1. Introduction

Schizophrenia is a severe psychiatric disorder, developing between early teens and early thirties (1) and affecting approximately 1% of the population worldwide (2). Three major symptoms groups, positive and negative symptoms and cognitive deficit have been categorized in schizophrenia (3). Positive symptoms include hallucinations, delusions, conceptual disorganization and thought disorder and have been hypothesized to reflect the elevation of dopamine (DA) function in the associative neural system of the striatum (4). Negative symptoms encompass social withdrawal, diminished motivation, anhedonia, emotional blunting, and impoverished talk. The similar aspects to negative symptoms of schizophrenia appear in other psychiatric disorders such as mood disorder, major depressive disorder, and autism spectrum disorder (5); and those aspects have heterogeneous neurobiological underpinnings. Cognitive deficit represents affection in working memory, attention, and executive function. Functional alterations of glutamate- and γ-aminobutyric acid (GABA)-mediated neurotransmission have been hypothesized to underlie the cognitive performance of schizophrenia (6). As an accompanying phenomenon with the above three major symptom groups, an impairment of prepulse inhibition (PPI) in auditory startle response has been identified in patients with schizophrenia (7). Impaired PPI has been thought to reflect the deficits of pre-attention processing in the sensorimotor gating system (8). Most current therapeutic drugs for schizophrenia have efficacy for positive symptoms and impaired PPI and weak efficacy for negative symptoms and cognitive deficit (9). Especially, negative
Negative Symptoms in Schizophrenia Model

Negative symptoms are resistant to the treatment of antipsychotic drugs, and a few aspects of negative symptoms related to the deficits of cognitive performance (10). Therefore, there is a need for development of new effective drugs for the negative symptoms of schizophrenia, and it is necessary to have comprehensive animal models to understand the neurobiological alterations of their symptoms.

In general, it is important that the comprehensive animal models associated with the human symptom fit three main criteria: face, construct, and predictive validities. Face validity phenomenologically mimicked the fundamental symptoms found in the patients. Construct validity conforms to theory in pathophysiology and etiology proposed for the disorder. Taken together with diagnosis, these predictive validities would be to reveal the information for the symptom and novel therapeutic drugs. Therefore, animal models should be developed based on the construct validity of schizophrenia, which are evaluated by the face and predictive validities for the negative symptom. These validities have been evaluated by phenotype screening methods for the other psychiatric disorders (Table 1). For example, social withdrawal is assessed by decreased contact time in the standard reciprocal social interaction and three chamber social interaction tests, which was developed to estimate the impaired communication ability as the core symptom of autism (11). Depression-like diminished motivation is investigated by increased immobility time in the forced swimming and tail suspension tests. Anhedonia (inability to feel pleasure) and anxiety-like mood (state of feeling nervous) are checked by decreased drinking preference for sucrose in the sucrose preference test and anxiety-like mood (state of feeling nervous) are checked by decreased drinking preference for sucrose in the sucrose preference test and decreased time spent in the open arms of the elevated plus-maze test, respectively. Thus, symptoms of animal models in those behavioral assays are considered to be useful for the development of new therapeutic drugs to improve the negative symptoms of schizophrenia. In this review, we represent the behavioral phenotypes in available animal models of schizophrenia for drug discovery, focusing on negative symptoms, i.e., depressive aspects, of schizophrenia.

2. Pharmacological animal models

In the 1950s, the first animal model of schizophrenia, amphetamine model, has been developed on the basis of pharmacological evidences. The model was based on the DA hypothesis of schizophrenia being due to the hyperfunction in DAergic neurotransmission in the mesolimbic neuronal system, since a DA D2-receptor antagonist relieved the positive symptoms seen in patients with schizophrenia. Single amphetamine administration for animals induces enhancement in locomotor activity and stereotyped movement, repeated amphetamine administration develops sensitization in locomotor activity, and then exaggerating hyperlocomotion caused by amphetamine re-challenge after withdrawal (12). Those repeated amphetamine-induced locomotor impairments reflect hyperactivation of the DAergic response in the striatum. However, even repeated amphetamine administration does not induce deficits in social interaction (13). An unlikely amphetamine model, another pharmacological animal model based on the glutamate hypothesis of schizophrenia being caused by hypofunction in glutamatergic neuronal system in the prefrontal cortex, the N-methyl-D-aspartate (NMDA)-type ionotropic glutamate receptor–antagonist model presented a psychosis encompassing the negative symptoms of schizophrenia as well as the positive symptoms and cognitive deficit (Table 2).

2.1. NMDA-receptor antagonist model

Pharmacological evidences for the glutamate hypothesis that the hypofunction of the glutamatergic neuronal system in schizophrenia were increased in the past half a century. For example, in healthy humans, the blockade of the NMDA receptor by treatment of phencyclidine (PCP), dizocilpine (MK-801), or ketamine induces the positive symptoms seen in patients with schizophrenia.

| Table 1. Behavioral assays applied in the research of negative symptoms of schizophrenia |
|------------------------------------|-------------------------------------|-------------------------------------|
| Negative symptom index | Behavioral assay | Original analysis disease |
| Sociability | Reciprocal social interaction test | Sociophobia (Social anxiety) |
| | 3-chamber social interaction test | Autism |
| Motivation | Forced swimming test | Depression |
| | Tail suspension test | Depression |
| Anhedonia | Sucrose preference test | Depression |
| Mood | Elevated plus-maze test | Anxiety |
| | Light/dark box test | Anxiety |
| | Open field test | Anxiety |
including hallucinations and delusions (14, 15), and PCP at low doses produces psychotic symptoms, resembling the negative symptoms of schizophrenia such as progressive social withdrawal and impoverished talk (16). Furthermore, in patients with schizophrenia, PCP aggravates the positive symptoms, and both single and repeated PCP treatment also occur the deficits of cognitive performance (17).

Table 2. Behavioral alterations in pharmacological and neurodevelopmental animal models of schizophrenia

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Sociability</th>
<th>Motivation/Anhedonia</th>
<th>Cognition</th>
<th>Locomotor activity/ Sensorimotor gating</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>Social interaction ±</td>
<td>ND</td>
<td>Attention ↓</td>
<td>Locomotor activity, Locomotor sensitization to psychostimulant ↑ Prepulse inhibition ↓</td>
<td>12, 13</td>
</tr>
<tr>
<td>PCP (single)</td>
<td>Social interaction ↓</td>
<td>Forced swimming ±</td>
<td>Latent learning, Working memory ↓</td>
<td>Locomotor activity ↑ Prepulse inhibition ↓</td>
<td>18 – 21, 28</td>
</tr>
<tr>
<td>Prenatal MAM</td>
<td>Social interaction before puberty ↓</td>
<td>ND</td>
<td>Spatial learning, Novel object recognition ↓</td>
<td>Locomotor activity ↑ Prepulse inhibition after puberty ↓</td>
<td>40, 43</td>
</tr>
<tr>
<td>Neonatal ventral hippocampal lesion</td>
<td>Social interaction ↓</td>
<td>Aggression ↑</td>
<td>ND</td>
<td>Working memory, Spatial memory ↓</td>
<td>Locomotor activity ± Prepulse inhibition ↓</td>
</tr>
</tbody>
</table>

↑: Significant increase, ↓: Significant decrease, ±: No significance, ND: No data in references

administration, repeated administration of PCP in rodents produced enhanced immobility in the forced swimming test (23). This enhanced immobility in the task is thought to reflect behavioral despair and is attenuated by general antidepressant drugs. Pretreatment with typical antipsychotic drugs, haloperidol, chlorpromazine, levomepromazine, and pimozide, failed to reverse the repeated PCP-induced enhanced immobility (22, 24). Alternatively, this is reversed by pretreatment with the atypical antipsychotic drugs clozapine, risperidone, and quetiapine, without affecting immobility in the control group (22). Interestingly, the tricyclic antidepressant drugs imipramine, chlomipramine, and desipramine failed to attenuate the repeated PCP-induced enhanced immobility (23).

Furthermore, resembling single PCP administration, repeated PCP administration in rodents also induced deficits in social interaction (21), which is inhibited by pretreatment of both typical and atypical antipsychotic drugs haloperidol and clozapine (13). However, repeated PCP administration failed to induce any significant change in the sucrose preference test. This task is used to evaluate change in rewarding properties and thought.
to relate to anhedonia (25). Anhedonia is thought to result in the dysfunction of neuronal processing in the rewarding system, which is involved in the mesolimbic and mesocortical neuronal pathways. Thus, repeated PCP administration does not seem to alter the natural rewarding system in spite of the administration of an addictive drug. Furthermore, in the prefrontal cortex of repeated PCP-administered animals, both basal and forced swimming stress–induced DA release were decreased (24, 26), and the same stress-induced 5-HT release was increased in the neurochemical analysis. These neurochemical changes support the therapeutic strategy of current antipsychotic drugs which is going to improve the unbalance between the DAergic and 5-HTergic neuronal systems.

Also, in the administration of another NMDA-receptor antagonist in animals, several reports indicated that MK-801 and ketamine affects emotional behaviors. Single ketamine administration at low dose decreased social interaction and caused anxiety-like behaviors in rats (27). This ketamine-induced deficits in social interaction were reversed by both clozapine and risperidone, but not haloperidol (27). After a withdrawal period from repeated ketamine administration, drug-washout rats showed decreased aggressive behaviors (sniffing, following, and grooming the partner and social play) (28). Thus, the administration with single and repeated ketamine as well as PCP is likely to induce depressive aspects relevant to the negative symptoms of schizophrenia.

These findings suggest that the NMDA-receptor antagonist model, especially repeated PCP model, observed not only the positive symptoms and cognitive deficit, but also the negative symptoms seen in patients with schizophrenia (26). Thus, the repeated PCP model is useful to evaluate the pharmacological effects of new drugs for the negative symptoms of schizophrenia, and antipsychotic drugs with 5-HT2A receptor agonistic properties are effective deficits in social interaction in schizophrenia.

3. Neurodevelopmental animal models

In human epidemiological studies, infection, malnutrition, or hypoxia in the fetus is a risk factor for the onset of schizophrenia (29). This hypothesis of the development of schizophrenia is that exposure to a harmful event in early stage of life triggers alterations of neurodevelopment in the brain and subsequently results in the expression of schizophrenia-like phenomena in young adulthood. Neurodevelopmental animal models of schizophrenia are generated by control of exposure to a harmful substance or environmental situation during the prenatal period, which is the phase to produce functional and structural changes in brain development. Prenatal or maternal immune activation, disruption of neurogenesis during gestational period, lesion in the neonatal ventral hippocampus or post-weaning social isolation has caused the appearance of some behavioral alterations associated with schizophrenia. Thus, neurodevelopmental animal models produced by the various stresses in early stage of life have advanced face and construct validities based on epidemiological findings associated with schizophrenia (30). For instance, the neurodevelopmental animal models for schizophrenia have been reported by the injection of polyriboinosinic-polyribocytidilic acid (polyI:C) and methylazoxymethanol acetate (MAM) in the prenatal period and the excitatory toxic lesion in the ventral hippocampus in the neonatal period (Table 2).

3.1. Prenatal polyI:C injection model

PolyI:C is a synthetic analogue of double-stranded RNA, and its treatment in animals leads to immune activation by the pronounced induction of pro-inflammatory cytokines and interferons in short-term period (31). Prenatal injection to polyI:C by treatment of pregnant rodent dams causes the expression of schizophrenic-like phenomena in their adolescent offspring. The resultant offspring showed enhancement of locomotor sensitivity to psychostimulant (e.g., amphetamine and MK-801) without spontaneous hyperlocomotion (32, 33) and impaired PPI in auditory startle responses (34). Gestational treatment of polyI:C also caused selective impairment in non-spatial memory processing such as novel object recognition, but not novel location learning in the Morris’s water maze test, in resultant offspring (33, 35). Offspring from gestational immune-activated mouse dams also exhibit social withdrawal and anhedonia (36). With these behavioral alterations, neurochemical alterations such as reduced DA and glutamate levels in the prefrontal cortex and hippocampus were observed in these offspring (36).

In the studies using the prenatal polyI:C injection model, the pretreatment with antipsychotic or antidepressant drug, clozapine or fluoxetine, respectively, during the periadolescent stage prevented the positive symptoms-like behavioral abnormalities (enhanced locomotor sensitivity to psychostimulant and impaired PPI) (37), and the 2-week pretreatment with clozapine, but not haloperidol, reversed cognitive deficit, i.e., reduced performance to recognize a novel object (33). On the other hand, there have been no investigations on the efficacy of antipsychotic drugs on the negative symptoms-like behavioral abnormalities.

3.2. Prenatal MAM injection model

Administration of a neurotoxin MAM, which inhibits
DNA synthesis in mitosis, at a gestational point of time to rat dams does not affect litter size and body weight (38, 39), but interferes with behavioral phenotypes in their offspring (38, 40). Prenatal MAM injection from pregnant dams, in the offspring, enhanced spontaneous locomotor activity and locomotor sensitivity to the psychostimulant amphetamine (40). Furthermore, the offspring show behavioral impairments such as the reduction of social interaction before puberty and deficits of PPI, spatial learning, and cognitive function after puberty (39, 40). However, for these behavioral schizophrenia-like symptoms in the prenatal MAM injection model, the pharmacologic improvement with the currently available therapeutic drugs has hardly been investigated. Thus, it is not clear whether the prenatal MAM injection model has good predictive validity. Paradoxically, this neurodevelopmental model could be useful to develop the novel antipsychotic drugs based on a new therapeutic strategy, since existing medicines is almost ineffective against the negative symptom of schizophrenia.

The prenatal MAM injection model exhibited not only impaired behavioral phenotypes associated with schizophrenia, but also increased microdialysate DA levels in the nucleus accumbens, but not the frontal cortex (39). Supporting the increase of DAergic neurotransmission in the mesolimbic neuronal system, an electrophysiological study has shown that enhanced spontaneous firing rate of DA neurons in the ventral tegmental area, which is an origin part of DA neurons to project on the nucleus accumbens, is observed in the offspring of MAM-administrated dams (41). Moreover, in the prenatal MAM injection model, an inactivation by tetrodotoxin microinjection in the ventral hippocampus completely reversed the enhanced activity of DA neurons in the ventral tegmental area, and also normalized the enhanced locomotor sensitivity to amphetamine (41). This observation suggests that the hyperactivation of the mesolimbic DAergic neuronal system is consequently due to the excessive neuronal activity in the ventral hippocampus (42, 43). In turn, for excessive activity of the glutamatergic neurotransmission in the ventral hippocampus, prenatal MAM injection may lead to the loss of parvalbumin-containing GABAergic interneurons in the same region (44), one of the neurochemical features seen in patients with schizophrenia (45).

3.3. Neonatal ventral hippocampal lesion model

Structural and functional alterations in the hippocampus of schizophrenia are consistently demonstrated in post-mortem and neuroimaging studies. Therefore, the ventral hippocampal lesion animal model was developed based on pathological evidence for the presence of ventricular enlargement and hippocampal atrophy seen in patients with schizophrenia (46, 47). Neonatal lesion of the ventral hippocampus in rodents (corresponding to the anterior hippocampus in human) by microinjection of the excitatory toxin ibotenic acid induces abnormal behavioral phenotypes after puberty (46).

The neonatal ventral hippocampal lesion in rodents appears to cause some behavioral changes with progressive development. For example, impairments in spatial learning and working memory appear around postnatal day 25 (48), and social withdrawal and increased aggression occur by postnatal day 35 (49). Then, hypersensitivity to stress or the psychostimulant amphetamine (50) and NMDA-receptor antagonist PCP and MK-801 (51) in locomotor activity and deficits in PPI appear after around prenatal day 56 (52). Interestingly, the neonatal ventral hippocampal lesion model also shows enhanced acquisition of sucrose in the sucrose preference test (47), indicating increase in rewarding properties that is an opposite phenomenon to anhedonia seen in patients with schizophrenia. In addition, the deficits in social interaction and increase of aggression in this animal model are independent of sexual maturity (53). These deficits in social interaction and aggression are observed only by the lesioned model in the ventral, but not in the dorsal hippocampus (53). In behavioral analysis in response to therapeutic drug, administration of the atypical antipsychotic drug clozapine in the prenatal ventral hippocampal lesion model reversed amphetamine-induced hyperlocomotion (47) and failed to improve the deficits in social interaction (49). In neurochemical analysis, the prenatal ventral hippocampal lesion model shows reduced potassium-induced glutamate release from the ex vivo slices of the prefrontal cortex (50) and unaltered basal DA levels in the nucleus accumbens under normal condition by the in vivo microdialysis method (47). Thus, this experimental approach to generate an animal model of schizophrenia is insufficient in predictive validity and therefore would be inaccurate to clarify the neurobiological processes of schizophrenia, which would be required for complete understanding of the disease. However, similar to the aforementioned the prenatal MAM injection model, the prenatal ventral hippocampal lesion model may provide useful clues for how to treat the negative symptom of schizophrenia.

4. Genetic animal models

The twin studies of schizophrenia have demonstrated that this psychiatric disorder is a predominant genetic disorder with heritability estimated to be approximately 80% (54). However, the family studies of schizophrenia have shown that the neurobiological effects of single
genetic alteration are inadequate to explain the complex heterogeneous symptoms of schizophrenia (55). Studies to discover the genes associated with schizophrenia have been carried out globally by meta-analyses, and many risk factor molecules such as the NMDA receptor subunit 1 and 2A (NR1 and NR2A), disrupted in schizophrenia 1 (DISC1), neuregulin 1 (NRG1), dysbindin, and reelin have been proposed to date. About those genes, genetically modified mice have been generated to assess behavioral and neurobiological phenotyping (Table 3).

### 4.1. NMDA receptor subunit

Importance of the NMDA receptor in the neuro-pathology of schizophrenia is supported by reduced expression of essential NR1 subunit forming the NMDA receptor in postmortem tissue from patients with schizophrenia and enhanced expression of NR1 subunit by repeated treatment with antipsychotic drug (56). In animals, the NR1 subunit is disrupted by systemic traditional and conditional genetic manipulation, and these result in several impairments (hyperlocomotion, stereotyped movement, reduced social interaction, cognitive dysfunction, reduced PPI, and abnormal brain development) resembling various symptoms of schizophrenia (57, 58). Furthermore, these abnormalities are partially improved by antipsychotic drugs, haloperidol clozapine, and quetiapine. Consistent with the glutamate hypothesis of schizophrenia, which is the dysfunction of the NMDA receptors, the postnatal NR1 deletion in the corticolimbic interneurons exhibit distinct schizophrenia-like symptoms after adolescence, including deficits in novelty-induced hyperlocomotion, working memory and impaired PPI (59). In addition, deficits in social interaction, anhedonia, and anxiety-like behavior are also observed in the mutant mice (59). However, post-adolescent NR1 deletion mice did not reveal the above behavioral abnormalities. Thus, these observations suggest that postnatal inhibition of NMDA-receptor activity contributes to the pathophysiology of schizophrenia-related disorders.

Genetica dysfunction of the NMDA receptor in animals is also induced by the deletion of other subunits composing the receptor. For example, mice lacking NR2A subunit are exhibited schizophrenia-like abnormal behaviors such as hyperlocomotion in a novel environment, which is reversed by administration of antipsychotic drugs haloperidol and risperidone (60), and deficits in spatial learning in the Morris’s water maze test (61, 62). Further studies revealed that mice lacking the NR2A subunit also showed both reductions in anxiety-like and depression-like behaviors (63).

### 4.2. DISC1

The DISC1 gene was originally identified in a Scottish pedigree in which a balanced translocation involving chromosomes 1 and 11 (1;11)(q42.1;q14.3) was strongly linked to psychopathology including schizophrenia, major depressive disorder and bipolar disorder. DISC1, a synaptic protein expressed in early developmental stages, plays an important role in neurogenesis, neuronal migration, and synaptic plasticity of prenatal and post-

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Sociability</th>
<th>Motivation/Anhedonia</th>
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<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR1</td>
<td>Social interaction ↓</td>
<td>Sucrose preference ↓</td>
<td>Social memory, Spatial working memory ↓</td>
<td>Locomotor activity ↑ Prepulse inhibition ↓</td>
<td>57 – 59</td>
</tr>
<tr>
<td>DISC1</td>
<td>Social interaction ↓ (Q31L mutant)</td>
<td>Forced swimming, Sucrose preference ↓ (Q31L mutant)</td>
<td>Working memory ↓</td>
<td>Locomotor activity ↑ (L100P mutant) Prepulse inhibition ↓</td>
<td>66, 67, 69</td>
</tr>
<tr>
<td>NRG1</td>
<td>Social interaction ±</td>
<td>ND</td>
<td>Contextual learning, Working memory ↓</td>
<td>Locomotor activity ↑ Prepulse inhibition ↓</td>
<td>71 – 73, 75</td>
</tr>
<tr>
<td>Dysbindin</td>
<td>Social interaction ↓</td>
<td>ND</td>
<td>Reference memory, Novel object recognition ↓</td>
<td>Locomotor activity ↑ Prepulse inhibition ↑</td>
<td>79, 80</td>
</tr>
<tr>
<td>Reelin</td>
<td>Social interaction ↓</td>
<td>ND</td>
<td>Working memory ↓</td>
<td>Locomotor activity ↑</td>
<td>85, 87</td>
</tr>
</tbody>
</table>

↑: Significant increase, ↓: Significant decrease, ±: No significance, ND: No data in references

Table 3. Behavioral alterations in genetic animal models of schizophrenia
nated neurons. Since there is a positive linkage between the mutation of DISC1 gene and the enhancement of susceptibility for schizophrenia (64), it is thought that DISC1 is implicated in the onset of schizophrenia (65).

Several behavioral changes in many DISC1 mutant mice mimic partially symptoms of schizophrenia. Transgenic mice with the DISC1 L100P amino acid mutation (66) and expressing constitutive CaMK-dominant negative (DN) DISC1 (67) showed spontaneous hyperlocomotion in a novel environment, whereas other some transgenic mice did not show altered locomotor activity (e.g., DISC1 Q31L transgenic mice in ref. 66 and females in ref. 68 and 69). The DISC1 L100P transgenic mice (66), constitutive DISC1 knockdown mice (67), and in utero DISC1 knockdown mice (69), but not mice expressing inducible CaMK-DN DISC1 (68), also showed subtle impaired PPI that is attenuated by the antipsychotic drugs haloperidol and clozapine (66). Working memory in the DISC1 L100P transgenic mice (66) and in utero DISC1 knockdown mice (69) was diminished, while spatial memory in the Morris’s water maze test, cognitive function in the novel object recognition test, and contextual learning in the fear-conditioning test were not impaired in various DISC1 mutant mice (66 – 68, 70). Similar phenotypical discrepancies were observed in sociability: one study reported the reduction of social interaction in the DISC1 Q31L transgenic mice (66), whereas other studies reported no change in social interaction (67, 68). In addition, among several DISC1 mutant animals, the DISC1 Q31L transgenic mice only exhibited anhedonia in the sucrose preference test (66). These findings suggest that characteristic behavioral phenotypes in DISC1 mutant mice depended on the method used for gene manipulation to produce the malfunction of DISC1.

4.3. NRG1 and ErbB-4

NRG1 and its binding receptor EebB-4 are also thought to reflect on the onset of schizophrenia (71, 72). NRG1 is a growth factor containing an epidermal growth factor-like domain and has at least six major isoforms, types I – VI, with different N-termini by alternative splicing. Activation of EebB-4 by NRG1 is critically involved in brain development such as neuronal migration and neurite outgrowth. Some NRG1 isoforms are abnormally expressed in patients with schizophrenia and EebB-4 gene is identified as being disrupted by micro-deletions or microduplications in patients with schizophrenia (71, 72).

Several NRG1 mutant mice were generated by expression control of all or each NRG1 isoform. Heterozygous all NRG1 isoforms knockout mice had some abnormal behaviors including spontaneous hyperlocomotion and impaired PPI. The former, but not the latter, abnormality was reversed by the antipsychotic drug clozapine (73). Moreover, NRG1 isoforms types I – III knockout mice showed selective deficits in social novelty, but not normal social interaction and spatial learning (70, 74). On the other hand, heterozygous EebB-4 knockout mice displayed reduced spontaneous locomotor activity (75) and reduced social interaction (76).

4.4. Dysbindin

Linkage mapping study of Irish multiplex families has suggested that dysbindin (Dystrobrevin Binding Protein 1: DTNBP1) is implicated in patients with schizophrenia (77). Furthermore, postmortem studies reported that reduced expression of dysbindin in the dorsolateral prefrontal cortex and hippocampus occurs in patients with schizophrenia, and clinical genetic studies in schizophrenia indicated associations between dysbindin and the negative symptoms of schizophrenia symptoms (78, 79). Dysbindin is a synaptic protein to regulate exocytosis and vesicle genesis in synaptic neurotransmitter release (79, 80).

Dysbindin knockout mice showed increased spontaneous locomotor activity, reduced social contact in a dyadic social interaction test (74, 81), impaired spatial reference memory, and declined object recognition, but enhanced contextual learning (70, 81). Unfortunately, there is no data on the reversal effect of antipsychotic drug on behavioral alterations in dysbindin-knockout mice.

4.5. Reelin

“Reeler” mice are a spontaneous mutant animal with heterozygously disrupted reelin gene. Reelin, an extracellular matrix glycoprotein, functions to regulate the processes of neuronal migration and neuronal positioning in brain development and is involved in synaptic formation and plasticity in the adult brain (82). Reelin mRNA is dramatically reduced in the hippocampus and prefrontal cortex of patients with schizophrenia, along with a decrease in and GABA synthase glutamic acid decarboxylase 67 mRNA, in these individuals (83).

Although the reeler mice showed normal social interaction and working memory (84, 85), mice with epigenetic hypermethylation of reelin promoter region by methionine exhibited reduced social interaction in a novel environment (86). Moreover, the reeler mice showed reduced spontaneous locomotor activity responding to treatment with olanzapine, deficits in working memory, and impaired PPI, but no alteration of spatial learning (85, 87).
5. Combination animal models

The pathogenesis of schizophrenia has multiple epidemiological risks and also involves their interactions (88). Approaches to develop a more useful animal model of schizophrenia have been demonstrated by the interactions between susceptibility genes and environmental factors after the birth (89, 91, 92). The combination of gene mutation and viral infection before birth, i.e., prenatal treatment with polyI:C in the inducible DISC1 knockout mice induced more apparent phenotypes for animal models of schizophrenia, such as reduced social interaction, depressive-like behavior, and anxiety-like behavior, compared with either single operation (89). In addition to combination of polyI:C and DISC1, there is the combination of gene mutation and exposure to social stress. The social defeat stress, in which the animal is chronically subjected to physical or non-physical signs from a more aggressive animal, is used to develop an animal model of major depressive disorders. Animal exposed by social defeat stress shows social withdrawal and anxiety-like behavior (90). The exposure to social defeat stress for 3 weeks of adulthood in the DISC1 L100P and Q31L transgenic mice, respectively, has been investigated (91). The former resulted in additive and synergistic effects on social withdrawal and anxiety-like behavior in each single operation and disappearance effect on hyperlocomotion and PPI deficit induced by the genetic manipulation. The latter failed to cause any abnormality in each single operation. Thus, the combination animal models seem to possess more appropriate phenotypes of schizophrenia by gene-environment interactions, but it is necessary to confirm their predictive validity via evaluating the responses to available antipsychotic drugs.

6. Conclusion

It is the ideal that the animal models used to develop therapeutic drugs possess all of the face, construct, and predictive validities for the targeted clinical disorder. In animal models for schizophrenia, appropriate behavioral symptoms, neurobiological alterations, and similarity of therapeutic efficacy, such as hypersensitivity to stress, social withdrawal, cognitive deficits, mesolimbic DAergic hyperfunction, cortical glutamatergic hypofunction, and neuronal dysconnectivity in the prefrontal cortex-hippocampus pathway and improvement by antipsychotic drugs, should be appear after puberty. However, as mentioned in this review, the past studies have hardly demonstrated the depressive aspects in animal models associated with the negative symptoms of schizophrenia, excluding social interaction. The similarity of the clinical efficacy of antipsychotic drugs has been also been little inspected. Thus, the previous studies would be insufficient to understand the heterogeneous neurobiological foundations of schizophrenia in face and predictive validities, in spite of the fact that we did not describe construct validity including neurochemical and structural defects in this review. Therefore, in many animal models of schizophrenia, particularly the genetic models, additional studies to demonstrate the three validities are necessary. In addition, the development of suitable animal models for heterogeneous neurobiological disorders such as schizophrenia would be difficult in a single management method in pharmacology, environmental epidemiology, and gene technology. Therefore, by combining pharmacological, neurodevelopmental, and genetic manipulations, it may be able to prepare a more appropriate animal model, in which the weakness in each separate model would be compensated for. The application of combination approaches to schizophrenia study would enhance the knowledge and reliability of preclinical research.

In summary, until there is a better understanding of the neurobiological mechanisms underlying the negative symptoms of schizophrenia, we cannot remove the uncertainty regarding the preclinical studies for the development of new therapeutic drugs. The development of new preclinical animal models for negative symptoms and new behavioral tests with greater translational relevance to the negative symptoms would be a major breakthrough in the understanding of the neurobiological processes of clinical symptoms.

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