On The Cover

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Psychology, Psychiatry and the Mind-Body Problem: Going Back to the Future to Investigate the Biological Foundations of Schizophrenia

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Abstract

Schizophrenia is a debilitating disorder affecting approximately 1% of the population and imposing a significant burden on society. Despite its high degree of heritability, genetics alone cannot account for disease-susceptibility. It appears that an interaction between genetic and environmental factors precipitates disease onset by affecting the expression of certain genes. The most consistent evidence across patient cohorts and brain regions is a deficit in the expression of the gene encoding glutamic acid decarboxylase 67kDa (GAD67). GAD67 is the primary enzyme responsible for producing GABA, the brain's main inhibitory neurotransmitter. GABAergic interneurons are a diverse group of cells that mediate aspects of brain function via inhibitory influence on pyramidal cells and other interneurons in various brain regions. Cholecystokinin (CCK) is a molecular marker identifying a subclass of interneurons and is itself downregulated in schizophrenia. A correlation between decreased CCK and GAD67 mRNA in the same brain region suggests that CCK+ interneurons are among those that are dysfunctional in schizophrenia. CCK+ interneurons are concentrated in brain regions that are implicated in the negative and cognitive symptoms of schizophrenia which are not well managed by current antipsychotic treatments. Empirically determining the functional consequences of GAD67 downregulation in specific types of interneurons using novel mouse models will reveal differential or additive influences of these cells on aspects of behavior and hopefully translate into improved treatment options for patients suffering from schizophrenia.

Keywords: *schizophrenia, gene expression, GAD67, CCK, interneurons, mouse behavior*

Introduction

The mind-body problem has intrigued philosophers for thousands of years. At its center is the question of whether the mind is a separate, nonphysical entity or a product of bodily processes. An individual's disposition on this issue is the foundation for the way he/she views the functions of the brain and by extension, disorders of the brain. Schizophrenia is a disorder that has intrigued psychiatrists and neuroscientists for hundreds of years. Two of the people who have influenced schizophrenia research and treatment from the beginning had different interpretations of its mind-body problem relationship. Emil Kraepelin referred to it as dementia praecox, or "premature madness" linking it to other dementias, such as Alzheimer's dementia, that have defined neuropathology. He was convinced that schizophrenia was a disorder of the brain and devoted himself to looking for pathogens and/or pathology that might explain its symptoms[1]. In contrast Eugen Bleuler described it as schizophrenia, or "split mind", and believed connecting with patients individually to understand their illness was more beneficial than studying neurobiology[2]. Psychosis, a defining feature of schizophrenia, is an impairment in distinguishing reality amongst hallucinations and delusions. This presents a problem for researchers interested in mental illness. Is it possible to scientifically study "split mind" as a disorder of reality? Interestingly, a revolution in experimental psychology was taking place at about the same time Kraepelin and Bleuler were consolidating their observations. J.B. Watson detailed his displeasure with the existing study of the mind in an article published in 1913[3]. In his view, psychological processes can be studied as a science only when subjective processes of introspection, consciousness, and the mind are excluded[3]. Watson's "behaviorism" would later be extended by scientists like B.F. Skinner to incorporate those complicated "internal" processes that have quantifiable outcomes such as value judgments, motivation, and decision-making, which are now also thought to be dysfunctional in schizophrenia. No causal pathology exists for schizophrenia[4]. However, alterations in neural connectivity and gene expression are being identified and advances in molecular biology have made it possible to mimic these insults in genetic mouse models. Although we will never recapitulate psychosis in a mouse, incorporating classical views of schizophrenia and behavior with modern molecular biology allows for the empirical analysis of molecular genetic dysfunction and its effect on the brain and on behaviors associated with complex human disorders like schizophrenia.

Schizophrenia

Schizophrenia is a debilitating disorder affecting approximately 1% of the population[5]. Symptoms fall into three domains: positive symptoms including hallucinations and delusions, negative symptoms including social withdrawal, anhedonia, avolition, etc. and cognitive symptoms including deficits in working memory, disorganized thought, and attention. In addition to the behavioral impact, cardiovascular disease and metabolic syndromes, including weight gain and diabetes among others, contribute to a mortality rate that is 2.5 times higher in schizophrenia than the general population[6-8]. Perhaps more alarming is that schizophrenics are 13 times more likely to commit suicide, adding to the already devastating emotional impact on the families of those afflicted[7]. Patients typically first experience symptoms in adolescence or early adulthood and as many as 60% experience impairment throughout life[9]. The duration and severity of the illness represent a significant financial and emotional burden to the patient, his/her family, the healthcare system, and society at large. In 2002, schizophrenia cost the United States an estimated $62.7 billion in direct healthcare costs, alternative housing, and lost productivity[10]. Adding to the negative impact on society and the healthcare of the individual, schizophrenics make up a large proportion of the homeless population, albeit difficult to quantify[10,11]. The financial and emotional toll of the illness reaches from patient to population and working towards a better understanding of its cause(s) and the biological basis of its dysfunction will enable more effective treatments to alleviate that strain.

While the exact cause(s) of schizophrenia remain elusive, heredity was quickly identified as a major factor and Kraepelin devoted a section of his early textbook to the topic[12]. Family studies have revealed a 46% risk for diagnosis of a monozygotic (identical) twin when the other is affected

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and nearly the same numbers for children with two schizophrenic parents[13]. The risk for a dizygotic (non-identical) twin is 17% when the other is diagnosed which is similar to the risk for children with one schizophrenic parent[13]. Monozygotic twins are genetically identical and would therefore share 100% of the risk if it was a purely genetic disease[13]. It is now appreciated that genetics may confer schizophrenia susceptibility while a combination of multiple factors including environmental influences may be necessary for disease manifestation[14,15]. "Environment" is a broad term that can refer to the internal surroundings that affect a particular cell and/or the external surroundings affecting an organism. Environmental factors exert this influence directly by affecting specific cellular processes (i.e. toxins) or indirectly by manipulating the expression of genes (i.e. hormones, drugs, immune system activation, etc.)[16]. This interaction between genetics and environment, through which a genetic predisposition is revealed, can explain how individuals with identical genetics (i.e. monozygotic twins) differ in subtle aspects of their appearance or personality and in some cases in drastic aspects of their physical and mental health. In the absence of gross pathology seen in other neuropsychiatric disorders[4,17], many researchers have focused their attention on the role that gene expression plays in the disease process of schizophrenia.

GABA-Associated Deficits in Schizophrenia

The involvement of GABA dysfunction in schizophrenia was established as a theoretical possibility in the 1970s based on data from animal models regarding GABAergic control of dopamine release in striatal and mesolimbic circuits[18]. The following decade foreshadowed the emergence of GABA-associated deficits as a major factor in schizophrenia with findings implicating reduced GABA content in the amygdala[19], suggesting deficient synthesis; radioligand-binding studies suggesting increased GABA receptor[20,21] and decreased GABA transporter[22] protein levels; and reduced interneuron densities[23] in post-mortem brain tissue from patients with schizophrenia. In 1995, Akbarian and colleagues published the first studies of gene expression in post-mortem schizophrenic brain tissue and found a decrease in GAD67 mRNA in prefrontal cortex that could not be accounted for by cell loss[17]. GAD67, or glutamic acid decarboxylase 67kDa, is the enzyme primarily responsible for the production of GABA in the brain[24]. The GAD67 expression deficit has become one of the most consistently replicated gene expression findings in schizophrenia across many different brain regions, patient cohorts, methods, and investigators[25-31] which is remarkable given the diverse genetics and presentation of symptoms seen in schizophrenia. Subsequent efforts have focused on illuminating the impact of GAD67 downregulation on other cell signaling pathways already implicated in schizophrenia, on the function of the brain, and ultimately on behavior.

How might GAD67 gene expression deficiency occur in schizophrenia and how might it fit with what is known about the neurobiology of schizophrenia? Interestingly, data from animal models suggest that GAD67 expression can be reduced by chronic dopamine D2-receptor stimulation[32,33] or with acute NMDA receptor antagonism[34] in multiple brain regions. These data mirror the ability of chronic dopamine stimulation[35] and acute NMDAR antagonism[36,37] to precipitate psychosis in humans. Thus, the NMDA hypofunction hypothesis, the dopamine hypothesis, and the GABA dysfunction hypothesis of schizophrenia could be integrated with GAD67 deficiency being a key player in each[29]. In addition, studies of the gene that encodes GAD67, GAD1, have yielded a number of single nucleotide polymorphisms (SNPs) that are found more frequently in schizophrenic patients than in normal individuals[38-40]. The majority of SNPs in each study was found in gene regulatory sequences not coding parts of the gene, suggesting a role in gene expression and not protein function. An analysis of patients with one of the genetic variants confirmed decreased GAD67 mRNA levels post-mortem[38]. A third mechanism that may contribute to decreased GAD67 expression in schizophrenia is epigenetics. Genes can be downregulated when promoters or other regulatory sequences are methylated, causing changes in chromatin structure that prevent transcription[41]. Methylation is carried out by enzymes called DNA methyl transferases. One such enzyme, DNMT1, is overexpressed in GABAergic interneurons of schizophrenic patients[41] correlating with a GAD67 mRNA decrease in the same cells[42]. Huang *et al.*, linked those findings by reporting an 8-fold increase in the methylation of the GAD67 gene promoter in schizophrenic patients[43]. Even more interesting is that nicotine has been shown to reverse the methylation status of the GAD67 promoter which may explain in part the high incidence of cigarette smoking among individuals with schizophrenia[44]. The fact that several different mechanisms can lead to a decrease in GAD67 gene expression illustrates how diverse genetic and environmental insults can affect the brain through a common mechanism and might explain a portion of the complexity of schizophrenia etiology.

CCK+ GABAergic Interneurons, Schizophrenia, and Behavior

At about the same time Kraepelin and Bleuler were defining schizophrenia, Santiago Ramón y Cajal was astounded by the "prodigious abundance and unusual wealth of forms of the so-called neurons with short axon"[45]. GABAergic interneurons are so diverse in fact that creating a nomenclature for their numerous defining characteristics continues to be a tedious task[46]. It is now appreciated that interneurons can be grouped based on morphological, molecular, and physiological properties[46]. Classification is important because subtypes of interneurons are involved in facilitating different processes in the brain and are concentrated in brain regions that mediate different behaviors. Therefore dysfunction of particular classes of interneurons could generate diverse pathophysiology and behavior. Cholecystokinin (CCK) is a so-called brain-gut peptide that identifies a particular class of interneurons found primarily in limbic and frontal circuits[47-49]. A correlation between decreased CCK and GAD67 mRNA in the same brain region suggests that CCK+ interneurons are among those that are dysfunctional in schizophrenia[50]. They are defined by morphology as either large multipolar/basket cells or small bipolar cells[51]. These two subclasses are further divided based on electrophysiological properties[52] highlighting the diversity within this class of interneuron. Functionally, they contribute to gamma oscillations[53,54]. Neural oscillations are the coordinated firing of neurons in particular brain regions and define the signal-to-noise ratio of neural communication[55]. Gamma oscillations are a particular type that is disrupted in schizophrenia and may underlie learning and memory deficits seen in patients[25]. Distinct from other interneuron types, CCK+ interneurons express M3 muscarinic receptors[56] and alpha7 nicotinic receptors[57] further linking them to cognitive symptoms of schizophrenia since both are promising new clinical targets for cognitive improvement[58,59]. They also express the endocannabinoid receptor CB1[57,60] which has been linked to psychosis onset and outcome[61] and the $5HT_3$ receptor which may be involved in providing "emotional, motivational, or other state dependent tuning" in a fast, temporally bound manner[62]. The role CCK+ interneurons play in behavior has yet to be assessed directly. Defining this role will contribute to an improving understanding of the biology of schizophrenia and direct treatments, like the M3 and alpha7 drugs, towards particular types of symptoms in individual patients.

When one considers the role CCK+ interneuron dysfunction plays in schizophrenia pathophysiology, brain regions concentrated with CCK+ interneurons are naturally implicated. CCK+ cells are found primarily in medial frontal cortex including limbic, orbitofrontal, and anterior cingulate

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cortices and in the amygdala and hippocampus indicating a similar concentration of CCK+ interneurons by proxy[63] although there is additional low-level CCK expression in some pyramidal cells[47,51]. This distribution is remarkably similar in mice[47], nonhuman primates[63], and humans[48,49]. These regions are linked functionally through their participation in motivation, reward, social behavior, decision-making, self-regulation, affect, and learning and memory: also remarkably similar in rodents, nonhuman primates, and humans[64,65]. In fact, many of these processes have been studied most extensively using rodents as an experimental model. Psychiatric research has begun to mirror these studies, providing evidence of dysfunctional amygdalocortical and corticolimbic circuits in psychiatric illnesses including schizophrenia[66]. Basolateral amygdala (BLA) interactions with orbitofrontal (OFC) cortices encode the value of stimuli in a dynamic way[64,67]. The OFC uses this information for decision-making along with other frontal cortical structures like the anterior cingulate (ACC). Continuous updating of these representations is necessary for adaptive learning and cognition[68]. The cortical and amygdalar contributions to this behavior are dysfunctional in schizophrenic patients[69,70] in a manner that can be reproduced in mice[65]. CCK+ interneurons may be involved in coordinating this behavior since they represent a large percentage of the interneurons in the BLA[71] and in layer II/III of medial frontal cortex[63] where BLA afferents project[72]. This situation has a clear connection to clinical data correlating decreased CCK levels with negative symptoms[73] and theoretically with cognitive symptoms. Although it is not clear if peptide expression indicates interneuron function per se, other evidence linking CCK+ interneurons specifically in the integration of the "emotional, motivational and general physiological state of the animal"[74] supports a role for dysfunction of these cells at the interface of negative and cognitive symptoms of schizophrenia.

Modeling Schizophrenia in Mice

Mouse models offer the ability to study the causal influence of genetic and environmental manipulations and their interactions on cellular, molecular, and behavioral processes that are dysfunctional in schizophrenia. Discussed here are dysfunctional GABAergic processes. An important caveat when comparing mouse and human interneurons, besides obvious differences in cortical size, is that the proportion of interneurons in the human cortex is much greater than in the mouse[75]. However, this discrepancy may also lend itself to the interpretation that findings of interneuron studies in mice could have a much more robust effect in primates. On the behavioral level, psychosis is excluded from these studies because hallucinations, delusions, disorganized speech, and thought disorder are not quantifiable in mice regardless of their validity or lack thereof. That limitation does not diminish the utility of the mouse since negative and cognitive symptoms can be modeled and represent a tremendous clinical need as they are consistent predictors of prognosis and are not well managed with current medications[76,77]. The neurobiology and neuropsychology of dysfunction in these symptom domains is beginning to merge with longstanding behavioral neuroscience research into the neural basis of normal social, affective and cognitive processes[64,67,69]. Furthermore, the neural dysfunction responsible for positive symptoms is becoming better understood[78-82]. As a result, we may be able to employ mouse models in the near future to interrogate the underlying circuitry molecularly and physiologically even if we cannot directly measure analogous behaviors. After all, the goal is not to manufacture murine schizophrenia, but to gain knowledge of the ways dysfunctional circuitry changes the behavior of an organism, however that may be, and use that knowledge to guide hypotheses that can be taken back to the clinic.

Conclusion

There are many current hypotheses of schizophrenia and even more candidate genes. It is important to note that these hypotheses are not mutually exclusive. Bleuler noted the diversity of presentation of the illness from the beginning, naming the disorder not schizophrenia, but the "group of schizophrenias" in 1911[83]. Genetic susceptibility to schizophrenia is conferred by a large number of genes that may interact with environmental factors in a number of possible combinations[15] to produce pathophysiology and symptoms. This complexity contributes to the difficulty in elucidating the neurobiology of the illness. Converging evidence is beginning to identify certain pathophysiological processes that represent the final common pathway(s) of the disorder. Experimentally defining the manifestation of dysfunction in these pathways will be the next step towards understanding how the pieces fit together to form a complex and severe mental illness. Our laboratory has developed novel mouse models that downregulate GAD67 in specific classes of interneurons[84] which will contribute to an empirical understanding of how each class may participate in the pathophysiology and behavior of schizophrenia or to particular symptom domains. These studies join a movement towards clinical research that will be more amenable to translation than qualitative descriptors of symptoms[85]. Advancing research in these areas seems to have moved the field "back to the future" of psychiatry and experimental psychology by revisiting Kraepelin's search for the biology of psychosis[1] and Watson's exclusion of the "mind" to study behavior scientifically[86,87]. While it is impossible to fully recreate schizophrenia in an animal model, the combination of a Kraepelinin search for biological underpinnings of psychiatric illness and the application of a (modern) behaviorist point-of-view to defining symptoms of the illness will permit a more thorough comparison of advanced genetic models and permit their use to develop better treatments for psychiatric illness: exactly as Kraepelin himself suggested nearly a century ago.

"However little it may be possible to identify human with animal brain-functions and illnesses, yet, from the effects produced by particular noxae in the brains of animals, conclusions can be drawn as to the issue of like processes in man." –E.Kraepelin, 1919[1]

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