Testing the glutamate hypothesis of schizophrenia

Joshua A Gordon

A study in this issue presents a new mouse model that directly tests the glutamate hypothesis of schizophrenia. The study reports that a decrease in NMDA receptor signaling during a particular developmental window in interneurons can induce cellular and behavioral changes similar to those seen in schizophrenia.

Modeling psychiatric illness in mice is a tricky business, especially for diseases such as schizophrenia, which is marked by disturbances in cognition, social function and reality testing. Schizophrenia disrupts functions at the core of what seems to make us human, and even the notion of producing a model of schizophrenia in an animal as lowly as the mouse can offend our sensibilities. However, evidence indicates that schizophrenia is, at least in part, a genetic disease. Thus, the promise of genetic mouse models to enrich our understanding of the neurobiology of schizophrenia is tremendous, as is the number of mouse models of schizophrenia presented in the literature. Can they all be valid? How is one to evaluate whether any given animal is a good model of schizophrenia? Although some models, such as those with the strongest genetic validity, might more faithfully represent the causality of schizophrenia than others, attempts to define a 'good' model often miss the point. Science uses models not to represent diseases in all their complexity, but rather as tools to test hypotheses. In the current issue, Belforte *et al.*[1] present a mouse model that tests an important hypothesis about a specific aspect of schizophrenia pathogenesis. The study is compelling as much for its approach as for its findings. It reminds us that the most important question is not whether this is a good model, but instead, what is this model good for.

The author is in the Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York, New York, USA, and the Department of Integrative Neuroscience, New York State Psychiatric Institute, New York, New York, USA.
e-mail: jg343@columbia.edu

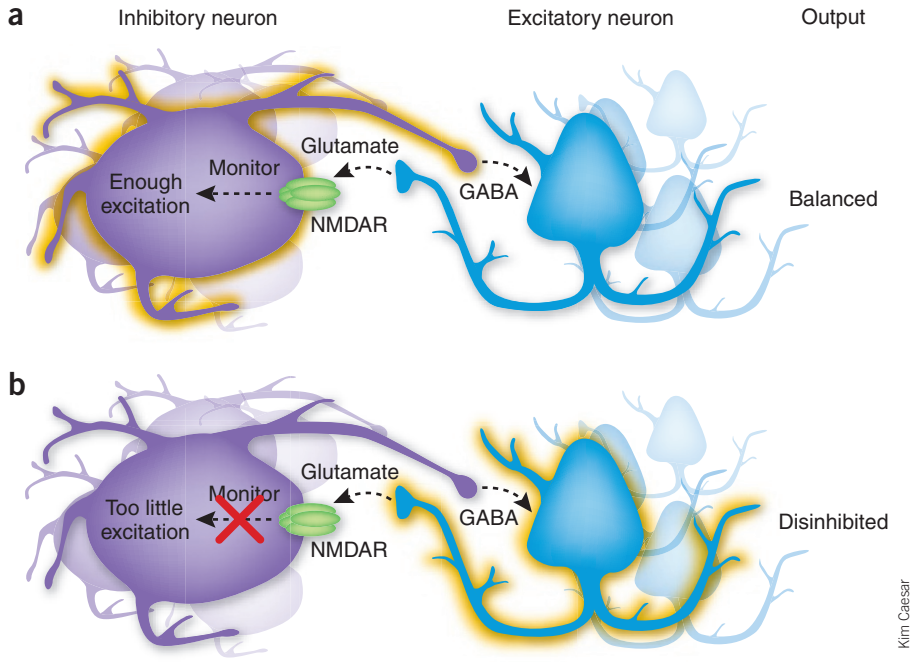

Figure 1 Glutamate hypothesis of schizophrenia pathogenesis. (**a**) Inhibitory neurons monitor levels of excitatory activity via NMDA receptor (NMDAR) signaling. Normally, the inhibitory neuron maintains sufficient GABA release to balance inhibition with excitation. (**b**) In the cortex of individuals with schizophrenia, decreased NMDA receptor signaling disrupts this monitoring function, fooling inhibitory neurons into acting as if there is insufficient excitatory activity. The inhibitory neurons downregulate their output, disinhibiting the excitatory neurons.

The starting point for the Belforte *et al.*[1] study is the glutamate hypothesis, which posits that a deficit in glutamate neurotransmission underlies a substantial portion of the dysfunction seen in schizophrenia. Glutamate is the principal excitatory neurotransmitter in the mammalian CNS. Glutamate binds three major types of receptors, one of which, the NMDA receptor, is important in the hypothesis. The NMDA receptor is most famous for its importance in learning and memory, but its lesser-known direct role in excitatory neurotransmission was the focus of the study. Suspicions that the NMDA receptor is involved in schizophrenia were first raised by the realization that certain drugs of abuse, namely PCP and ketamine, cause a constellation of symptoms that are typically seen in schizophrenia, including psychosis, social withdrawal and working-memory deficits[2]. These drugs were shown to be NMDA receptor antagonists. Evidence from a variety of clinical data gathered since then have extended these initial observations[3],

lending further credence to the notion that glutamate hypofunction is involved in schizophrenia. It should be noted that the glutamate hypothesis is not based on genetics *per se*, despite reasonable attempts to integrate the hypothesis with existing genetic findings[3,4]. There is little evidence, for example, of genetic linkage between schizophrenia and the genes for the NMDA receptor subunits or other components of the glutamatergic system. Instead, it is a pathophysiologic hypothesis that attempts to understand what might be going wrong in the brains of individuals with schizophrenia in a comprehensive fashion.

Indeed, what makes the glutamate hypothesis so attractive is its ability to tie together disparate pieces of evidence from the literature into a unified theory of schizophrenia pathogenesis. Consider, for example, one of the most widely accepted pathological findings in the cerebral cortex of individuals with schizophrenia, namely that there is altered staining for various components of the machinery required for proper function of GABA, the principal inhibitory neurotransmitter in the brain[5,6]. A host of biochemical abnormalities have been found in a particular subtype of GABA-containing neurons, the so-called fast-spiking interneurons that express the calcium-binding protein parvalbumin. These include decreases in the expression of the GABA-synthesizing enzyme GAD67 and parvalbumin itself. Collectively, these and other abnormalities suggest that there is diminished activity, synthesis and release of GABA. Complementary increases in the expression of GABA receptors at postsynaptic sites innervated by these interneurons also suggest that the GABA system is hypoactive. Notably, similar changes can be induced by chronic administration of NMDA antagonists, which are thought to preferentially diminish activity in interneurons[4,5]. These and other findings suggest that, in parvalbumin-positive neurons, the NMDA receptor serves as a kind of a monitor of overall excitatory activity in the cortex. Decreased NMDA receptor signaling would be interpreted by the interneuron as too little excitatory activity and cause it to reduce its own activity in an effort to restore the balance between inhibition and excitation required for proper circuit function (**Fig. 1**). Thus, the GABA deficits found in schizophrenia could be the direct results of diminished NMDA receptor activity specifically in the cortical interneuron population[4].

Previous studies have shown that decreased expression of the NR1 subunit, which is required for normal NMDA receptor function, causes a range of schizophrenia-like phenotypes[7,8], consistent with the broader glutamate hypothesis. To directly test the hypothesis that NMDA receptors in interneurons are important, Belforte *et al.*[1] used a conditional knockout strategy. They used a line of mice expressing the Cre bacterial recombinase in a subset of inhibitory interneurons in the hippocampus and cortex. These mice were mated with mice that have an altered gene for the NR1 subunit, in which the recognition sites for the Cre recombinase had been inserted. In the resultant hybrid mice, NR1 protein expression was undetectable in approximately half of the inhibitory interneurons in the cortex and hippocampus; the great majority of these were indeed parvalbumin-containing neurons. Notably, the authors confirmed that deletion of the NR1 subunit completely eliminated NMDA receptor–mediated currents in affected interneurons. NR1 expression in pyramidal neurons, and in interneurons in other brain regions, was unaffected.

It should be emphasized that these mice are not a model of schizophrenia *per se*. There is no genetic evidence, for example, implicating the human *GRIN1* gene (which encodes NR1) in schizophrenia, nor is there direct evidence of decreased expression of NR1 in interneurons in postmortem tissue. Interpreting behavioral phenotypes in these mice must be done with caution. Indeed, interneuron-specific ablation of NR1 subunits resulted in a broad variety of behavioral abnormalities, some of which may have little relevance to schizophrenia. Nonetheless, disruptions were found in pre-pulse inhibition of the acoustic startle reflex and spatial working memory, two reasonably well-validated behavioral tests that are often used to model classic schizophrenia-associated deficits in sensorimotor gating and prefrontal cortex–dependent cognition. Risperidone, an effective antipsychotic, reversed the working memory deficits seen in the mutants. Although controversial, some studies have observed similar pro-cognitive effects of the drug in individuals with schizophrenia[9,10].

Less clearly relevant findings were obtained in a variety of additional behavioral tests. Nest building, mating frequency and social short-term memory were disrupted by NR1 ablation, suggestive of social deficits seen in the disorder. Increased anxiety-like behavior and decreased preference for sweets were also seen, consistent with emotional disturbances that can be seen in some individuals with schizophrenia. Several of these behavioral disturbances were exacerbated after social isolation stress. Although many might argue that these social and emotional behaviors are of unclear relevance to schizophrenia, the data are at least consistent with the notion that an interneuron-specific decrease in NMDA receptor function could contribute to the behavioral abnormalities observed in schizophrenia.

However, the utility of this model lies not in how well it recreates the behavioral abnormalities seen in schizophrenia, but rather in how it informs our understanding of schizophrenia pathogenesis. Thus, it is the question of whether the interneuron-specific manipulation of NMDA receptor function recapitulates the schizophrenia-associated deficits in GABA neurotransmission that is of greatest interest. To this end, the authors measured the expression levels of GAD67 and parvalbumin. Crossing the mutant mice to an additional reporter line, they found that GAD67 and parvalbumin levels were decreased only in NR1-deleted interneurons. They further confirmed that these decreases affected inhibitory function by recording from putative excitatory neurons during awake exploration. Activity in these neurons was increased in the NR1-deleted mice, consistent with a decrease in inhibitory tone. These results indicate that interneuron-specific reductions in NMDA receptor signaling can alter the expression of GABA-related markers and affect circuit function as predicted by the glutamate hypothesis. They further suggest that additional studies using these mice might be useful for refining the glutamate hypothesis.

Indeed, the study proceeds to do just that, taking advantage of conditional knockout technology to ask when in the life of the animal NR1 deletion exerts its effects. Using an additional mouse line with a more difficult to recombine allele at the NR1 locus, the authors were able to derive mice in which the NR1 deletion was not complete until adulthood. Tested as adults, these mice had normal levels of pre-pulse inhibition, spatial working memory, and GAD67 and parvalbumin expression. Moreover, activity of excitatory neurons was normal. These findings suggest that deletion of the NR1 subunit during development is crucial for the emergence of both its behavioral and pathophysiological consequences. This extension of the glutamate hypothesis, that NMDA receptor hypofunction during development might cause the bulk of dysfunction in schizophrenia, is at once important and troubling. It is important because it reminds us of the potential importance of developmental processes and critical periods in schizophrenia. It is troubling because it suggests that by the time

patients are identified later in life, the damage caused by NMDA receptor hypofunction may already have been done. Restoring NMDA receptor signaling may not be sufficient to reverse the pathophysiology and reduce the symptoms of the disease.

The glutamate hypothesis of schizophrenia has survived this test. We now know with a fair degree of confidence that an interneuron-specific decrease in NMDA receptor signaling can induce changes in the GABA system similar to those seen in schizophrenia. We further suspect that this reduced signaling is particularly influential during a particular developmental window. Whether or not this reduction in NMDA receptor signaling actually happens in individuals with schizophrenia, the model can be used to answer a host of important questions. Will restoring NMDA receptor function during adulthood reverse these changes, or is the system already locked in to its fate? Are the changes in pathophysiology and behavior causally related? Is NR1 deletion in any specific cortical region particularly important in the syndrome? And, perhaps most importantly for patients, can therapies, new or extant, reverse the behavioral consequences of NR1 hypofunction? Each of these questions can be addressed as the NR1 deletion model is studied in greater depth, as long as each study is designed with an appropriate hypothesis in mind.

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Ion pumps get more glamorous

David L Glanzman

A new study shows that the Na^+/K^+ ATPase can function as an integrator of spike activity and interacts with a K^+ conductance to provide a cellular short-term memory of locomotion in the *Drosophila* larval motor circuit.

I suspect I am not the only behavioral neuroscientist who finds the subject of ion pumps to be sleep inducing. Naturally, the importance of ion pumps to basic neuronal function is appreciated. By constantly pushing Na^+ ions out of and K^+ ions into neurons, Na^+/K^+ pumps generate the difference in charge across the neuronal cell membrane that is the basis of the resting membrane potential. Nevertheless, I have long regarded the subject of ion pumps in much the same way I regarded botany as an undergraduate student: I recognized its importance, but was content to leave the study of this subject to others, as its scientific attractiveness to me was scant. But now a new study by Pulver and Griffith[1] suggests that a lowly ion pump can have a more glamorous mechanistic role than I had imagined. The authors show that a Na^+/K^+ pump in motor neurons of fruit fly larvae can subserve motor memory, albeit over a short span of time.

Drosophila larvae advance through waves of contractions of their body wall musculature that are segmentally coordinated by a central pattern generator (**Fig. 1a**). The central pattern generator causes motor neurons in the ventral ganglion of larvae to fire in high-frequency, rhythmic bursts (**Fig. 1b**,**c**); this rhythmic firing then drives the peristaltic contractions of the body wall that produce larval locomotion[2]. Each action potential burst in the motor neurons is followed by an afterhyperpolarization (AHP; **Fig. 1d**), a negative membrane potential (~20 mV below the resting potential) that typically lasts for 15–20 s[1].

Pulver and Griffith[1] first sought to identify the intrinsic membrane currents that underlie the AHP. Electrophysiologically recording from a pair of motor neurons, the authors found that the AHP was abolished by blocking sodium channels in the neurons with the neurotoxin tetrodotoxin. In contrast, potassium currents

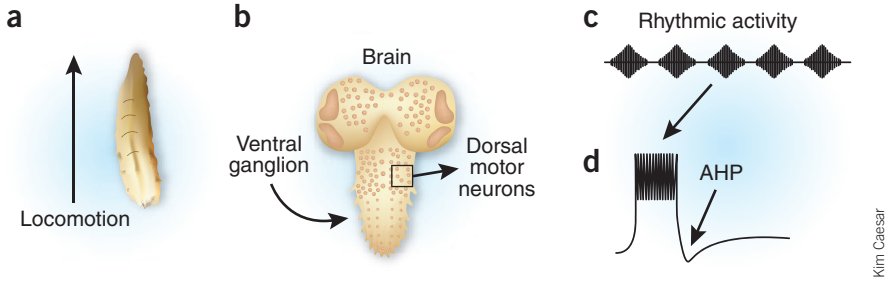

Figure 1 Neural activity related to locomotion in *Drosophila* larvae. (**a**) Movement of the larva is produced by peristaltic waves of contraction of the body wall musculature. The rhythmic contractions of the body segments are coordinated by a central pattern generator. (**b**) The CNS of the *Drosophila* larva. The motor neurons that drive locomotion are located in the dorsal region of the ventral ganglion. (**c**) Rhythmic pattern of firing of the motor neurons during locomotion, as recorded extracellularly from the ventral nerve. The pattern of motor neuron firing is generated by the central pattern generator. (**d**) A single burst of action potentials in a dorsal motor neuron during locomotion, as recorded with a whole-cell patch electrode. An AHP (arrow) follows the burst. The AHP lasts for several seconds and is produced by the Na^+/K^+ ion pump. The amplitude of the AHP encodes the number of action potentials in a motor neuron burst, independent of the pattern of firing. In addition, the AHP interacts with an A-type K^+ current to provide a cellular memory of previous activity in the motor neurons by modulating the onset of the first action potential in a burst.

The author is in the Department of Physiological Science, University of California Los Angeles (UCLA) College, and the Department of Neurobiology and Brain Research Institute, David Geffen School of Medicine at UCLA, UCLA, Los Angeles, California, USA.
e-mail: dglanzman@physci.ucla.edu