Schizophrenia: an integrative approach to modelling a complex disorder

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The discovery of candidate susceptibility genes for schizophrenia and the generation of mice lacking proteins that reproduce biochemical processes that are disrupted in this mental illness offer unprecedented opportunities for improved modelling of this complex disorder. Several lines of evidence indicate that obstetrical complications, as well as fetal or neonatal exposure to viral infection, are predisposing events for some forms of schizophrenia. These environmental events can be modelled in animals, resulting in some of the characteristic features of schizophrenia; however, animal models have yet to be developed that encompass both environmental and genetic aspects of this mental illness. A large number of candidate schizophrenia susceptibility genes have been identified that encode proteins implicated in the regulation of synaptic plasticity, neurotransmission, neuronal migration, cell adherence, signal transduction, energy metabolism and neurite outgrowth. In support of the importance of these processes in schizophrenia, mice that have reduced levels or completely lack proteins that control glutamatergic neurotransmission, neuronal migration, cell adherence, signal transduction, neurite outgrowth and synaptic plasticity display many features reminiscent of schizophrenia. In the present review, we discuss strategies for modelling schizophrenia that involve treating mice that bear these mutations in a variety of ways to better model both environmental and genetic factors responsible for this complex mental illness according to a "two-hit hypothesis." Because rodents are able to perform complex cognitive tasks using odour but not visual or auditory cues, we hypothesize that olfactory-based tests of cognitive performance should be used to search for novel therapeutics that ameliorate the cognitive deficits that are a feature of this devastating mental disorder.

La découverte de gènes candidats pour la prédisposition à la schizophrénie et la production de souris sans protéines reflétant les processus biochimiques perturbés dans le cas de cette maladie mentale offrent des possibilités sans précédent d'améliorer la modélisation de ce trouble complexe. Plusieurs sources de données probantes indiquent que des complications obstétriques et l'exposition fœtale ou néonatale à une infection virale sont des événements qui prédisposent à certaines formes de schizophrénie. Il est possible de modéliser ces événements environnementaux chez des animaux et de produire certaines des caractéristiques de la schizophrénie, mais on n'a pas encore mis au point de modèles animaux qui englobent les aspects tant environnementaux que génétiques de cette maladie mentale. On a identifié un nombre important de gènes candidats pour la prédisposition à la schizophrénie qui encodent des protéines mises en cause dans la régulation de la plasticité synaptique, la neurotransmission, la migration des neurones, l'adhésion cellulaire, la transduction des signaux, le métabolisme de l'énergie et l'excroissance des neurites. Pour appuyer l'importance de ces processus dans la schizophrénie, les souris ayant des concentrations réduites ou complètement inexistantes des protéines qui contrôlent la neurotransmission glutamatergique, la migration des neurones, l'adhésion des cellules, la transduction des signaux, l'excroissance des neurites et la plasticité synaptique présentent de nombreuses caractéristiques qui rappellent la schizophrénie. Dans cette critique, nous discutons de stratégies de modélisation de la schizophrénie qui consistent à traiter des souris porteuses de ces mutations de diverses façons afin de mieux modéliser les facteurs tant environnementaux que génétiques à l'origine de cette maladie mentale complexe en fonction d'une « hypothèse double ». Comme les rongeurs peuvent exécuter des tâches cognitives complexes en se servant d'indices olfactifs mais non visuels ou auditifs, nous posons en hypothèse que des tests de performance cognitive fondés sur l'olfaction devraient servir à chercher des agents thérapeutiques nouveaux qui atténuent les déficits cognitifs caractéristiques de ce trouble mental dévastateur.

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Introduction

Schizophrenia is a debilitating mental illness characterized by symptoms that may be positive (delusions and hallucinations, disorganized speech) and negative (affective flattening, avolition, impoverishment of speech and language, social withdrawal) in nature, as well as cognitive deficits (attention deficits, impaired executive functions such as planning, abstract thinking, rule flexibility, and inhibition of inappropriate actions and irrelevant sensory information, as well as short-term and long-term memory deficits).[1–5] Cognitive deficits associated with working memory and executive functioning are becoming increasingly recognized as central to both the diagnosis and amelioration of this mental illness.[6–9] Although antipsychotic drugs relieve the positive symptoms of schizophrenia, these drugs have limited utility in the treatment of the negative symptoms and cognitive deficits associated with this disorder.[10] The discovery of a large number of candidate schizophrenia susceptibility genes (SSGs) further supports the heterogeneous nature of this disorder, indicating that a single animal model will not accurately reflect this diverse mental illness.[11,12] In the present review, we propose an alternative approach based on the "two-hit hypothesis" of schizophrenia[13] to develop relevant animal models in which a specific environmental insult is performed on genetically altered mice that may be reflective of the predisposing effects of various mutations for schizophrenia. Given the strong epidemiological evidence implicating obstetrical complications and viral infection as risk factors in schizophrenia and the large literature supporting immunological alterations in at least some patients with this mental illness, we propose that experimental manipulations that mimic the effects of these risk factors may reproduce environmental events that place genetically predisposed individuals at high risk. If this assumption is correct, it may be possible to more accurately model certain aspects or subtypes of schizophrenia by combining such manipulations with predisposing genetic alterations. Because rodents perform particularly well on complex cognitive tasks when odours are used as discriminative stimuli,[14–16] but poorly when auditory or visual stimuli are used,[17–19] we further propose that it may be possible to use this sensory modality in rodents to assess the potential efficacy of treatments for executive function deficits in schizophrenia. By encompassing both environmental and genetic risk factors in a single model and using olfactory-based tests to probe higher cognitive processes such as transitive inference,[20] it may be possible to provide a mechanism for the identification of novel treatments for this devastating mental disorder.

Genetic and environmental risk factors: implications for disease modelling

The worldwide prevalence of schizophrenia is about 1% and is generally similar in most ethnic populations that have been studied.[21] In comparison with the 1% risk of schizophrenia in the general population, the probability of developing this disorder is elevated significantly in the relatives of people with schizophrenia, ranging from 6% in parents to nearly 50% in identical twins.[22] Schizophrenia, therefore, has a large genetic component; however, the significant discordance between identical twins points to the importance of environmental factors.[23,24] Epidemiological studies have implicated severe maternal malnutrition, exposure to influenza virus, repeated psychological stress, obstetrical complications (hypoxia/ischemia) and exposure to adverse intrauterine events as possible environmental risk factors.[25–28]

Pathophysiology of schizophrenia

Schizophrenia is a complex disorder with a poorly defined cause and pathophysiology.[29] The most consistent finding in schizophrenia is an enlarged ventricular system accompanied by an overall reduction in brain volume, with regional decreases in the hippocampus, thalamus and frontal lobes.[23,30,31] Neuroimaging and postmortem analyses have demonstrated subtle, but distinct, abnormalities in the schizophrenic brain particularly in the hippocampus and neocortex.[32,33] Neurons in these regions appear reduced in size with abnormal dendritic arborization and synaptic organization, which is suggestive of reduced synaptic connectedness as a result of developmental disturbances of synaptogenesis during gestation and early childhood and/or synaptic pruning during adolescence.[34–37] McGlashan and Hoffman,[38] using a computer-simulated model of synaptic connectivity, have proposed that schizophrenia does not result from a simple loss of neurons but, rather, from abnormally elevated levels of synaptic pruning during development.[39] This excessive pruning may account for the mild abnormalities in social and cognitive function that patients with schizophrenia display at an early age before the emergence of the full positive, negative and cognitive deficits that define the disorder at adulthood.[39,40] Several animal models support this neurodevelopmental hypothesis by demonstrating that an early insult can produce at adulthood some of the behavioural, morphological and neurochemical alterations resembling those observed in patients with schizophrenia.[41–43]

Neurodevelopmental animal models of schizophrenia

The neurodevelopmental hypothesis of schizophrenia postulates that an early event disrupts normal brain maturation, resulting in the obvious appearance of clinical symptoms at puberty or young adulthood.[44] Rodent models involving experimental insults during the fetal or neonatal period result in the appearance of behavioural abnormalities, imbalances in central neurochemistry and morphological changes in the brain at these developmental stages that resemble some, but not all, clinical aspects of schizophrenia.[28] Behavioural abnormalities that have been reported in these perinatal or postnatal models for schizophrenia consist of impaired prepulse inhibition (PPI), increased sensitivity to the locomotor-stimulant effects of dopaminergic agonists, social withdrawal and a variety of cognitive deficits that reproduce some of the features of schizophrenia. The various animal models that

have been developed for schizophrenia have been the topic of numerous excellent reviews.[24,42,45–48] The following is a brief description of the more common models that have been employed, as well as new models that are based on strong epidemiological evidence implicating obstetrical complications, impaired neurogenesis and viral infection as potential risk factors.

Neonatal lesion and MK-801 models

Adult rats that have sustained bilateral lesions (ibotenic acid) or transient inactivation (tetrodotoxin) of the ventral hippocampus as neonates represent popular neurodevelopmental models of schizophrenia that have been the subject of several excellent reviews.[24,42,49] Typically, experimental manipulations are performed at postnatal day 7, resulting in either a transient[50] or permanent[41] inactivation of the ventral portion of the rat hippocampus. These treatments result in behavioural abnormalities, altered neurochemistry and gene expression, and working memory deficits that are apparent at early adulthood.[41,50–53] The hypersensitive locomotor response of such animals to novelty, stress and pharmacological stimulation of dopamine receptors is reversed by antipsychotic drugs.[54] Furthermore, these animals display social withdrawal that is not reversed by long-term low dosing with atypical antipsychotic drugs.[55]

Systemic injection of the *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 at the same time point produces neuronal apoptosis throughout the brain.[56] Whereas this model has been reported to lead to the emergence of PPI deficits and morphological changes reminiscent of those observed in patients with schizophrenia,[57] there is no general agreement on the optimal parameters for producing these behavioural alterations.[57,58] In summary, these models have proven particularly useful in demonstrating that neonatal injury may impair neurodevelopment and perhaps place genetically predisposed individuals at risk for schizophrenia. Their utility is limited, however, by the severe nature of these interventions, namely, excitotoxic lesions that destroy the ventral hippocampus or promote widespread neuronal apoptosis in the central nervous system (CNS), that contrast with the modest neuropathological changes reported post mortem in patients with schizophrenia. More etiologically relevant interventions that do not rely on bilateral lesions or widespread neuronal cell death may better model the more subtle neuropathological events that place a genetically predisposed individual at risk of developing schizophrenia.

Obstetrical complication model

A role for obstetrical complications in increasing the risk of developing schizophrenia is supported by numerous epidemiological studies, including a longitudinal population-based cohort study conducted in Sweden of all children born between 1974 and 1993.[59] These complications include inappropriate maternal nutrition, repeated psychological stress and hypoxic/ischemic events during labour.[25] Multicentre studies and meta-analyses in the psychiatric literature show that the risk of schizophrenia is multiplied by 2 if pregnancy is complicated by these factors.[60] It is possible to mimic some of the cellular damage or alterations in neuronal pathways that are seen in the brains of patients with schizophrenia through the use of animal models of obstetrical complications. One model involves intrauterine anoxia or prolonged hypoxia, leading to behavioural abnormalities resembling schizophrenia in affected offspring.[61,62] In other models, pups receive anoxia during cesarean section birth,[63] unilateral carotid artery ligation combined with transient exposure to a low-oxygen environment on postnatal day 7–14,[64] or simply exposure to a low-oxygen environment.[28] Alterations in the brain dopamine system following early hypoxic or ischemic events have been outlined by these models;[28] however, the particular pattern of events that causes these long-term changes in the CNS has not been elucidated.

Animal models of obstetrical complications suggest that an adverse event in utero may lead to abnormalities in brain development and behaviour that are reminiscent of schizophrenia. These models do not, however, produce deficits representing the altered brain structure, neurochemistry and behaviour seen in patients with schizophrenia. For example, abnormalities in these animal models appear to be limited to the positive (dopamine-related) symptoms of schizophrenia, such as increased locomotor activity.[28,65]

Polyriboinosinic–polyribocytidilic acid model

The hypothesis that influenza may be a causative factor in schizophrenia was first proposed by Karl Menninger in 1922 who noted that infection of the mature brain may be followed by symptoms of schizophrenia.[66] Subsequent studies showing that the incidence of schizophrenia is elevated in individuals exposed to influenza epidemics while in the second trimester support this proposal. These studies include the Finnish cohort studies with long-term follow-up and a study in the southern hemisphere.[67–69] Subsequent epidemiological studies have shown that in addition to influenza viruses, several other viruses that include cytomegalovirus, herpes simplex virus 1, rubella and Borna disease virus have been implicated as potential causative or predisposing agents for schizophrenia.[70–73] An association between CNS infections in childhood and adult-onset schizophrenia has also been reported.[26] These findings have given rise to detailed animal studies of the effects of various types of viruses on neurodevelopment that have shown that early exposure to viral infections can lead to abnormalities relevant to schizophrenia.[74] Neonatal or prenatal inoculation with cytomegalovirus, herpes simplex virus type 1 or human influenza virus leads to disrupted PPI, social withdrawal and alterations in temporolimbic morphology that model deficits observed in schizophrenia.[74] Recent studies have shown that these alterations are more likely the result of the maternal response to infection rather than the virus itself.[75] Support for this theory has come mainly from studies reporting behavioural, neurochemical and morphological abnormalities in the offspring of rodent dams injected with polyriboinosinic–polyribocytidilic acid (poly I:C), a synthetic double-stranded RNA used to

mimic viral exposure. Poly I:C elicits a maternal immune response similar to that observed during viral infection, but it offers an advantage in that it mounts a nonspecific immune response through the induction of proinflammatory cytokines without the use of infectious virus.[76] Furthermore, poly I:C produces a transient cytokine response that lasts only 48 hours. Studies by Zuckerman and colleagues[76] have demonstrated that pregnant rats injected with poly I:C at gestational day 15–17 give birth to pups that, while appearing normal at birth and through adolescence, develop several behavioural abnormalities reminiscent of schizophrenia upon entering adulthood. These animals display deficits in PPI, increased sensitivity to the locomotor-stimulating effects of amphetamine and MK-801, and excessive switching, a specific cognitive deficit characterized by disrupted latent inhibition and enhanced reversal learning.[77] In addition, many of these behaviours were reversed by treatment with antipsychotic drugs such as haloperidol or clozapine, which is consistent with the clinical pharmacology of schizophrenia.[76,77] Whereas the mechanisms responsible for the emergence of these abnormalities following immune activation during pregnancy are unclear, it is likely that increased inflammatory cytokine production plays a pivotal role. Maternal poly I:C exposure during pregnancy increases maternal levels of tumour necrosis factor-α (TNFα) in the plasma, placenta and amniotic fluid,[75] suggesting that the fetus is exposed to high levels of this cytokine. Inflammatory cytokines have been shown to decrease the survival of serotonergic, dopaminergic, hippocampal and cortical neurons, and TNFα inhibits the dendritic development of cultured cortical and hippocampal neurons consistent with neuropathological observations in schizophrenia.[75,78] Accumulating evidence indicates that inflammatory cytokines and neurotrophins interact in the CNS, suggesting that altering cytokine levels in the developing brain may increase the risk for neurodevelopmental disorders like schizophrenia by disrupting neuronal survival and connectivity.[79,80]

Methylazoxymethanol acetate model

Transient disruption of neurogenesis has also been suggested as a model of schizophrenia.[81–84] Disruption of neurogenesis in the fetal brain is achieved by systemic administration of the mitotic inhibitor methylazoxymethanol acetate (MAM) to pregnant rats.[84] MAM treatment during gestation induces neuroanatomical alterations and behavioural deficits in the offspring that become apparent during early adulthood, mimicking certain aspects of schizophrenia.[83,84] However, as Leng and colleagues[84] point out, some of these behavioural effects have been found to be weak and inconsistent, obscuring the validity of the MAM model.

Genetic disease models

Genetic linkage and association studies suggest that multiple chromosomal regions contain candidate SSGs.[11] Within these regions, a variety of genes have been identified that encode proteins implicated in biological processes such as synaptic plasticity, neurotransmission and neurodevelopment — all of which are known to be disrupted in schizophrenia. The discovery of candidate SSGs and elucidation of their function have further implicated abnormalities of neurodevelopment (neurogenesis, neural cell migration and adhesion, synapse formation) and synaptic function (synaptic plasticity, neurotransmission [hypoglutamatergic and hyperdopaminergic]) in this disorder.[11,35,85] Notable among these SSGs are those encoding catechol-*O*-methyltransferase (COMT), dysbindin-1, D-amino acid oxidase (DAO), its activator DAOA (previously known as G72), regulator of G-protein signalling 4 (RGS4), calcineurin, neuregulin 1 (NRG1) and Disrupted in Schizophrenia 1 and 2 (DISC-1 and DISC-2).[11,86–92] The fact that many of these genes encode proteins that regulate synaptic function, neurotransmission and neurodevelopment suggest that schizophrenia may be a genetic disorder of the synapse.[11]

A number of knockout (gene deletion or inactivation) mice for candidate SSGs or genes that encode proteins implicated in processes disrupted in this mental illness, such as synaptic plasticity, neuronal migration, neurotransmission, neurogenesis and cell adhesion, have been generated. Because the phenotype of these animals has been reviewed elsewhere,[43,48,93] we have focused on those genetic models that have received the most attention and in our opinion hold the most promise for yielding insights into the complex nature of schizophrenia.

Calcineurin-null mice

Calcineurin is a calcium- and calmodulin-dependent protein phosphatase comprised of a regulatory and catalytic subunit that plays a significant role in neurite extension, synaptic plasticity, and learning and memory.[94] Decreased expression in the hippocampus of isoforms of the catalytic calcineurin subunit has been reported in patients with schizophrenia, suggesting that reductions in this protein may underlie reduced synaptic plasticity in this brain region.[86] In line with these findings, forebrain-specific calcineurin deletion results in a variety of deficits in electrophysiological measures of synaptic plasticity, as well as decreased performance in behavioural assays of episodic memory (delayed matching-to-place task, radial maze task) but, interestingly, not in tests of reference memory (contextual fear conditioning, Morris water maze).[95] These mice also displayed increased locomotor activity, impaired PPI, decreased social interaction and deficits in latent inhibition.[95,96] Although it is not known whether any of these aberrant behaviours in forebrain-specific calcineurin knockouts are ameliorated by antipsychotic drugs, the identification of calcineurin as a candidate SSG[86] coupled with the behavioural phenotype of mice lacking this phosphatase suggests that they may serve as a useful genetic disease model for schizophrenia.

Neuregulin-hypomorphic mice

Linkage studies from diverse populations provide compelling evidence that neuregulin 1 (*NRG-1*) is a susceptibility gene for schizophrenia.[90,97–100] This work indicates a strong correlation between polymorphisms in the 5′ noncoding end of

the *NRG-1* gene and schizophrenia. Because no mutations in the region coding for *NRG-1* have been found, *NRG-1* polymorphisms associated with schizophrenia should not alter the biological activity of the encoded protein but may alter its expression.[101] Consistent with this hypothesis, the ratios of 3 NRG-1 isoforms are altered in the dorsolateral prefrontal cortex of patients with schizophrenia.[102] NRG-1 acts as a trophic and differentiation factor in the CNS, where it regulates neurodevelopmental processes such as neuronal migration and survival, neurogenesis and synaptic plasticity that have been implicated in schizophrenia.[101,103–106] NRG-1 and its ErbB tyrosine kinase receptors (ErbB 1–4) are abundant in the adult rodent and human brain.[107–110] Gene-expression profiling studies provided the first evidence that NRG-1–ErbB signalling is altered in schizophrenia by demonstrating that ErbB3 expression is reduced by 58% in the prefrontal cortex of individuals with schizophrenia relative to controls.[111,112] Further evidence for a link between NRG-1 and schizophrenia comes from mice lacking 1 copy of the *NRG-1* gene that have behavioural abnormalities resembling schizophrenia, such as hyperactivity that is reduced by clozapine, as well as latent inhibition and PPI deficits.[113–115] Alternative splicing of the *NRG-1* gene results in 15 isoforms, all of which contain an epidermal growth factor–like (EGF-like) domain that is necessary and sufficient for NRG-1 to activate ErbB2, 3 and 4. Three types of NRG-1 proteins are distinguished by different domains located N-terminal to the EGF-like domain. Types I and II have immunoglobulin-like (Ig-like) domains, whereas type III has a cysteine-rich domain (CRD). Nearly all type I and II NRG-1 proteins have at least 1 transmembrane (TM) domain (for review, see Buonanno and Fischbach[116] and Falls[117]). Mice with homozygous deletions in either the EGF-like or TM domains of the *NRG-1* gene produce embryonic lethality due to malformations of the heart.[97,118,119] Mice that lack the CRD perish at birth from lack of Schwann cells.[120] Heterozygous animals with targeted mutations of type I or II NRG-1s are healthy, survive until adulthood and display mild behavioural deficits that reproduce some features of schizophrenia. *EGF-NRG-1* (+/–) mice show locomotor hyperactivity and PPI deficits, the latter not being reversed by a low dose of clozapine (1 mg/kg).[113] By contrast, *Ig-NRG-1* (+/–) mice do not display elevated locomotion relative to wild-type littermates but do have latent inhibition deficits.[114] *TM-NRG-1* (+/–) mice have been generated, but their behavioural phenotype, reported to include PPI deficits, has only been reported in abstract form.[121] Given the strong evidence implicating the *NRG-1* gene in schizophrenia, *NRG-1* heterozygous null mice may represent genetically relevant models for this disease.

Close homologue of L1-null mice

Neural cell adhesion molecule 180 (NCAM-180) is a cell-surface glycoprotein that is expressed in neurons and glial cells throughout the developing and mature nervous system.[122] Cell adhesion molecules regulate many functions essential for development of the CNS such as cell adhesion, axon guidance and synaptic plasticity.[123] NCAM-180 has been implicated in the control of neurite outgrowth, neuronal migration, cell survival and synaptic plasticity.[124] Conditional ablation of NCAM-180 produces abnormalities in cell interactions in the developing and mature brain that impair performance in behavioural tests of synaptic plasticity.[125] Despite the fact that genetic studies suggest that NCAM-180 is unlikely to play a prominent role in schizophrenia,[126] mice lacking this particular adhesion molecule as well as others such as Close Homologue of L1 (CHLI) have received attention as putative animal models of schizophrenia.[127,128] Initial findings of PPI deficits in NCAM-180-null mice[127] have not been replicated,[129] perhaps because of differences in the parameters used for the PPI tests in these studies or the fact that early behavioural studies with NCAM-180-null mice did not take into account the possibility that strain differences between NCAM-180-null and wild-type controls can influence PPI sensitivity.[129] Mutations in *CHL1* have been linked to schizophrenia.[130–132] Mice that lack CHL1 display neuronal positioning and dendritic growth abnormalities of pyramidal neurons in the developing neocortex[133] and deficits in PPI, suggesting that they may represent a putative genetic disease model for some forms of schizophrenia.[128] The effects of antipsychotic drugs on PPI deficits in *CHL1*-null mice have not been reported.

NR1-hypomorphic mice

Short-term administration of NMDA receptor antagonists such as phencyclidine and ketamine can produce positive and negative symptoms as well as cognitive deficits in subjects with no psychiatric disorder, suggesting that impaired NMDA receptor–mediated neurotransmission may be a contributing factor in schizophrenia.[134–137] Furthermore, administration of NMDA antagonists such as phencyclidine promotes social isolation in rats that mimics certain aspects of the negative symptoms characteristic of schizophrenia (for review see Sams-Dodd[138]). Genetically altered mice that express 5%–10% of the normal level of the Nr1 subunit gene expression of the NMDA receptor display behavioural abnormalities that have been related to schizophrenia, many of which are ameliorated by clozapine.[139] These include enhanced locomotor and stereotyped behaviour when placed in a novel environment, social deficits and reduced copulatory behaviour; however, neither dopamine metabolism nor dopamine release was altered in the striatum.[139] Administration of clozapine normalized all of these behavioural deficits. More recently, deficits in PPI have been reported for these NR1-hypomorphic mice that are not improved by clozapine.[140]

STOP-null mice

Neuropathological studies suggest that alterations in synaptic structure and function in schizophrenia are associated with disturbances in cytoskeletal function.[141] The recent generation of mice deficient in STOP (stable tubule only polypeptide) has revealed that this cytoskeletal-associated protein plays a critical role in synaptic plasticity, neurogenesis and neurotransmission.[142] STOP (–/–) mice have no obvious deficits in brain anatomy but display synaptic defects characterized by depleted synaptic vesicle pools and

deficiencies in both long-term potentiation and long-term depression in CA1 synapses of the hippocampus.[142] There are no other obvious morphological abnormalities in the CNS of STOP-null mice. Moreover, STOP-null mice display a profound behavioural phenotype characterized by increased sensitivity to the locomotor-activating effects of mild stress and dopaminergic stimulants (amphetamine), nurturing deficits, inability to perform object recognition tasks and social withdrawal.[142,143] The few behavioural studies performed to date on these animals indicate that the hyperlocomotor activity and nurturing deficits are considerably improved by long-term administration of antipsychotic drugs.[142,143] Neurochemical and electrophysiological measurements suggest that increased mesolimbic dopaminergic neurotransmission is responsible for the enhanced sensitivity of STOP mice to mild stress and amphetamine.[143] STOP-null mice also display PPI deficits that are not responsive to clozapine.[140] Given that cytoskeletal abnormalities likely contribute to schizophrenia[141] and that STOP-null mice display neurochemical and behavioural features characteristic of this mental illness (some of which are reversed by long-term but not short-term administration of neuroleptics), it has been proposed that these animals may serve as a useful model for psychosis.[142,144]

The human *STOP* gene (GeneBank accession number AP000588), located at position 11q14, lies within a region that has been linked to major mental diseases including schizoid disorders.[145–147] In addition, the candidate SSG, *DISC-1*, encodes a microtubule-associated protein that has been implicated in a number of diverse neuronal processes such as mitochondrial function, cell adhesion, signal transduction, neuronal migration and neurite outgrowth that are thought to be impaired in schizophrenia.[123,148–153] The most common mutation in *DISC-1* associated with schizophrenia results in a balanced ([1:11][q42.1:q14.3]) chromosomal translocation that may also impair *STOP* gene function by positional effects on 11q14 where the *STOP* gene is located.[89,142] However, the clinical relevance of this speculation awaits linkage studies.

Behavioural correlates in animal models of clinical symptoms

In the previous sections, we have outlined several environmental and genetic models that reproduce many of the features reminiscent of schizophrenia; however, it is still unclear whether these abnormalities are linked to the symptoms and disease process in schizophrenia.[154] Disturbances in information processing are considered to be an important feature of schizophrenia reflected by insufficient sensorimotor gating in the form of impaired PPI.[155] However, deficits in PPI as well as latent inhibition and spatial memory are observed in other neuropsychiatric disorders such as Gilles de La Tourette's syndrome, Huntington's disease, dementia, obsessive–compulsive disorders and mania.[154,156,157] Moreover, whether antipsychotic drugs actually improve PPI deficits in patients with schizophrenia has been controversial,[158,159] casting doubt on the validity of PPI-based screening for the detection of putative antipsychotic drugs using animal models. In this regard, it is relevant that whereas antipsychotic drugs reduce novelty-induced hyperlocomotion in *NR1*-hypomorphic, *STOP*-deficient and *EGF-NRG-1* (+/−) mice, a test associated with mesolimbic dopaminergic hyperactivity that may model positive symptoms in schizophrenia, these drugs do not ameliorate their PPI deficits. This suggests that such animals may reflect disease processes that operate in schizophrenia. Latent inhibition or poorer performance on a learning task involving a previously pre-exposed nonreinforced stimulus is disrupted in rodents by dopaminergic stimulants that also exacerbate positive symptoms in individuals with schizophrenia, effects that are reversed by antipsychotic drugs (for review see Weiner[160]). Consequently, latent inhibition may be considered a behavioural measure for at least some aspects of positive symptoms that has validity in both rodents and humans. With respect to negative symptoms, the situation is less clear. Social interactions have been considered a behavioural correlate of negative symptoms in schizophrenia.[154] Consistent with the weak effects of antipsychotic drugs against negative symptoms, this behavioural measure is not improved in at least one neurodevelopmental model (neonatal ventral hippocampal lesions), whereas mixed results have been observed in genetically altered mice (*NR1*-hypomorphic mice, improved; *STOP*-null mice, partial effects) treated with antipsychotic drugs. Until therapeutics with good efficacy against negative symptoms are developed, it will be difficult to determine the degree to which social interaction tests in rodents model these clinical features of schizophrenia.[154] In terms of cognitive deficits, it is also uncertain whether behavioural tests of spatial navigation memory or contextual fear conditioning in rodents are relevant to mental processes disrupted in schizophrenia. Consequently, there is considerable need to devise behavioural tests that measure the same mental processes in both rodents and humans.

Olfaction: a sensory modality to assess cognitive processes in rodents with human relevance

Although nonhuman primates may better model several aspects of human physiology, making it easier to devise tests that model the same mental processes in humans, the challenges associated with producing precise and inducible alterations in gene expression in the primate CNS limits this animal model. By contrast, the inducible deletion of specific genes in select cell populations in the brain may be achieved using Cre/loxP technologies in the mouse. Temporally and spatially controlled gene ablation can be achieved in the mouse brain using the Cre/loxP system.[161–163] This technical approach is based on the ability of the Cre recombinase to catalyze the recombination between 34-bp loxP recognition sequences.[164–167] The loxP sites are engineered around crucial exons by gene targeting in such a way as not to affect normal gene function. Expression of Cre recombinase in specific tissues catalyzes both excision of the DNA segment between the loxP sites and then ligation of the resulting fragments into an intact DNA strand. This approach is targeted to a crucial exon that depending on the normal function of the deleted exon results in either a loss or gain of gene function in that

tissue.[168] Typically, transgenic mice have been generated in which site-specific recombination in the brain has been produced by expression of the Cre recombinase under control of regulatory elements in neuron-specific genes such as calcium-calmodulin-dependent kinase IIα and Thy-1.[161,163] More recently, mice have been generated in which a ligand-inducible system under the control of a neuron-specific promoter is used to permit both spatial and temporal control of Cre recombinase activity.[163] These mice that express Cre recombinase are then mated with mice engineered with loxP sites to permit inducible gene ablation.

The availability of such transgenic mice necessitates the development of behavioural tests for complex mental processes such as executive function in the rodent. The olfactory system is particularly well suited to the investigation of complex cognitive processes in rodents.[169–171] Our rationale for using olfactory paradigms to examine the nature of cognitive deficits in animal models of schizophrenia is based on 3 major findings. First, olfactory identification is consistently impaired in both individuals with newly diagnosed schizophrenia and those with chronic schizophrenia managed effectively with antipsychotic drugs.[172,173] Second, olfactory processing is mediated by many of the same medial temporal lobe areas of the brain that have been implicated in schizophrenia.[29,174] Projection neurons from the olfactory bulb travelling along the olfactory tract enter the ipsilateral anterior ventromedial temporal lobe where they synapse with pyramidal cells. The majority of these afferents terminate in the piriform cortex, a structure thought to be responsible for initial odour perception.[169] A minority of projections from the olfactory bulb terminate posteriorly in the entorhinal cortex, considered to be the primary entry point to the hippocampus. The piriform cortex and entorhinal cortex send projections to the amygdala that may provide a neuroanatomical basis for linking olfaction to emotion and memory, both of which processes are disrupted in schizophrenia. Patients with schizophrenia have reduced cortical volumes in brain regions that receive afferents directly from the olfactory bulb, suggesting that deficits in olfactory function are related to structural abnormalities in these regions.[175] Third, a number of odour-based paradigms for rodents are available to model cognitive processes such as learning set formation, working memory and associative learning that are impaired in individuals with schizophrenia.[2,6,169,170,176,177] Olfactory testing paradigms may therefore offer a means by which to determine whether genetic and neurodevelopmental models of schizophrenia reproduce deficits in cognitive function observed in patients.[171,178–181]

Transitive inference

Transitive inference requires remembering the relations among items and the ability to make inferences about the orderly relations between items not presented together during training.[20,182] To determine whether an animal is capable of performing transitive inference, the animals are trained in a series of discriminations between 5 distinct odours, termed A–E, which are presented as pairs. Adult rodents can be successfully trained to choose A over B (A > B), B over C (B > C), C over D (C > D) and D over E (D > E). To demonstrate transitive inference, rodents are required to select odour B over odour D (B > D), despite the fact that these 2 odours had not been paired together during training. When tested in probe trials, rodents are able to correctly choose odour B over odour D, demonstrating transitive inference. This test is particularly relevant given that transient inference, a functional operation of relational memory organization, has been shown to be impaired in schizophrenia.[183]

Future directions

According to the "two-hit hypothesis" proposed by Bayer and colleagues,[13] a patient at risk is a carrier of a mutant candidate gene (first hit) that during fetal development receives a second hit in the form of an environmental factor. Based on this hypothesis, we propose that by exposing genetically altered mice during the perinatal or neonatal stage of development to an experimental manipulation that mimics the effects of environmental risk factors associated with schizophrenia, it may be possible to better model some forms of this mental illness. Because there are a large number of possible mutations that may genetically predispose an individual to the deleterious effects of a variety of environmental risk factors, the potential number of environmental and genetic combinations is prohibitive. One strategy to deal with this problem is to expose a genetically altered mouse with a weak but pro-schizophrenic-like behavioural phenotype (*NR1* knockdown, *STOP* [+/–], *EGF-NRG-1* [+/–], *Ig-NRG-1* [+/–], calcineurin [+/–] or *CHL1* [+/–]) during development to a treatment that mimics an environmental risk factor (intrauterine hypoxia, poly I:C or MAM). We propose that such a strategy may represent an integrative approach for modelling both the environmental and genetic factors that result in schizophrenia. Because cognitive deficits are a major liability and unmet medical need for schizophrenia, we further propose that olfactory-based tests be used to identify novel therapeutics capable of improving performance in complex cognitive tasks such as transitive inference. By combining genetic and environmental models with traditional and more sophisticated behavioural testing, it should be possible for neuropharmacologists to develop new ways to model schizophrenia and enable development of the next generation of antipsychotic drugs.

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