Adolescent Onset of Cortical Disinhibition in Schizophrenia: Insights From Animal Models

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Schizophrenia and related mental disorders are common and devastating conditions for which we have a limited understanding of their origin and mechanisms. Although this apparent lack of progress despite vast research efforts could be due to difficulties in reproducing the disease in animals, animal work is now providing important insight onto possible pathophysiological changes in the brain. Postmortem studies of human brains have provided data indicating altered local inhibitory circuits in the cerebral cortex in schizophrenia and different developmental, pharmacological, and genetic animal models converge in revealing deficits in cortical interneuron function that can be associated with neurophysiological and behavioral alterations resembling aspects of the disease. Schizophrenia pathophysiology has a complex developmental trajectory because overt symptoms become evident during late adolescence despite earlier events contributing to the disease. The late incidence of schizophrenia can be explained by the protracted maturation of brain circuits implicated in the disease, particularly during adolescence. Excitatory and inhibitory processes in cortical circuits are tightly modulated by dopamine (DA), and many aspects of DA function in cortical regions acquire their adult profile during adolescence. This maturation fails to occur or is abnormal in several different rodent models of schizophrenia, yielding a number of functional and behavioral deficits relevant to the disease. Thus, periadolescent changes in cortical inhibitory circuits are a critical developmental stage likely implicated in the transition to schizophrenia. These observations provide the foundation for novel research-based therapeutic approaches and perhaps will even lead to ways to prevent the progression of the disease in predisposed subjects.

Key words: GABA/interneuron/prefrontal cortex/hippocampus/review/dopamine/adolescence/development

Adolescent Maturation of Cortical Circuits and Schizophrenia

The development of brain circuits during adolescence is of obvious importance for schizophrenia and other major psychiatric disorders including bipolar disorder and major depression. Although there is a clear genetic component and/or early developmental and environmental factors contributing to schizophrenia, symptoms do not manifest fully until late adolescence. A possible explanation for this delay is that developmental alterations affecting immature brain systems may not have a strong impact on their function and the behaviors, these systems support until they complete their maturation. The prefrontal cortex (PFC) and hippocampus stand out as major regions affected in schizophrenia, and the transmitters involved include dopamine (DA), glutamate, and gamma amino butyric acid (GABA). DA is critical for modulating both excitatory glutamate transmission and local inhibition mediated by GABA. It has indeed been proposed that the modulation of excitation-inhibition balance in the PFC and hippocampus by DA matures during adolescence. Here, I will review recent studies addressing adolescent maturation of cortical circuits and data showing altered maturation in animal models of schizophrenia.

Several groups have identified changes in the DA modulation of GABA interneuron function and pyramidal neuron ability to sustain persistent activity during adolescence. DA receptors reach stable adult levels by the end of the adolescent period, and the density of DA fibers in the primate PFC increases during adolescence to be later pruned to adult levels. D1 receptors increase their density postnatally to peak during adolescence and decrease later in rodents and humans. The PFC also shows a delayed anatomical maturation, as evidenced in imaging studies in humans and characterized by cortical thinning during adolescence that correlates with intellectual ability. Electrophysiologically, D1 agonists...
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The pathophysiology of schizophrenia has been an elusive target of research. An important factor in such slow progress has been the difficulty in reproducing many aspects of the disease in animals. Over the past few decades, several attempts at modeling the disease were made using pharmacological approaches, lesions, environmental manipulations, and more recently genetic manipulations. Although progress was initially slow, converging findings across models suggest altered development of cortical circuits as a possible key factor in schizophrenia pathophysiology.

What do we look for in an animal model of schizophrenia? The consensus is that such models must have some extent of “face,” “construct,” and “predictive” validity. Face validity is the reproduction of major outcomes of the disease, such as agitation, hypersensitivity to stress, hallucinations, social withdrawal, etc. This is certainly very difficult if not impossible to obtain with the positive symptoms of the disease (ie, hallucinations and delusions) but manageable with negative symptoms (such as social withdrawal and anhedonia) and cognitive symptoms (ie, working memory deficits, altered executive functions, etc.). Because cognitive neuroscience has blossomed with sophisticated tools to analyze the neurobiological underpinnings of complex behaviors, this realm of the disease has become amenable to study in animal models. Predictive validity is usually present when outcomes of a model are treated with drugs that are effective in the disease. Although certainly important for positive symptoms, this approach does not allow advances in our understanding of cognitive deficits because this symptom cluster is poorly treated by typical and atypical antipsychotic drugs. Construct validity refers to a model reproducing “etiology” or “mechanisms” possibly associated with the disease, and here is where many models have shown a remarkable convergence of findings that can be related to observations in human subjects. The concept of construct validity is limited in schizophrenia because we do not really know the origin or mechanisms of the disease and, therefore, we cannot reproduce them in an animal model. However, several animal models have been built to test the consequences of known risk factors or hypothesized pathophysiological conditions. This line of research can enhance N-methyl-D-aspartate (NMDA) receptor-dependent function in the PFC, an interaction likely relevant to the persistent activity involved in working memory mechanisms. The D1-NMDA interaction in the rodent PFC becomes more robust during adolescence, allowing the emergence of persistent depolarizations in adult brain slices when D1 and NMDA receptors are coactivated. This enhanced glutamatergic efficacy in the adult PFC is balanced by an increase in the DA recruitment of local inhibitory processes. In juvenile rats, D1 agonists increase GABA interneuron firing, whereas D2 agonists have either a weak inhibitory effect or no effect. In the adult PFC, however, D2 agonists become strongly excitatory over fast-spiking interneurons, allowing a more efficient attenuation of cortico-cortical synaptic responses in the adult circuit (figure 1). Thus, as the control of excitation-inhibition balance within the cortex by DA (and likely by other modulators as well) is refined during adolescence, interneuron activation becomes more critical for cognitive performance in adults. Furthermore, early deficits in cortical glutamate, GABA, and DA interactions would not become apparent until the periadolescent maturation brings these interactions online. This hypothetical scenario is supported by human imaging data indicating behavioral adolescent traits are related to complex developmental trajectories of prefrontal regions and reward systems, and it can be directly tested using animal models in which early development is altered and functional and behavioral anomalies emerge during adolescence.

Can We Model Psychiatric Disorders in Rodents?

Fig. 1. Periadolescent maturation of the dopamine (DA) modulation of prefrontal cortex (PFC) circuits. Top: schematic representation of a juvenile circuit; left: DA receptor effects in pyramidal neurons (D1 receptors increase cell excitability and glutamate responses; D2 receptors decrease cell excitability and glutamate responses) and gamma amino butyric acid (GABA) interneurons (D1 agonists enhance cell excitability and D2 agonists have a weak inhibitory effect). Right: levels of activity in a hypothetical network of PFC pyramidal cells are displayed with darker being higher. In this illustration of a juvenile network, the more strongly activated neuron (arrow) stands out from the rest. Middle: similar schematic representation of an adult PFC circuit. In the adult, D1 receptors increase NMDA responses in pyramidal neurons more effectively and D2 receptors increase GABA interneuron activity. The result is a higher contrast between strongly activated and weakly activated units (right). Bottom: In a circuit in which interneurons fail to be properly activated by DA, such as what is observed in animal models of schizophrenia, the contrast between strongly and weakly activated units is blurred, and there may be even units that are improperly enhanced (downward arrow), thereby the encoding of goals and decision making by the PFC becomes inefficient.
has provided important information regarding functional circuits that would not have been possible with human studies. As a consequence, schizophrenia research is on the verge of a highly significant advance because decades of animal work have changed the meaning of construct validity: animal models have provided a wealth of information suggesting possible pathophysiological scenarios for human mental disease, which can now be directly tested in patients.

An important consideration for all animal models is the timing of appearance of anomalies. Because the onset of florid symptoms typically occurs during late adolescence, it is critical that schizophrenia-relevant phenomena emerge at a similar developmental stage in animal models. Adolescence is a well-characterized period in rodents. Several studies revealed behavioral and neural changes occurring during and after the hormonal changes that characterize puberty. In short, gonadal maturation takes place by the end of the first month of life in rats, and the second month (adolescence) is characterized by risk-taking and impulsive behaviors,25 as well as many immature anatomical and physiological features.4 Because this time period is brief and amenable to studies, the knowledge gained in recent years on different aspects of rodent adolescent maturation is proving very important for the development of models relevant to schizophrenia.

The Early Days: Pharmacological Models

In the 70’s and 80’s, the primary approach to modeling complex psychiatric conditions was to use pharmacological tools known to induce psychosis in humans. Amphetamine and least significant difference are capable to induce psychotic episodes, but their characteristics differ from what is seen in the disease.26,27 When used in animal research, these agents have been important for elucidating several aspects of monoamine involvement in psychosis. Indeed, current effective therapeutic approaches involve blockade of DA D2 receptors, and therefore, the main thinking about schizophrenia pathophysiology focused on altered monoaminergic transmission for several decades. But the development of the DA hypothesis has not been a planned discovery process. In fact, antipsychotic drugs were discovered by accident due to the efficacy of agents designed as antiallergic28; the clinical efficacy of these drugs was later shown to correlate with their affinity for D2 receptors.29 There is no doubt DA systems are affected in schizophrenia and are perhaps responsible for positive symptoms but no pathophysiological scenario can put all the pieces of the schizophrenia puzzle together based on an etiological process involving a DA alteration. Other pharmacological tools have been more illuminating in this regard. Phencyclidine (PCP) and its congeners (eg, ketamine) are noncompeting NMDA receptor antagonists, and they have been shown not only to induce psychotic states in normal subjects but also to bring back symptoms in schizophrenia patients in remission.30,31 These agents have been used extensively in imaging studies to elucidate brain regions affected in the disease. They are also the best approach at bridging human and animal studies because they can be used in parallel to assess behavioral and neurophysiological outcomes and their relationship with neurobiological mechanisms. Noncompeting NMDA antagonists have provided us with a possible scenario in which DA alterations are secondary to abnormal information processing in cortical regions. Animal work using NMDA antagonists reveals a disinhibited cerebral cortex. The pioneering work of Bita Moghaddam showed increases in rat PFC pyramidal cell firing and decreases in fast-spiking interneuron firing32 as well as increase in glutamate levels33 with these agents, suggesting their effect may be located primarily in NMDA receptors of local inhibitory interneurons. A preferential effect of NMDA agonists on GABA interneurons is easy to explain. NMDA receptors are voltage-sensitive ligand gated ion channels that are blocked by Mg2+ at the negative membrane potential most pyramidal neurons exhibit at rest. It takes a significant depolarization (around 20 mV) to remove the Mg2+ blockade. GABA interneurons, on the other hand, are continuously depolarized and exhibit high levels of firing,12 therefore likely to escape Mg2+ blockade. NMDA antagonists can bind to all NMDA receptors, but their impact will be felt on those that are active, primarily those located in inhibitory interneurons. Indeed, PFC interneurons exhibit NMDA receptors with higher proportion of NR2B subunits,34 but they also express NR2C and 2D subunits, which yield NMDA receptors with a lower degree of Mg++ blockade.35 Although these reports of high-density NMDA receptors in PFC interneurons contrast with recent studies showing negligible NMDA currents in this cell population,36,37 it is possible that either noncompeting NMDA antagonists affect a subpopulation of interneurons or the slice preparation loses a critical input that drives cortical interneurons and does not reflect well the in vivo condition. The consequence of NMDA antagonism on PFC circuits will likely be a reduced local inhibition and altered excitation-inhibition balance in the cortex that can result in a noisy pattern of activity in cortical ensembles. Thus, interneurons are a critical element affected by NMDA blockade, and the excitation-inhibition imbalance thereby produced could be an important factor in the psychotomimetic effect of NMDA antagonists.

Developmental Models Reveal Loss of Interneuron Function

More recently, a variety of models were created to assess whether developmental alterations could yield phenomena relevant to schizophrenia. The best characterized among these models include the prenatal administration of the antimitotic methylazoxymethanol (MAM) acetate and
the neonatal ventral hippocampal lesion (NVHL). Markers of interneuron function were found abnormal in practically every developmental model tested. Whether it is loss of parvalbumin (PV) immunoreactivity or electrophysiological deficits in interneurons or interneuron-dependent functions, the data indicate inhibitory cortical interneurons are a vulnerable population that could be affected at many different developmental stages with a variety of manipulations.

The NVHL model is the most extensively studied with nearly 100 published articles and more than 20 laboratories using it. Behaviorally, adult rats with a NVHL show hyperlocomotion, increased response to novelty, abnormal social behaviors, exaggerated responses to stress, stimulants and NMDA antagonists, and increased liability for addictive behaviors. Several studies identified cognitive deficits in this model, including working memory deficits, perseveration, sensorimotor gating deficits, and object recognition deficits in primates with a similar lesion. Thus, this model produces anomalies that could be related to all symptom domains of schizophrenia. Recent work has focused on identifying pathophysiological changes that may be relevant to the behavioral deficits in the NVHL model. Neurons in the medial PFC and nucleus accumbens in anesthetized NVHL rats respond with exaggerated firing to stimulation of the ventral tegmental area (VTA), the source of dopaminergic projections to these regions. Recordings in brain slices revealed increased excitability of pyramidal neurons and a deficient activation of fast-spiking GABA interneurons by D2 agonists in the PFC of NVHL rats. Many electrophysiological and behavioral features of the NVHL model can be ameliorated with antipsychotic drug treatment. The critical role of altered PFC information processing in this model is further shown by deficits being reversed by an adult PFC lesion. As with the human condition, most alterations observed in NVHL rats emerge during adolescence. An important caveat in this model is the presence of a (small) lesion, which is not observed in the brains of schizophrenia patients. Because most of the behavioral and physiological data relate to PFC function, the NVHL is not likely to model hippocampal pathology in schizophrenia, but the deleterious effects of affecting hippocampal activity at a critical stage in development, which ultimately can yield subtle alterations in PFC circuitry. In summary, the data obtained with NVHL rats indicate that impaired activation of PFC interneurons by DA is a critical feature of this model, and the functional consequences of such impairment emerge during adolescence. Thus, it is likely that although circuit anomalies were driven early in development by the neonatal lesion, deficits only emerge when PFC circuits would mature during adolescence. Therefore, what this and other models may have in common is a failure in the periaDOlescent maturation of circuits responsible for excitation-inhibition balance in the PFC and perhaps other cortical regions.

Other models have relied on perinatal manipulations to reproduce environmental factors that could confer a predisposition for the disease such as perinatal stress and maternal infection. Several groups have used prenatal exposure to viral particles or bacteria-associated endotoxins to induce behavioral changes in rats with some face validity for schizophrenia. Injection of the bacterial endotoxin lipopolysaccharide in the ventral hippocampus of rat pups causes electrophysiological deficits in PV interneurons in the adult PFC, characterized by loss of their activation by DA D2 receptors, a finding identical to what was observed in NVHL rats. This result indicates that in order to obtain loss of adult PV interneuron function by an early hippocampal manipulation, a lesion is not required, suggesting that the lesion component in the NVHL model is not critical for its outcome. It is worth noting that the NVHL procedure is effective during a limited critical window (postnatal day [PD] 6–9), and preliminary observations using DiI injections in the ventral hippocampus indicate that this is the time hippocampal afferents are arriving into the PFC (Calhoon and O'Donnell, unpublished observation). Thus, interfering with hippocampal function during that critical period could result in altered development of PFC circuits. Furthermore, the prenatal administration of MAM yields adult rats with fewer interneurons expressing PV and loss of high-frequency oscillations. An interesting approach combining NMDA antagonism with a developmental time frame showed that early postnatal PCP reduces the number of adult PV+ neurons in the PFC. Another developmental model that addresses environmental factors is rearing animals in social isolation. The hippocampus of isolation-reared rats shows reduced number of PV+ cells and recognition memory deficits are alleviated by a metabotropic glutamate 2/3 (mGluR2/3) agonist, which reduces excess glutamate release. It is therefore possible that during development, the role of interneurons on cortical circuitry can be affected by several factors, and if disrupted could lead to abnormal behaviors and other measures related to the disease in the adult animal.

Emerging Genetic Models Also Reveal Loss of Interneuron Function

Because schizophrenia has a clear genetic component, a large number of candidate genes have been identified as conferring predisposition for the disease. Several mouse models have been developed to express gene variants associated with the human condition. Although this line of work is still in its early stages, in many instances, interneuron function was found altered in adult mice expressing those gene variants. Furthermore, genes associated with risk for schizophrenia are critical for cortical
interneuron development. For example, neuregulin and its receptor ErbB4, both implicated in schizophrenia, are important for the development of chandelier cell synapses onto pyramidal neurons and for excitatory synapses onto GABAergic interneurons. ErbB4 knock-out mice exhibit a reduction in PV interneurons and loss of power of kainate-induced gamma oscillations. The disrupted-in-schizophrenia 1 (DISC1) gene is truncated in a family with high incidence of major psychiatric disorders. Mice with a dominant negative DISC1 present reduced PV in the medial PFC. In-utero injections of sh-RNA that transiently knock down DISC1 yield altered adult, not juvenile PFC function. In that study, we observed abnormal DA modulation of adult PFC circuits compatible with impaired interneuron function. Thus, several genetic manipulations converge in revealing abnormal interneuron function that emerges during late adolescence.

Cortical Disinhibition as a Common Final Pathophysiological Condition for Schizophrenia Models

What would be the consequences of altered interneuron function in all these models? PFC interneurons are critical for cognitive functions and they contribute to high-frequency oscillations detected in electroencephalographic and field potential studies. Loss of interneuron function, in particular of the PV population, would yield a desynchronized and disinhibited cortex, which could result in exaggerated and inefficient activity such as what is found in imaging studies. But the most dramatic effect of interneuron dysfunction would be evident in epochs during which their activity should be enhanced by modulators and other inputs. For example, VTA stimulation silences most rat PFC pyramidal neurons, likely via activation of fast-spiking interneurons. In awake behaving rats, high-frequency oscillations in the PFC emerge during decision-making epochs in which VTA DA neurons are activated. This finding suggests that phasic DA release in the PFC engages local inhibitory processes. Loss of interneuron function would yield a poor recruitment of inhibitory processes by DA, excessive pyramidal cell firing, and impaired response selection. In NVHL rats, we have recently shown loss of transient increase in beta oscillations and excessive pyramidal cell firing during the decision instance of a choice task. In that study, NVHL rats showed deficits in reversal learning, a process that correlated with the increase in beta power in controls. Furthermore, reducing glutamate levels with a mGluR2/3 agonist improved performance in NVHL rats. Thus, a rodent model that causes altered DA activation of interneurons in the adult PFC exhibits cognitive deficits that can be associated with disinhibition.

If interneurons are a primary site of alteration in schizophrenia, manipulations that selectively impair interneurons in the PFC and/or hippocampus should yield schizophrenia-related phenotypes. Indeed, mice in which NR1 subunits of the NMDA receptor were knocked out selectively in PV+ cortical interneurons yield sensorimotor gating deficits, working memory deficits, hyperlocomotion, enhanced response to stimulants, and many other schizophrenia-related phenomena. Pharmacological blockade of GABA-A receptors within the PFC in rats also yields cognitive anomalies that are related to schizophrenia, indicating proper inhibitory function within the PFC is required for cognitive functions. Thus, multiple etiologies could produce a common set of symptoms by affecting the development of excitatory-inhibitory balance in cortical circuits.

Conclusions

A disinhibited cortex seems to be a central feature of many different animal models of schizophrenia. Loss of adult PV interneuron function is a common observation that can be produced with several manipulations produced at various developmental times. Thus, the diverse model approaches currently in use converge in ultimately affecting cortical GABA interneurons. The diverse set of possible causes of interneuron dysfunction may have relevance to schizophrenia because the disease likely involves multiple etiologies. Why are cortical GABA interneurons affected by such diverse manipulations? Their increased vulnerability compared with pyramidal neurons may stem out of their increased baseline activity and firing. Indeed, fast-spiking PV positive interneurons present high firing rates and their membrane potential sits at a relatively depolarized value. This increased electrical activity may result in enhanced metabolic demand, which could yield to the higher vulnerability. Recent work by Margarita Behrens yielded interesting information regarding cellular processes that may affect interneuron function. Noncompeting NMDA antagonists affect inhibitory interneuron function by altering redox mechanisms, an effect that depends on interleukin 6 and other cytokines. Thus, blockade of NMDA function in interneurons could elicit changes similar to those reproduced in immune activation models and similar to the loss of glutamatergic inputs that may be occurring in the NVHL model, yielding interneurons affected by oxidative stress (figure 2). As interneuron recruitment by DA is not efficient in juvenile stages (prior to PD45 in rats) and in vivo inhibitory processes in prefrontal regions are weak at that age, the presence of “sick” interneurons at early developmental stages would not have dramatic functional consequences. Perhaps altered preadolescent PFC circuits could yield “prodromal” cognitive deficits, which are consistent but relatively mild and not too different from what some unaffected children may show. It is during the periadolescent period, when interneurons acquire the strong modulation by DA reviewed above, that an altered interneuron population...
would yield overtly abnormal physiology and behavior. The ongoing changes in cortical inhibitory circuits during adolescence are also likely to provide vulnerability to the deleterious effects of stress and other environmental influences that could trigger pathological changes in a vulnerable circuit. Human neurophysiological studies are consistent with this idea of a vulnerable period during adolescence, as neural synchrony (a neurophysiological readout of interneuron function) during a Gestalt perception task was transiently reduced during late adolescence, an effect that correlated with loss of performance. The maturation of cortical circuits and local inhibitory processes during adolescence is therefore susceptible to environmental influences that could put into evidence preexisting developmental anomalies. The strong data suggesting disinhibition in animal models of schizophrenia parallels the highly replicated observation of interneuron deficits in schizophrenia patients detected in postmortem studies. These observations have led to the development of novel therapeutic approaches aimed at restoring excitation-inhibition balance in cortical circuits. A promising initial observation is the efficacy of a mGluR2/3 agonist, which by acting on presynaptic receptors reduces glutamate release, in a clinical trial. Remarkably, benzodiazepines are heavily used as adjuvant treatment in schizophrenia, supporting a beneficial role of enhancing GABA-A receptor activity. The possible role of oxidative stress in schizophrenia has led to testing antioxidants as adjunct treatment, with some success. All these efforts are still on their early stages; allosteric modulators for metabotropic glutamate or GABA-A receptors, partial agonists for DA D1 or D2 receptors, and redox modulators will likely provide greater benefits when they become available. Also, the notion that cortical circuits are abnormal during development may warrant the search of adequate biomarkers that, combined with epidemiological information, can help identify an at-risk population to be treated preventively. The identification of cellular, synaptic, and circuit elements that are responsible for the adolescent maturation of cortical circuits should provide important information that could help identifying possible targets to alleviate or even prevent the conversion to the disease in vulnerable subjects. These are all promising possibilities that have opened up thanks to animal studies. Further work is obviously needed to precisely indentify novel targets and approaches. The answer to the question of whether we can model schizophrenia in rodents may be “no” if one expects reproducing all symptoms of the disease in animals, but it is clear that the available models have advanced the views of pathophysiological mechanisms to a point in which new intervention strategies can be developed with a strong rationale behind.

**Funding**

National Institutes of Mental Health (R01 MH60131).

**Acknowledgments**

The Authors have declared that there are no conflicts of interest in relation to the subject of this study.

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