

Schizophrenia, DISC1, and Animal Models

by John J. Medina, PhD



After a lecture I delivered at last year's Psychiatric Congress ("Myths and Facts, Part I: Infant Brain Development," Orlando, October 2007), I was chatting with a group of mental health professionals about understanding the distance between a gene and a behavior. I cautioned against the sometimes overly enthusiastic application of behaviors observed in animal models to issues relating to human mental health. Then I mentioned that I would address the subject in a future *Molecules of the Mind*.

This month's column is an example of the perils and the promises of using an animal model to ferret out one of psychiatry's most intractable problems: the genetic substrates mediating schizophrenia and depression. The subject concerns what some describe as the "schizophrenic mouse," an animal carrying a disrupted gene called "disrupted in schizophrenia 1" (DISC1), and its utility in illuminating the human molecular machinery behind this most frustrating disorder. It is a hopeful tale that illustrates the great progress of the research community in characterizing this tragic illness. It also represents a cautionary tale in the interpretation of results.

The goal of this column is to describe some of the biology of this interesting gene and its potential links to mental health in light of this caution. I will start with some of the general concerns about using animal

models to describe anything about human behavior. Then I will move on to the isolation of specific genetic sequences in a famously troubled Scottish family. I will end with a description of some truly remarkable progress associated with laboratory rodents carrying mutated DISC1 genes and add a quick comment about exactly what this animal model may (and may not) reveal about the disorder.

General objections

Let me begin by summarizing some of my comments from Orlando, which are obvious appeals for caution and a reason for excitement. The first comment has to do with the comparative validity of applying findings among creatures with the same obvious neurological structural and functional differences, as is seen in humans and laboratory animals. The human cortex, if stretched out fully, is

about the size of a baby blanket. The mouse cortex, if stretched out fully, is about the size of a postage stamp. The bewilderingly connective nature of the human brain allows us to build things such as complex skyscrapers. The not-as-bewildering connective nature of the mouse brain allows it to build things such as simple nests.

My grumpy objections even carry over to the use of primates. It is not as if macaque monkeys merely write symphonies badly and we write them well. We do not, in the end, share the same cognitive universe with any other creature in the world, and so we must be conservative in our interpretations, even with near genetic neighbors.

My second comment has to do with the nature of studying human behavioral pathologies. In my admittedly reductionist research universe, the most basic diagnostic forms of psychiatric disorders can be distilled to a list of symptoms. Some of these lists possess symptoms that are unique to a given disorder, some possess symptoms that are not unique, and some possess considerable overlap with other diagnostic categories. Even when the categories seem on sure footing, many symptoms are best described as a continuum of behaviors in which severity may differ from one person to another.

These exigencies limit much of

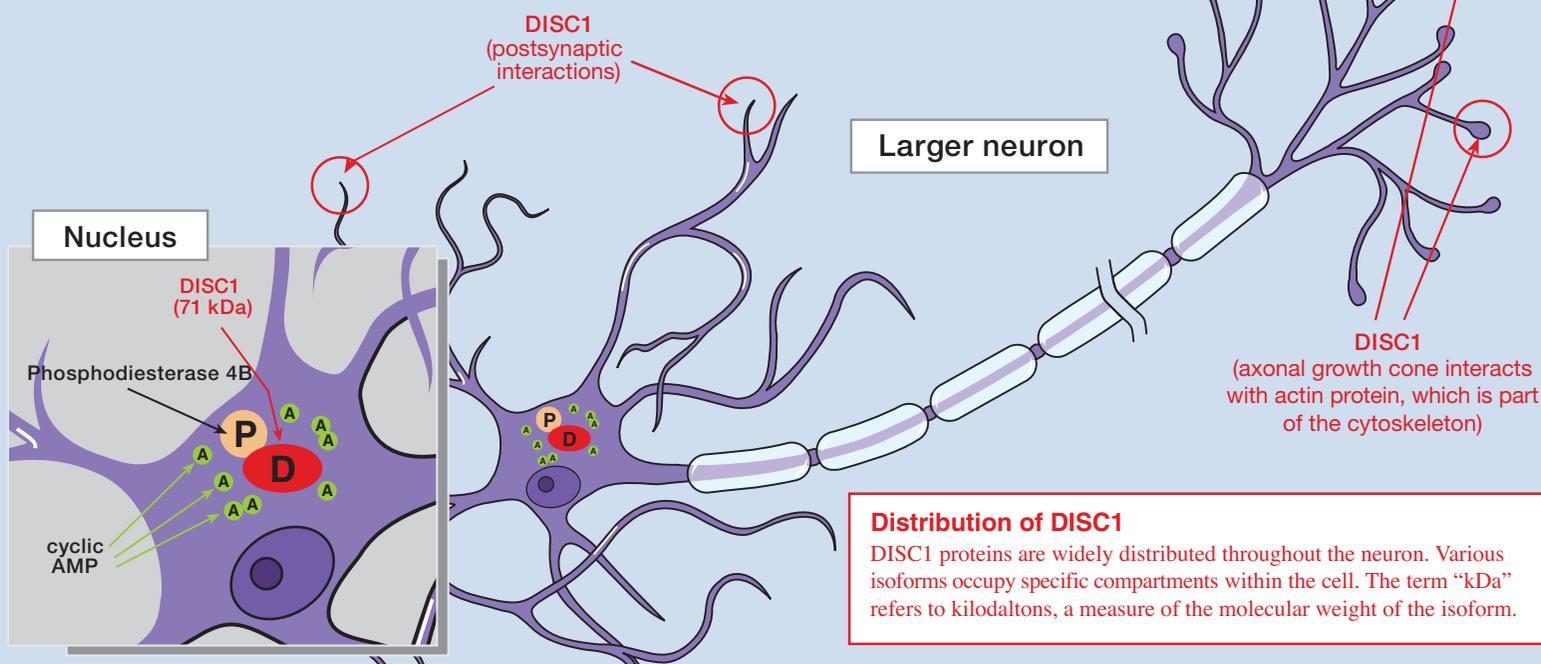
what can be said about interpreting animal data in light of human experience. The observable data can help, but most behavioral findings from animal models incompletely describe the full-blown human experience. Rather, they seem to be mimicking certain specific *characteristics* of a psychiatric illness. It is here that the presence of animal models finds its greatest utility, and I can perhaps be a bit less finicky. I think of using animal models in the same way that I think of using a flashlight to discover items in a darkened closet. Flashlights are very useful for revealing certain characteristics as we peer into the unknown but no one ever confuses the presence of the flashlight with the items the flashlight reveals. The same is true of animal models. They make great flashlights that are fully capable of showing us the way to find the mysterious biological objects hiding in our behavioral wardrobes. However, one must never confuse these insights uncovered by animal models with human behaviors. The animal models simply inform us about which shelves we should examine first.

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Figure

Molecular distribution of DISC1

Shown below is the distribution pattern of some of the isoforms of disrupted in schizophrenia 1 (DISC1) proteins and various associated molecules. The inset box shows the nucleus of the neuron depicted in the larger drawing.



Animal Models

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That is what my comments at the Psychiatric Congress were all about. I am now ready to talk about some truly exciting findings that point to some very important molecules sitting on our behavioral shelves.

A long history

The molecular biology of schizophrenia, and, to a lesser extent, depression, has frustrated researchers for many years. More than 130 candidate genes have been found that predispose humans to these disorders. However, many of these findings were not successfully replicated, and to date there is no gene or combination of genes capable of providing an overarching molecular description of schizophrenia or major depression.

That doesn't mean progress has been slow, however. One of the most promising findings came from the investigation of a Scottish family. A break point at chromosome 1q42 was found to cosegregate with a prominent presentation of mental illness, including major depression, bipolar disorder, and schizophrenia. There was a gene disrupted by this translocation aptly named DISC1. There are a number of protein isoforms of DISC1 that are localized to different intracellular compartments within the neuron. (Isoforms are multiple versions of the same protein in which amino acid sequences may be different, but overall function is the same [Figure]). Another sequence, called DISC2, was also isolated and appeared to regulate the expression of DISC1.

Linkage studies from laboratories investigating other families soon confirmed the association of mutations in DISC1 with a predisposition for schizophrenia (and the other mood disorders mentioned). The gene became linked to reduced gray matter density, reduced gray matter volume, abnormal hippocampal structure/function, and impaired memory. No one knew what DISC1 did at the time (we still do not know), but whatever it did, the brain and its attendant psychiatric disorders appeared to be involved.

After the isolation of DISC1, researchers began to unravel some of the molecular interactions of this mysterious gene. DISC1 protein was found to be expressed in specific areas of the mammalian brain (mouse brain) during 2 critical developmental stages. The first occurred in utero—during early brain development. The second occurred ex utero—with the onset of puberty. There is a particu-

larly large elevation of protein in the developing hippocampus. Such regional specificity in an expression profile has been found to be highly conserved, showing that important flashlight feature of working with animal models. This expression pattern has been demonstrated in nonhuman primates and even in human brain tissues.

This conserved specificity led researchers to seek its role at the cellular and molecular levels, and once again, a developmental story emerged. The protein predicted from the amino acid sequence had a specific shape—a helical tail domain and a globular head. That finding may immediately throw up a red flag. This is the canonical structural motif of proteins deeply involved with intracellular scaffolding, which is huge in brain development. Such scaffolding plays vital roles in regulating neurite outgrowth and in mediating intracellular signals that guide neuronal migration while the brain is forming. Consistent with this scaffolding function, DISC1 has been shown to interact with a wide variety of proteins, many associated with the neural cytoskeleton. (The cytoskeleton is a dynamic internal structure that compartmentalizes the cytoplasm into functional domains and helps maintain cellular shape. Controlling neural growth at the molecular level also requires control over cytoskeletal remodeling and is an important developmental process.)

One of the most important molecules with which DISC1 interacts is a protein called phosphodiesterase 4B (PDE4B, see Figure). PDE4B appears to be functionally regulated by DISC1 and is also involved in the signal transduction pathways mediated by a canonical molecule, cyclic AMP (cAMP). cAMP mediates a wide variety of adult and developmental intracellular functions; it is the classic signal transduction molecule and is involved in a wide variety of cytosolic processing, including compartmentalizing the same sets of molecules through which DISC1 interacts.

These interactions appear to have psychiatric consequences. Similar to DISC1, disruption of the gene that encodes PDE4B is an independent risk factor for schizophrenia. The molecular functions of the PDE4B isoforms protein can be inhibited by rolipram, a drug currently in use as an anti-inflammatory medication that also possesses antipsychotic properties. In laboratory animals, PDE4B can mediate both memory and mood. Indeed, the ability of rolipram to inhibit the molecular action of PDE4B has caused a number of laboratories to investigate its potential as an

antidepressant.

Another important protein with which DISC1 interacts, although in a complex form, is a gene called lissencephaly 1 (LIS1). As its name implies, mutations in the LIS1 gene cause severe brain malformations in humans, a group of disorders often called the "lissencephalies." These deformities are a direct result of aberrant neuronal migration in the developing neocortex.

Taken together, the DISC1 protein plays a powerful role in a number of neurologically related developmental processes. However, what are the consequences of carrying a DISC1 mutation in an intact animal, whether rodent or Scottish family member? As mentioned, the jury awaits the final verdict about the real function of DISC1. One important step toward fulfilling that goal is to examine or even to breed for naturally occurring DISC1 mutations in laboratory animals and look for the presence of altered behaviors. One could speed up the process by creating deliberate mutations in an intact animal's genes and then investigating the same gene-to-behavior relationship.

To the present

Creating mutations is exactly what teams of researchers from Canada, Japan, and the United Kingdom have done. Three types of animals were examined, each possessed disruptions in DISC1 genes. One was a naturally occurring deletion mutation. Deletion mutations occur from missing nucleotides and often result in the aberrant reading of a gene. This can either result in no protein or one with altered biological function. Two of the mutations were created using a chemical mutagenesis program involving *N*-ethyl-*N*-nitrosourea, a toxic mutagen capable of introducing point mutations once every 1.5 Mb. The 2 deliberately mutated animals resulted in missense variants in the DISC1 gene. Missense mutations are nonsynonymous point mutations in which the alteration in a single nucleotide results in an amino acid substitution. These substitutions can also result in a protein with altered biological function.

Consistent with previous data, these mutations created abnormal brain pathologies. One of the missense mutants had a smaller brain volume and displayed behaviors remarkably different from the controls. The protein produced by this mutant was associated with reduced PDE4B activity when compared with controls. These animals had prolonged swim immobility, decreased latent inhibition, and showed a significant reduction in their ability to socialize.

These altered behaviors are evocative of some of the information-processing anomalies observed in patients with mood disorders and psychoses (such as generally impaired processing, anhedonia, depression). Perhaps most interesting, their abnormal behavior could be markedly improved with the administration of clozapine (Clozaril) and haloperidol (Haldol).

The second missense mutation was unlike the first. Although the animal also had a reduced overall brain volume, it presented with a different set of behavioral anomalies. These included alterations in sensorimotor gating reminiscent of patients with schizophrenia—prepulse and latent inhibition—with an increase in overall horizontal activity. These behaviors could also be altered with medications that have been given to humans. The prepulse inhibition, for example, could be reversed with the administration of the PDE4 inhibitor, rolipram. Interestingly, this mutant produced a protein that was not associated with a reduction in the activity of PDE4B.

Conclusion

These data represent a remarkable achievement, even when one keeps in mind the cautions mentioned previously. The results unambiguously reveal an important role for DISC1 in the normal development of the mammalian brain. They also uncover aspects of DISC1's important association with PDE4B, and suggest what parts of the DISC1-PDE4B interaction mediate specific brain functions.

Most important, these mutations create behavioral alterations in animal models that (fragmented though they may be) are reminiscent of the observations in psychiatric clinics. The ability of known psychiatric medications to address specific mutant-generated behaviors is especially helpful. Another important finding is that a common genetic etiology appears to exist between "fragments" of schizophrenia and fragments of depression. There is a similar suggestion that the human counterparts of these disorders share a similar common genetic ancestry.

There is every reason to be excited. As long as one is careful about how the data are interpreted, from the phylogenetic distance between rodents and humans to the potential ambiguities of the diagnostic criteria, we should expect greater excitement to come. May such animal research have a long and well-funded future.

Dr Medina is a developmental molecular biologist and private consultant, with research interests in the genetics of psychiatric disorders. □