetic mutations found in humans with neurodegenerative disorders have been used to develop genetic animal models that permit identification of the biochemical consequences of these gene defects. Dramatic

advances in genetics have led to the identification of genetic mutations that cause a variety of neurodegenerative disorders, and knowledge of these mutations provides the basis for a host of different types of genetic animal models. This seemingly simple approach is actually far more complex. Genetic models can include knocking out the gene that is defective in humans, knocking in a mutant gene, inducing chromosomal rearrangements, and inserting conditional gene modifications (that limit expression of the mutant gene to specific brain regions and at selected times). Genetic background also influences the effects of specific genetic manipulations (Heiman-Patterson et al. 2011). Knockouts may demonstrate behaviors unrelated to the specific genetic-related behavior in humans with development of other phenotypes, like greater aggressiveness or blindness. Conditional expression of genes can be controlled by the presence or absence of a drug, such as the antibiotic tetracycline through the use of tet/on and tet/off mutations. Tet/on would express the mutation in presence of tetracycline whereas the tet/off would express the mutation in the absence of tetracycline. Knockin models can vary by the degree of expression, which can be even more complicated for modeling triplet repeat disorders (e.g., Huntington disease), in which the number of triplet repeats of CAG can vary from person to person. Thus, different genetic models of Huntington disease with varying numbers of triplet repeats may produce different biochemical consequences, an important issue to keep in mind when interpreting such studies (Pouladi et al. 2012). These models may, however, permit related changes in the physiology of pertinent circuits to be identified (Cepeda et al. 2013; Kravitz et al. 2010). In fact, optogenetic animal models may be useful in teasing out the specific function of relevant circuits whose dysfunction contributes to neurodegenerative disorders (Tye et al. 2013). Many genetic models do not exhibit behaviors that clearly correspond to the human condition, thereby challenging investigators to identify appropriate readouts of pathologic processes. Nevertheless exploration of relevant pathways, identification of cofactors that may contribute to development of pathology, and potential testing of interventions which could interfere with the etiopathology of disease demonstrate some of the incredible translational utilities of these animal models.

Animal models also permit us to test hypotheses about the relationship of known pathologic defects and brain networks. For example, optical imaging has been used to measure cortical network activity in wild-type mice and transgenic mice that exhibit abnormal amyloid beta (Aβ) deposition in brain (histologically visualized as plaques with no measure of soluble Aβ). These studies revealed a correlation between the amount of regional Aβ amyloid deposition (which accompanies Alzheimer disease in humans), changes in functional connectivity, and aging (Bero et al. 2012). Similarly, people with Huntington disease have loss of medium spiny striatal projection neurons. The consequences of loss of two different types of medium spiny neurons—those inhibited by D2 dopamine receptors or excited by D1 dopamine receptors—have been

investigated using animal models with optogenetic approaches that selectively activate one or another of these neuronal populations (Cepeda et al. 2013). This type of strategy can reveal changes in networks related to selected genetic defects with subsequent pathological defects relevant not only to Huntington disease but also to normal function of these basal ganglia circuits.

Once identified in animal model systems, pathologic circuits can be used to identify targets for therapeutic interventions or to determine target engagement by selected interventions. A rather straightforward example is the use of the multiple toxin models of nigrostriatal injury which produce behavioral abnormalities that vary in closeness to human Parkinsonism. The conceptual simplicity of such models, however, gives way to varying complexities, depending on the precise model and how it is used. Rodent models have motor behavioral deficits that have some similarity to human Parkinsonism, whereas nonhuman primate models recapitulate much more closely many of the motor and nonmotor features (Irwin et al. 1990; Tabbal et al. 2012; Brown et al. 2012, 2013; Schneider et al. 1988, 2013). Levodopa, commonly used to treat Parkinson disease in humans, may ameliorate some of the motor manifestations, but it can also exacerbate nonmotor features in nonhuman primate models, as it may do in human Parkinson disease, further validating this model system (Schneider et al. 2013). Multiple drugs to treat Parkinsonism were first tested in these animal models based on behavioral responses. In addition, these models can be used to screen other potential therapeutic agents by demonstrating pharmacologic target engagement or change in a measure of severity of brain pathway dysfunction. The key for these studies is to develop and validate appropriate measures (e.g., occupancy of the relevant receptor or changes in activity in a brain circuit). To do this, neuroimaging methods have been used with varying degrees of success. Although specific targets can be identified with diverse imaging methods in these models systems, care must be taken to ensure that the measures truly reflect the underlying pathologic processes; otherwise results obtained can be misleading (Ravina et al. 2005; Karimi et al. 2013; Brown et al. 2013). The circuit consequences of specific defects can be explored with a variety of methods, including MR-based resting state functional connectivity (Helmich et al. 2009; Hacker et al. 2012), PET-based covariance networks (Ma et al. 2012), and optogenetic methods (Ozden et al. 2013). Such changes in networks may provide a means to infer target engagement by a therapeutic agent, as has been suggested for changes in functional connectivity induced by memantine in rodents (Sekar et al. 2013), as a prelude to potential application to neuropsychiatric conditions. Changes in networks also may be used to investigate the mechanisms of a nonpharmacologic therapeutic modality like deep brain stimulation. The physiologic responses to deep brain stimulation in Parkinson disease have been investigated with multiple technologies, such as changes in ^{18}F-fluorodeoxyglucose (FDG) PET-based covariance identified networks (Asanuma et al. 2006) or optogenetic methods (Gradinaru et al. 2009). Premature application of

imaging methods can, however, lead to substantial errors in interpreting of the effects of interventions. For example, several studies to test new therapies to slow progression of Parkinson disease employed molecular imaging of presynaptic dopaminergic striatal neurons as a measure of disease severity, since these nigrostriatal dopaminergic neurons degenerate in this disease. In several studies, changes in the striatal uptake of these radioligands seemed to indicate slower progression of the disorder in one group of subjects, whereas clinical measures of progression provided evidence for a more favorable response in the other group (Ravina et al. 2005). These molecular imaging biomarkers had not been properly validated prior to implementation; that is, no one had determined "standard curves" to demonstrate that these measures truly reflect changes in severity of loss of nigrostriatal pathways until just recently. We now know that in a model system of toxin-induced damage to the nigrostriatal pathway, all of these striatal measures do not consistently reflect the severity of injury. Instead, these striatal measures approach zero as nigral cells decrease to 50%, yet motor Parkinsonism continues to progress as more nigral cell bodies die. Thus, previous studies using striatal measures of uptake likely found discordant results with clinical measures since striatal measures of more severely affected individuals were mostly noise (Karimi et al. 2013). Thus, validation in animal models prior to application in clinical studies seems prudent.

Animal models can also be used to develop and validate biomarkers that can reflect an underlying pathophysiologic process. The potential value of this type of biomarker is that it can help with diagnosis, if it:

- is found to be sufficiently specific and sensitive,
- measures disease severity,
- determines target engagement (which in this case refers to appropriate pathway modification) of a therapeutic agent (as discussed above),
- provides insight into mechanism of a therapy, or
- can be used, with the agreement of regulatory agencies (e.g., the U.S. Food and Drug Administration or the European Medicines Agency) as a surrogate endpoint for a clinical trial to potentially limit the number of participants needed for such a trial or to shorten the needed length of a trial.

These types of biomarkers frequently must be validated in appropriate animal models that permit careful control of the relevant variables and allow *in vitro* measures that may not be possible in humans to compare with the biomarker-based measures. Validation of biomarkers can be tricky and must include well-described reproducibility, accuracy, sensitivity, and specificity for the underlying biologic process. Without these critical steps, biomarker candidates may yield confounding results (Ravina et al. 2005; Karimi et al. 2013).

Identification of Circuit Dysfunction Due to Different Defects

A variety of approaches can be employed to define and modulate circuits in animal models that are relevant to the pathophysiology of human neurodegenerative disorders. Circuits or networks of coordinated brain regions can be identified with neuroimaging or electrophysiologic methods. Neuroimaging tools for this include structural imaging that can identify selected patterns of atrophy for specific dementing conditions (Kim et al. 2007), which may stimulate exploration in animal models of spread mechanisms for pathologic proteins (Walker et al. 2013). Functional imaging, with either resting state or activation-induced changes in regional metabolism or blood flow (Ma et al. 2012), provides an alternative strategy. These functional measures include molecular imaging-based calculations of resting state covariance patterns of regional activity (typically FDG or blood flow PET measures) or determination of patterns of regional activation induced by specific tasks or pharmacologic manipulations. MR-based methods that focus on blood oxygen level dependent (BOLD) include resting state and task/pharmacologic activation. Resting state data, particularly BOLD-based measures, can be analyzed using a variety of approaches, and each has its advantages and disadvantages. One common approach is seed-based analysis: seed regions in the brain are selected and all other brain regions which have resting state BOLD signals, correlating with the activity in the seed regions, are identified. Seeds can be based on a canonical standard set of brain regions (there are many such standard region sets): regions from the classic resting state networks, regions known to be affected by a specific pathologic condition (Hacker et al. 2012), or regions found in multimodal studies such as molecular imaging (Park et al. 2013) or MR-based diffusion imaging. For example, seed regions placed in the posterior putamen (i.e., the part of the striatum preferentially denervated in people with Parkinson disease) led to the identification of dysfunctional networks extending into thalamus, upper brainstem, and cerebellum (Hacker et al. 2012). Correlating the strength of these networks with severity of Parkinsonism, as measured by clinical rating scales, demonstrates face validity for the relevance of this circuit. Thus, this type of analysis could be used to investigate a variety of specific manifestations of Parkinson disease. Further studies in animal models may provide greater insights into underlying biochemical or physiologic changes that lead to these circuit changes. Resting state data may also be analyzed using dynamic functional connectivity approaches (Hutchison et al. 2013), graph-based methods (Wang et al. 2010a), or independent component analysis. Each approach has its advantages and limitations. In general, MR neuroimaging methods have high spatial resolution but poor temporal resolution. Molecular imaging has about a thousandfold greater sensitivity than MR for detecting changes in concentrations of chemical moieties (like a labeled ligand).

Electrophysiologic methods have much better temporal resolution than neuroimaging methods and can be used to investigate circuits in animal model

systems. These types of translational studies can be particularly relevant as electroencephalography (EEG), event-related potentials, magnetoencephalography (MEG), and local field potentials have been used to investigate neurodegenerative disorders in humans (Rossini et al. 2007; Oswal et al. 2013), and the animal model studies can help identify the mechanisms and pathophysiology underlying observed electrophysiologic changes. For example, local field potentials were used to identify changes in basal ganglia beta oscillations in various rodent models of Parkinson disease (Lobb et al. 2013); such animal studies provide important clues to the functional effects of deep brain stimulation in the treatment of Parkinson and related disorders. Another interesting example from a nonhuman primate model of Parkinson disease is the change in EEG synchronization of beta and gamma band frequencies after injury to nigrostriatal neurons, found with simultaneous scalp EEG and neuronal activity in pallidum and subthalamic nucleus (Gatev and Wichmann 2009). This approach revealed a marked disruption of basal ganglia-cortical connections.

Relevance to Neurodegenerative Conditions

These neuroimaging methods have been applied to humans with neurodegenerative conditions and then animal models of these disorders. Surprisingly, some of the standard resting state networks have been found in nonhuman primates or even rodents, despite the fact that the animals were anesthetized at the time of the imaging (Vincent et al. 2007; Mantini et al. 2011). The effects of anesthesia may not be trivial and remains a major point of investigation; some suggest that light sedation is better than deep anesthesia (Guilfoyle et al. 2013; Kalthoff et al. 2013). Alternatively, studies can sometimes be performed in awake animals. While this adds a substantial level of complexity and difficulty, it may prove critical for some types of studies. For example, levodopa-mediated circuits in nonhuman primates are completely altered by anesthesia (Hershey et al. 2000).

Each neuroimaging method has relatively limited temporal resolution compared to electrophysiologic methods such as MEG, which has poorer spatial resolution but much better temporal resolution (Hall et al. 2013). In fact, combining different imaging modalities may yield a more complete view of brain networks than any one individual method. For example, it may be possible to combine multiple imaging methods like molecular imaging (with thousandfold greater sensitivity compared to MR methods) with MR-based resting state connectivity, tractography, or both. Similarly, these methods could be combined with electrophysiology, which offers greater temporal resolution. Multimodal studies have been gaining increasing traction and will clearly play a greater role in future studies.

Once seemingly relevant and statistically significant networks have been found in animal models, there are still several criteria that must be met to

determine relevance to the human condition. Networks found in animal models that resemble those found in patients with specific neurodegenerative disorders suggest that the model may prove to be valid. Next, it would be helpful to know whether changes in the strength of network connectivity relates to a change in the relevant animal behavior. In this way, one could consider using network strength as a biomarker for testing the therapeutic efficacy of a new proposed intervention. The value of such a biomarker could include greater sensitivity compared to behavioral measures, potentially greater objectivity of the measure, and insight into the underlying mechanism of the intervention. In particular, a biomarker or change in network function that became abnormal prior to behavioral or symptomatic manifestations of a disorder could be particularly helpful in the development of an intervention that could forestall or prevent disease progression, or even the development of any manifestations. It is important to note that an intervention could have substantial behavioral benefit, even though its mechanism of action could bypass the function of the brain network proposed as a biomarker. A simple example of this would be the application of a molecular imaging of midbrain uptake of radiotracers for nigrostriatal dopaminergic neurons (reflecting severity of nigrostriatal injury that occurs in Parkinson disease and correlates with severity of motor Parkinsonism) to measure the efficacy of deep brain stimulation of the subthalamic nucleus (Tabbal et al. 2012; Brown et al. 2013). The stimulation effects are downstream of the component of the circuit measured with that particular molecular imaging biomarker (Hershey et al. 2000). In this case, the network biomarker would not be an appropriate measure of the efficacy of the intervention, even though it might be used to assess other interventions with different mechanisms of action. Thus, one must approach this type of investigation with caution and have clear goals before selecting the right biomarker.

The key steps in development and validation of a relevant biomarker for use in neurodegenerative diseases require an animal model system. The readout or biomarker applied to such an animal model must be robust, validated, and relevant to either the underlying mechanisms of disease or the behavioral manifestations of the disorder. Validation includes demonstration of a standard curve, just like any assay applied in a lab, especially if the biomarker is to be used to assess therapeutic interventions. To make a standard curve, one needs to be able to produce a graded deficit in the animal model system and then ensure that the measurement of the biomarker (or in this case, network function) reflects graded deficits in a clear manner. Alternatively, a biomarker or change in network function could be intact or not be intact—a categorical designation, potentially important for group classifications for research studies. This more limited descriptor still can have value if it is a more sensitive indicator of relevant dysfunction than overt behavior.

Once validated in an animal model system, a biomarker might serve several functions in human studies. If more sensitive than behavioral measures, it could provide an endophenotype for selecting patients for clinical trials (stratifying or

enhancing the homogeneity of patient groups) or genetic studies (distinguishing affected from unaffected subjects) (Racette et al. 2006). Another strategy is that analysis of various circuit abnormalities can provide sites for target engagement. For example, Bergman et al. (1994) used an MPTP-induced nigrostriatal deficit in nonhuman primates to identify increased single unit recordings with increased bursting in the internal segment of the globus pallidus. These direct electrophysiologic recordings provided rationale to further pursue pallidotomy and deep brain stimulation of either the subthalamic nucleus (which provides a direct glutamatergic input to globus pallidus) or direct stimulation of globus pallidus for treatment of Parkinson disease (Wichmann et al. 1994, 1999). In fact, much of the work on mechanisms of deep brain stimulation has come from studying changes in circuits in animal model systems (Miocinovic et al. 2013).

Investigations using multiple modalities may provide greater insights into the etiopathologic mechanisms of disease than studies which use a single metric of brain function. Examples include investigations of what causes regional vulnerability of selected brain regions to Aβ amyloid pathology, as identified by *in vivo* amyloid PET imaging in mouse models (Bero et al. 2012), or the relationship of various pathophysiologic changes that precede dementia onset in people with dominantly inherited mutation destined to develop the disease (Bateman et al. 2012; Benzinger et al. 2013). An important finding may be that these brain regions are key components of the default mode network and have high intrinsic brain activity (Sheline et al. 2010). Loss of intra- and internetwork resting state functional connectivity may accompany the progression of Alzheimer disease (Brier et al. 2012). Multimodality studies in animal models of abnormal amyloidosis may permit identification of the key pathologic dysfunctions that lead to these circuit abnormalities; however, these types of studies necessitate an analysis of the underlying changes in brain function, which requires careful *in vitro* biochemical analyses (Bero et al. 2011, 2012). Thus, the greatest insights into mechanisms of disease may arise from studies that integrate findings from multiple approaches to reveal what biochemical or physiologic processes underlie changes in abnormal brain circuits.

Caveats on Relevance of Animal Model to Human Neurodegenerative Disease

Several caveats must be considered when animal models are implemented to investigate human neurodegenerative disease. As noted, animal behaviors and neuroanatomical complexity frequently require extrapolation to corresponding human behavior or brain circuits. This is less true for nonhuman primates, particularly in neurodegenerative disorders, but clearly applies to rodents, flies or zebrafish. Imaging studies in animal models frequently employ anesthesia or sedation, which may or may not confound the interpretation of results. Some

resting state networks seem relatively impervious to this, but caution is clearly warranted as pharmacologic activation may vary substantially in nonhuman primates, depending on anesthesia (Hershey et al. 2000). Perhaps, most importantly, the selection of an animal model system must match the goals of an investigation. Some models offer the means to test potential symptomatic treatments (like toxin models), whereas others may be better suited to investigate underlying changes in biochemical pathways (like genetic models). These are not hard and fast rules but rather important guidelines to consider. The development and validation of model systems require an iterative process. Insights from human pathology, physiologic dysfunction, and genetics may provide the initial impetus for development of an animal model and investigation of changes in brain pathways and circuits. However, refinement of the model almost always yields a better tool. Finally, careful validation of these tools is critical to the interpretation of studies of either the model systems or biomarkers developed using these models. Each investigator that applies such tools needs to be responsible for understanding their advantages and limitations.

What Tools Do We Need?

Genetic models provide useful model systems to investigate underlying brain mechanisms. Advances in genetic engineering based on TALENs and CRISPR (Wang et al. 2013b) have made development of such models much easier. These studies can be done in mice, faster in zebrafish, and even faster in flies. However, behavior of animals such as zebrafish and flies may be difficult to align with human behavior, depending upon the complexity of the behavior. Development of sophisticated nonhuman primate genetic models could have a substantial impact on applicability of these model systems. Use of nonhuman primates is likely to be slower and more expensive, but the value and utility of such models may justify the additional effort for some applications (Hutchison and Everling 2012).

Additional work on functional connectivity methods remains to be done. Issues regarding preprocessing these types of data, including potential confounding factors such as the effects of global regression or tolerance for movement during data collection, need further evaluation. Clearer roles for different methods of data analysis will help. Substantial efforts to refine functional connectivity with resting state BOLD and anatomical connectivity with tractography through connectome projects offer important opportunities to compare connectivities (Blumensath et al. 2013; Ugurbil et al. 2013) in animal models; some have already started doing direct comparisons between human and nonhuman primates. Application of additional multimodality approaches will help integrate various measures and provide a more complete view of underlying pathology. This represents a particularly critical need, as multimodal approaches can take advantage of the greater sensitivity of molecular imaging,

the high spatial resolution of MR-based methods, and the faster temporal resolution of electrophysiology. In addition, further genetic studies in humans with neurodegenerative diseases will help provide genetic mutations upon which to base additional animal models.

The development of biomarkers that measure circuit function or other brain dysfunctions impervious to typical symptomatic treatments would be a major advance for development of interventions that could slow or halt disease progression for conditions like Parkinson disease. This would allow us to test such therapies in patients taking symptomatic therapy and offers two advantages: accuracy of diagnosis frequently increases with longer follow-up and recruitment of treated patients is far easier than recruiting those not yet treated. These advantages translate into substantially lower clinical trial costs. Of course, one must consider whether pathologic progression has exceeded the potential to reverse or slow disease processes at that point. This will depend on the degree of injury that occurs at the time of symptom onset and may vary greatly for different neurodegenerative diseases. Finally, enhanced educational opportunities, improved collaboration between basic investigators and clinician scientists, as well as greater funding for this type of research are all critical if progress is to be exacted. This poses a particular challenge in times of financial constraint.

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