Behavioral Phenotypes in Schizophrenic Animal Models With Multiple Combinations of Genetic and Environmental Factors

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Abstract. Schizophrenia is a multifactorial psychiatric disorder in which both genetic and environmental factors play a role. Genetic [e.g., Disrupted-in-schizophrenia 1 (DISC1), Neuregulin-1 (NRG1)] and environmental factors (e.g., maternal viral infection, obstetric complications, social stress) may act during the developmental period to increase the incidence of schizophrenia. In animal models, interactions between susceptibility genes and the environment can be controlled in ways not possible in humans; therefore, such models are useful for investigating interactions between or within factors in the pathogenesis and pathophysiology of schizophrenia. We provide an overview of schizophrenic animal models investigating interactions between or within factors. First, we reviewed gene-environment interaction animal models, in which schizophrenic candidate gene mutant mice were subjected to perinatal immune activation or adolescent stress. Next, environment–environment interaction animal models, in which mice were subjected to a combination of perinatal immune activation and adolescent administration of drugs, were described. These animal models showed interaction between or within factors; behavioral changes, which were obscured by each factor, were marked by interaction of factors and vice versa. Appropriate behavioral approaches with such models will be invaluable for translational research on novel compounds, and also for providing insight into the pathogenesis and pathophysiology of schizophrenia.

Keywords: animal model, behavioral phenotype, gene × environment interaction, environment × environment interaction, schizophrenia

1. Introduction

Schizophrenia is a severe and common psychiatric disease that has a lifetime prevalence of 0.5% – 1% globally (1). Epidemiologically, schizophrenia, like disorders such as ischemic heart disease, diabetes mellitus, and asthma, results from the cumulative effects of susceptibility genes and the environment (2). Genetic [e.g., Disrupted-in-schizophrenia 1 (DISC1), Neuregulin-1 (NRG1), Nuclear receptor related 1 (Nurr1) etc.] and environmental factors (e.g., maternal viral infection, obstetric complications, malnutrition, social stress, isolation) may act during the developmental period to increase the incidence of schizophrenia. It is unlikely that a single gene mutation or a single adverse life event is sufficient to increase the incidence of schizophrenia, and it is thought that it is the interactions between or within such factors (3). Thus, the interactions between or within factors have been hypothesized to be one mechanism underlying the complex heritability and variable phenotype of schizophrenia. In animal models, both genetic and environmental factors can be experimentally controlled in a way that is not possible in humans, and such models are therefore useful for investigating the interaction between or within factors in the pathogenesis and pathophysiology of schizophrenia (4). In order to evaluate the face validity (derived from phenomenological similarity between the behavior in the animal model and the specific symptoms of the human disease)
condition) of animal models designed to test interactions between or within factors for schizophrenia, the presence of schizophrenia-like behavioral phenotypes is investigated. These phenotypes include hyperactivity in a locomotor activity test as an index of positive symptoms, enhanced immobility in a forced swimming test, and social deficits in a social interaction test as indexes of negative symptoms, as well as sensorimotor gating deficits in a prepulse inhibition (PPI) test, and impairment of object recognition memory in a novel object recognition test as indexes of cognitive deficits. Here, we review recent animal models designed to investigate multiple combinations of genetic and environmental factors.

2. Behavioral phenotypes in putative models given the combined factors

2.1. Dominant-negative N-terminal human DISC1 transgene × immune activation

The DISC1 gene encodes a scaffold protein that has a multifaceted impact on neuronal developmental processes such as neuronal migration and dendritic arborization. The DISC1 gene is a candidate susceptibility gene for psychiatric disease that was originally discovered in a family with a chromosomal translocation severing this gene. Although the family members with the translocation have an identical genetic mutation, their clinical diagnosis and presentation vary significantly (5). To explore the involvement of truncated DISC1 in psychiatric disorders, transgenic mice expressing a dominant-negative N-terminal human DISC1 (DN-DISC1) under the expression control of CaMKII promoter were created (6). It has been reported that DN-DISC1 mice exhibited hyperactivity in a novel environment, increased immobility in the forced swimming test, and age (over 12-week-old)-dependent mild sensorimotor gating deficits in the PPI test (6, 7).

In contrast, clinical studies suggest that a serious viral infection in the central nervous system during the prenatal and postnatal stage is involved in the etiology of psychiatric disorders. Polyriboinosinic-polyriboctydilic acid (polyI:C) is a synthetic analog of double-stranded RNA, which is generated during viral infection as a replication intermediate for single-stranded RNA (ssRNA) or as a by-product of symmetrical transcription in DNA viruses (8). Neonatal polyI:C treatment at 5 mg/kg for 5 days (postnatal day 2 – 6) in ICR mice induced anxiety-like behaviors in the open field test, impairment of object recognition memory in the novel object recognition test, social deficits in the social interaction test, and sensorimotor gating deficits in the PPI test (9).

Ibi et al. (7) have investigated how the interaction between DN-DISC1 and neonatal immune activation during neurodevelopment affects phenotype in adulthood. Specifically, DN-DISC1 mice were injected with polyI:C during the neonatal stage, and their behavioral phenotypes were examined in adulthood. PolyI:C-treated C57BL/6N (wild-type) mice did not show characteristic behavioral phenotypes, including the phenotypes observed in PolyI:C-treated ICR mice (7, 9). Moreover, 8-week-old saline-treated DN-DISC1 mice did not show sensorimotor gating deficits in the PPI test (7) and also lacked other characteristic behavioral phenotypes that had been observed in such mice at 12 weeks of age (6, 7). However, PolyI:C-treated DN-DISC1 mice exhibited augmentation of MK801-induced hyperactivity, impairment of short-term memory in the Y-maze test, impairment of short-term object recognition memory in the novel object recognition test, social deficits (decreased sociability and aggressive behaviors) in the social interaction test, and impairment of hippocampus-dependent fear memory (contextual, but not cued learning) in the conditioned fear learning test after puberty (7) (Table 1, a). Nagai et al. (10) have investigated the effect of antipsychotics in neonatal PolyI:C-treated DN-DISC1 mice, which were established by Ibi et al. (7). Repeated treatment with clozapine (3 mg/kg), but not haloperidol (1 mg/kg), ameliorated impairment of object recognition in the novel object recognition test. Both antipsychotics suppressed the augmentation of MK801-induced hyperactivity, whereas they had no effects on impairment of short-term memory and hippocampus-dependent fear memory or social deficits (10). This mouse model may be useful for the screening of potential antipsychotics that could be more effective than clozapine in ameliorating negative symptoms and cognitive impairment in schizophrenia.

In a mouse model with inducible DN-DISC1 based on the Tet-Off system, prenatal immune activation by administration of polyI:C to pregnant dams at gestational day 9 resulted in social deficits in the social interaction test, exhibition of high levels of anxiety in the elevated plus maze test, and prolonged immobility in the forced swimming and tail suspension tests in offspring (11) (Table 1, a).

Although there are both differences and similarities in terms of the effects of neonatal and prenatal polyI:C treatment on behavioral phenotypes in adult DN-DISC1 mice, both neonatal and prenatal polyI:C treatments result in synergistic and additive effects on some behavioral phenotypes in adult DN-DISC1 mice. Thus, neonatal polyI:C treatment in DN-DISC1 mice may provide a model for schizophrenia that reflects gene-environmental interactions.
showed latent inhibition deficit, but only modest sensorimotor gating deficits and more depressive endophenotypes with prolonged immobility in the forced swimming test and a decrease of sucrose preference in the sucrose consumption test, which were reversed by antidepressant treatment (12, 13).

Regarding environmental adversity, numerous studies indicated psychosocial stressors in particular increase the risk of psychosis, especially with cumulative exposure (14). Within the domain of psychosocial stressors, social defeat refers to the sense of subordination experienced following an adverse social encounter and has been proposed as a key process linking social adversity with risk of psychosis, especially with cumulative exposure (14).

### Table 1. Behavioral phenotypes in putative models given the combined factors

<table>
<thead>
<tr>
<th>a. Dominant-negative N-terminal human DISC1 transgene (DN-DISC1) × immune activation</th>
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<th>Heterozygous Q31L mutant</th>
<th>Heterozygous Nurr-1</th>
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<th>Poly(ADP-ribose) polymerase (PARP)</th>
<th>Poly(ADP-ribose) polymerase (PARP)</th>
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<tr>
<td>MK801-induced hyperactivity</td>
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<td>Sociability in the social interaction test</td>
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<td>Aggressive behaviors in the social interaction test</td>
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<td>Contextual learning in the conditioned fear learning test</td>
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<td>Cued learning in the conditioned fear learning test</td>
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<td>Sensorimotor gating in the prepulse inhibition test</td>
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<td>Spontaneous locomotor hyperactivity in the novel environment</td>
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<td>Immobility in the forced swimming test</td>
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<td>Immobility in the tail suspension test</td>
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2.2. DISC1 point mutation × chronic social defeat

The involvement of DISC1 single point mutations in psychiatric disorders can be examined using two missense mutant mice: L100P (leucine to proline) or Q31L (glutamine to leucine) in the DISC1 mouse, which is developed by N-nitroso-N-ethylurea point mutagenesis (12). The L100P mutant mice exhibited schizophrenic endophenotypes with profound sensorimotor gating deficits in the PPI test and a latent inhibition deficit in the fear conditioning test that were reversed by treatment with an antipsychotic. In contrast, the Q31L mutant mice showed latent inhibition deficit, but only modest sensorimotor gating deficits and more depressive endophenotypes with prolonged immobility in the forced swimming test and a decrease of sucrose preference in the sucrose consumption test, which were reversed by antidepressant treatment (12, 13).

Regarding environmental adversity, numerous studies indicated psychosocial stressors in particular increase the risk of psychosis, especially with cumulative exposure (14). Within the domain of psychosocial stressors, social defeat refers to the sense of subordination experienced following an adverse social encounter and has been proposed as a key process linking social adversity with...
increased risk for schizophrenia (15). Animal stressed by social defeat showed social deficits in the social interaction tests and increased anxiety-like responses to open and bright space in the elevated plus maze test (16).

Haque et al. (17) have investigated how the interaction between single point mutation in the DISC1 gene and social adversity during adolescence produces behavioral phenotypes in adulthood using two lines of mice carrying different heterozygous DISC1 point mutations, L100P and Q31L, which were stressed by chronic social defeat (CSD) between 9 and 12 weeks of age. Heterozygous L100P mutant mice exhibited hyperactivity in the novel environment, which was diminished by the CSD (Table 1, b). Heterozygous L100P and Q31L mutant mice exhibited high anxiety in the elevated plus maze and CSD augments anxiety in the wild-type and heterozygous L100P mice but not Q31L mutant mice. Heterozygous Q31L mutant mice exhibited social deficits, and CSD inhibited sociability and social novelty in the wild-type and heterozygous L100P mutant mice. Heterozygous L100P mutant mice exhibited sensorimotor gating deficits in the prepulse-elicited reactivity deficit in the PPI test, but CSD failed to affect sensorimotor gating in any group. Heterozygous L100P and Q31L mutant mice exhibited latent inhibition deficit in the conditioned licking response test, but CSD failed to affect latent inhibition in any group. Although CSD stress to two heterozygous DISC1 point mutant mice resulted in complicated effects on behavioral phenotypes in adulthood, the interaction between single point mutations in the DISC1 gene and CSD was observed in some behavioral paradigms (17). These findings provide a model for investigating such interactions, with the potential to identify targets for treatments to rescue or prevent the effects of adverse environments when genetic vulnerability is present.

2.3. NRG1 heterozygous knockout × chromic social defeat

NRG1 regulates various neurodevelopmental processes, including neuronal migration, myelination, synaptic plasticity, and neurotransmitter function (18). The majority of NRG1 proteins are synthesized with a TM (transmembrane) domain. Although complete deletion of the TM domain region in the NRG1 (NRG1-TM) gene was lethal, NRG1-TM heterozygous knockout mice exhibited hyperactivity in the novel open field test, enhanced aggressive behaviors in the social interaction test, and sensorimotor gating deficits in the PPI test (19).

Desbonnet et al. (20) have examined the individual and combined effects of chronic social stress during adolescence and deletion of a schizophrenic risk gene, NRG1, on adult mouse phenotype. NRG1-TM heterozygous knockout mice were stressed by CSD during adolescence, and their behavioral phenotypes were examined in adulthood. NRG1-TM heterozygous knockout mice exhibited behavioral phenotypes similar to those reported by Kato et al. (19). CSD-stressed NRG1-TM heterozygous knockout mice exhibited impairment of short-term memory in the Y-maze test, whereas aggressive behaviors in the social interaction test were diminished (20) (Table 1, c). These results indicate CSD exposure in NRG1-TM heterozygous knockout mice results in synergistic and additive effects on some behavioral phenotypes in adulthood. Thus, behavioral phenotypes in CSD-stressed NRG1-TM heterozygous knockout mice may reflect a gene-environment interaction. The experience of psychosocial stress during adolescence may trigger further pathobiological features that contribute to the development of schizophrenia, particularly in those with underlying NRG1 gene abnormalities.

2.4. Nurr1 heterozygous knockout × immune activation

Nurr1 is a member of the orphan steroid hormone receptor family with pivotal functions in differentiation, migration, and survival of ventral mesencephalic dopaminergic neurons. Together with other seminal transcription factors such as Pitx3 and Lmx1b, Nurr1 also exerts a number of functions in postmitotic and mature mesencephalic dopaminergic neurons by regulating expression of tyrosine hydroxylase (TH) and dopamine transporter (DAT). The Nurr1 gene has been suggested to be a potential susceptibility gene in schizophrenia. This is based on the observation that two different missense mutations in the third exon of the Nurr1 gene, coupled with a 30%–40% reduction in the in vitro transcriptional activity of the Nurr1 dimers, are present in some people with schizophrenia. In addition, Nurr1 mRNA was found to be reduced in the prefrontal cortex in patients with schizophrenia. Nurr1-deficient mice displayed increased spontaneous locomotor activity and a potentiated locomotor reaction to systemic treatment with a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, dizocilpine (MK801). In addition, male, but not female, Nurr1-deficient mice showed significant sensorimotor gating deficits and prepulse-elicited reactivity deficit in the PPI test (21).

Vuillermot et al. (22) have investigated how the interaction between the Nurr1 gene deficiency and the prenatal immune activation produces phenotypic changes in adulthood. Nurr1 heterozygous knockout mice were injected with polyI:C at gestational day 17, and their behavioral phenotypes were examined in adulthood. PolyI:C-treated Nurr1 heterozygous knockout mice exhibited hyperactivity in the novel environment, sensorimotor gating deficits in the PPI test, latent inhibition
deficit in the conditioned freezing test, attention deficit in the two-choice visual discrimination test, and impairment of working memory in the dry maze test. Both hyperactivity and sensorimotor gating deficits were augmented by each combination (Table 1, d). These behavioral experiments highlighted that the combination of Nurr1 deficiency and immune activation could induce far more pronounced behavioral phenotypes than each factor alone, emphasizing the importance of gene–environment interactions in schizophrenic behavioral phenotypes (22). The present model may be helpful to study how gene–environment interactions shape vulnerability to cognitive deficits related to multifactorial and/or dopamine-associated psychiatric disorders such as schizophrenia and attention deficit/hyperactivity disorder.

2.5. Pituitary adenylate cyclase-activating polypeptide (PACAP) knockout × isolation rearing

PACAP belongs to the vasoactive intestinal peptide/secretin/glucagon superfamily, and it is widely distributed in the brain and the peripheral organs (23). Genetic analysis reveals that genetic variants of the genes encoding PACAP and its selective receptor, PAC1, are associated with schizophrenia. PACAP-knockout mice displayed remarkable hyperactivity in the novel environment, higher impulsivity in the cliff-avoidance test, sensorimotor gating deficits in the PPI test, and prolonged immobility in the forced swimming test, which were ameliorated by antipsychotics (24). Exposing mammals to early-life adverse events, including maternal separation or social isolation, profoundly affects brain development and adult behaviors and has been suggested to contribute to the occurrence of psychiatric disorders, such as depression and schizophrenia, in genetically predisposed humans. Rearing rat pups from weaning in isolation, to prevent social contact with conspecifics, produces reproducible, long-term changes including neophobia, impaired sensorimotor gating, aggression, cognitive rigidity, reduced prefrontal cortical volume, and decreased cortical/hippocampal synaptic plasticity (25).

To study how the interaction between the PACAP gene deficiency and adolescent social isolation produces phenotypic changes in adulthood, PACAP knockout mice were reared in isolation for 2 weeks from the age of 4 weeks, and their behavioral phenotypes were examined in adulthood (26). PACAP-knockout or isolation-reared mice displayed sensorimotor gating deficits in the PPI test. Isolation-reared PACAP-knockout mice exhibited early-onset hyperlocomotion in the novel environment, aggressive behaviors in the social interaction test, and worsened sensorimotor gating deficits in the PPI test (Table 1, e). The environmental factors during early, but not late, developmental periods influenced the expression of almost all behavioral abnormalities in PACAP-knockout mice, indicating that they were more sensitive to isolation stress than wild-type mice (26). These results support the hypothesis that gene–environmental interactions play an important role in the pathogenesis of psychiatric disorders.

2.6. Neonatal immune activation × adolescent phencyclidine (PCP) treatment

 Substance abuse is a potent risk factor for poor outcomes in schizophrenia and other serious psychiatric disorders (27). Exposure to chronic phencyclidine (PCP), a drug of abuse and a non-competitive NMDA-receptor antagonist, has been shown to induce severe psychoses, including schizophrenia-like symptoms and cognitive deficits in humans (28). The disturbance of organization and functions in the forebrain glutamatergic systems strongly implicates it in the pathophysiology of schizophrenia. We have reported that subchronic administration of PCP (10 mg/kg per day, s.c.) for 14 days, but not for 7 days, induces several behavioral abnormalities such as hyperactivity in a locomotor activity test, prolonged immobility in the forced swimming test, social deficits in the social interaction test, and impairment of recognition memory in the novel object recognition test (29).

We investigated whether the combination of neonatal immune activity and adolescent administration of drugs of abuse produces abnormal behavioral phenotypes in adulthood. Neonatal mice were repeatedly injected with polyI:C for 5 days on postnatal days 2 – 6 and then the adolescent mice were treated with PCP for 7 days on postnatal days 35 – 42. We found that neonatal polyI:C treatment markedly enhanced sensitivity to PCP-induced hyperactivity in adolescents. Mice treated with both neonatal polyI:C and adolescent PCP exhibited higher impulsivity in the cliff-avoidance test, social deficits, and exacerbated impairment of recognition memory in the novel object recognition test (Hida et al., unpublished data; Table 1, f). These results indicate that neonatal polyI:C-treated mice are more sensitive to PCP treatment than neonatal saline-treated mice. Thus, behavioral phenotypes induced by the combination of neonatal polyI:C and adolescent PCP treatments may reflect an environment–environment interaction. This model is useful for investigating the involvement of abnormalities in the glutamatergic system in several psychiatric disorders including schizophrenia, since the dysfunction of glutamatergic neurotransmission has been reported in neonatal polyI:C- or adult/prenatal PCP-treated mice (9, 29).
3. Conclusion

In this review, we provided an overview of animal models that allow investigation of interactions between or within factors for schizophrenia. These animal models show clear interactions between or within factors in terms of behavioral phenotypes. Namely, some normal and abnormal behaviors in animals with single factor were impaired and augmented or attenuated, respectively, by multiple combinations of genetic and environmental factors. The pathobiology of schizophrenia is fraught with complexity, hence the development of valid animal models for this disorder would present several challenges (20).

Epistasis genetic studies of schizophrenia have reported gene–gene interactions between candidate genes (30). The potential value of genetic models for exploring gene–gene as well as gene–environment and environment–environment interactions relating to schizophrenia should be seriously considered (31). Recently, the “two-hit” hypothesis has been highlighted in the pathology of schizophrenia (3, 32). In this hypothesis, the first hit consists of predisposing genetic factors and/or early environmental factors, such as genetic defects, viral infection, obstetric complications, or malnutrition, causing anomalous neural development and subtle changes in behavior. The second hit consists of one or more environmental factors, such as substance abuse or social stress. The effect of these later environmental factors (the second hit) may not produce clinical consequences in individuals that have not been primed by earlier genetic and/or environmental factors (the first hit) (32). To develop animal models with susceptibility genes and environments for schizophrenia, we should consider appropriate timing for exposure of genetic and/or environmental factors.

In the future, various combinations of susceptibility genes and environmental factors can be tested in mice to develop new models of schizophrenia. Appropriate behavioral approaches with such models would be invaluable for translational research on compounds with novel mechanism of action and also for providing new insight into the pathogenesis and pathophysiology of schizophrenia. However, in the animal models reviewed here, a number of factors must be taken into account when evaluating the validity of multifactorial animal models for schizophrenia: 1) the validity of the animal model for negative schizophrenic symptoms is unclear and 2) the effects of antipsychotics in animal models remain to be investigated (neonatal polyI:C treatment in DN-DISC1 mice is an example of an animal model for schizophrenia with better predictive validity). Thus, it is necessary to periodically assess the three validity (face, construct, and predictive), especially in terms of sensitivity to conventional therapeutic drugs.

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