Advances in Schizophrenia Research

Chapter 3

Animal Models of Schizophrenia: An Update

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Abstract

Schizophrenia is one of the most intriguing human diseases because it affects the most complex functions emanating from the brain. Bleuler defined the term in 1911 as a psychotic disorder characterized by four primary symptoms: "abnormal associations, autistic behavior and thinking, abnormal affect, and ambivalence" [1]. Developing an animal model that is expected to mimic deficits in such functions is an achievement in itself. However, it is an absolute necessity first because the pathology is devastating for patients and their families and, secondly and consequently, because there is a crucial the need to find more efficient treatments. Until now, many animal models have been developed by the manipulation of a sole factor (pharmacological, genetics, environmental etc.), while some others have tried to manipulate two of three factors. In this review we describe the main rodent models used in scientific research until now, and the new models more recently developed. Most of them can be considered as successful to trigger positive-like symptoms in animals (increased locomotion) and are useful to assess effects of antipsychotics (locomotion decreased to control level). Some of them also induce negative-like symptoms (decreased social interaction and anhedonia) and/or cognitive deficits (altered memory, attention, and sensorimotor gating), conferring an added-value to assess effects of existing psychotics and developing new ones.

Keywords: psychosis; rodents; stress; phenotype; social behavior; animal models

1. Introduction

Developing an animal model of schizophrenia is a real public health concern given the poor efficiency of current treatments, besides representing a considerable challenge. Indeed,

how assessing and validating an animal model of a psychotic disorder which is mainly diagnosed under certain guidelines in human? Until now, communication modes found in laboratory animals are far away from that of humans, and not really understood by us. Moreover, difficulty is increased owing to unknown etiology of the disease. Indeed, the biggest challenge is mimicking the disease in animals without knowing how it appears in humans? Lastly, and even more trickily, the huge variability of the symptoms encountered in patients induces a lack of validity criteria on which lean new preclinical developments. We are confronted to a disease which gathers symptoms that are not found in all patients, and which are, in a same patient, not permanent and constant but can evolve with time and evolution of the pathology. Despite these major obstacles that have to be kept in mind, several important advances have been made in neurosciences and psychiatry to understand how to provoke in animals behavioral alterations that are similar to some of those observable in patients with schizophrenia. In this review, we will relate main animal models used in experimental research on schizophrenia, remembering that there is no ideal model, as reported by [2]: "Any model will inevitably be a partial one" [2]. Each animal model, according to different criteria of validity, can however bring different contributions to the understanding of the human disease with the aim to improve treatments. Several approaches have been performed to model schizophrenia in animals (pharmacological interventions, brain lesions, environmental modifications, genetic alterations, etc.), most of the time independently, i.e. as a sole factor. We here discuss about the attempts that have been made to combine two or three factors to better fit with the multifactorial causes of the disease, and will relate animal species that are more and more used (e.g. fishes) to model behavioral alterations that can, at least partly, mimic some symptoms of the disease.

2. Altering Neurotransmission

The use of pharmacological tools to alter neurotransmission functioning is one of the first method used to design animal models of schizophrenia. Four neurotransmission systems have been targeted: dopamine, glutamate, serotonin, and endocannabinoïds. Dopamine is a monoamine mainly synthetized by substantia nigra neurons and released in cortex, striatum, and limbic system. It is involved in the control of motor and cognitive functions, reward and motivation and its dysfunction has been related to Parkinson's disease and psychosis. Based on the hypothesis of an increase in dopaminergic transmission system, the amphetamine model has been tuned and characterized in 1950's. Administered acutely in rodents, amphetamine induces an increase in locomotor activity, stereotyped movements and a deficit in sensorimotor gating; those effects are respectively considered to mimic positive and negative symptoms of the disease [3,4]. Thereafter, the absence of obvious cognitive deficits and the brevity of the effects lead to the preference of chronic administration of amphetamine and of other stimulating drugs targeting the dopaminergic system (L-DOPA, carbidopa, dopamine precursor [5] for example). Those models based on chronic administration are sensitive to haloperidol or clo-

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zapine, revealing a good predictive validity. After the dopamine story, the modulation of glutamatergic system arose. It was built on the observation of behavioral troubles after a misuse of glutamatergic drugs (non-competitive N-Methyl D-Aspartate NMDA receptors antagonists) such as phencyclidine (PCP) or ketamine by recreational users. Such observations motivated preclinical research to focus on this particular class of glutamatergic agents (cited above and others like dizocilpine, also called MK-801) to develop new animal models of psychosis, which was moreover reinforced by data reporting that a hypofunctioning of the glutamatergic system was the initial alteration in schizophrenia [6]. Glutamate neurotransmitter system is largely spread in the brain and is the most important excitatory cortical system. Although it is absolutely mandatory for a normal functioning of the brain, its over-activation is toxic for neurons. In rodents, the administration of the NMDA glutamate receptors antagonist, phencyclidine (PCP), triggers an increase in locomotor activity, a social withdrawal, an alteration in prepulse inhibition and in cognitive performances. Interestingly, social deficits can be alleviated by haloperidol [7], and cognitive deficits can also be counter-balanced by several atypical antipsychotics, such as clozapine [8], cariprazine or risperidone [9].

From these times, modulation of other neurotransmission systems has been considered, and particularly that of the serotonergic system. Serotonin is a monoamine synthetized by Raphe nuclei neurons and released in several brain regions. It has modulatory effects on other neurotransmission systems. This system is involved in the effects of hallucinatory drugs such as lysergic acid diethylamide (LSD), mescaline or psilocybin, which are agonist of serotonergic receptors, particularly 5-HT2A and 5-HT2C receptors. Hallucinations are one of the core positive symptoms of schizophrenia but are not easy to investigate in a reproductive manner in animals. Nevertheless, serotonergic agonists provoke behavioral effects in animals (sensorimotor gating deficits, altered locomotor activity, working memory, social behavior), mimicking psychotic-like symptoms, some of them, like head-twitch, being probably related to hallucinations.

More recently, the endocannabinoid system has been the object of several preclinical studies. The endocannabinoid system comprises lipid-derived messengers that act on G-protein-coupled cannabinoid receptors named CB1 and CB2. Its involvement in many different brain functions has been shown, such as motor coordination, memory, pain and social behavior [10]. The use of cannabinoid is model of psychosis mostly concerns the administration of either the most active component of the cannabis, the tetra-hydro-cannabinol (THC) or other cannabinoid agonists, during adolescence in rats and mice. Such a treatment provokes in adults several behavioral effects such as memory deficits, alteration in sensory filtering, in social relations, depressive-like symptoms, etc [11]. The sensitivity of this model to current antipsychotics remains to be characterized.

3. Perinatal Interventions

3.1. Immunological and pharmacological interventions

In the 80's, Mednick et al. suggested the existence of a link between maternal infection by virus type A2*influenza* and an increased risk for psychosis [12]. From these times, it has been specified that the inflammatory reaction induced by virus infection during pregnancy could induce disturbances in the development of the fetus, and particularly in the central nervous system, that could in turn be the cause of later psychiatric troubles. These clinical observations have been the starting point of new animal models based on the induction of an immune system reaction during pregnancy. The reaction is triggered either by infectious agents (virus *influenza*) or by other agents (for example, polyinosinic:polycytidylic acid) to trigger an immune system reaction which is similar to that of an infection. A behavioral phenotype characterized by an increased sensitivity to amphetamine, reduced social interactions, and altered cognitive functions appears in adult animals, together with anatomical and functional alteration of the brain [13,14]. In the same way, more invasive animal models have been developed, and consisted in brain lesion before or shortly after birth. One of the procedures consists in the injection of a toxin in new-born animals to affect the main brain structures involved in the pathology. One of the most frequently used models describes a bilateral injection with ibotenic acid inside the ventral hippocampus, which provokes a disconnection of the hippocampus during an important maturational period of brain development (see for review [15]). Besides brain alterations caused by the injection in adult brains (ventral hippocampus atrophy, enlargement of ventricles, frontal and temporal lobes alteration, etc.), the lesion also induces a dopaminergic hypersensitivity, and behavioral alterations such as memory deficits (affecting working memory and spatial learning) and a decrease in social interactions [15]. One of the important advantages of this model is the apparition of deficits at the puberty period, those deficits being related to the triad of symptoms of the disease i.e., positive and negative symptoms and also cognitive deficits. Other models have been tested, using other pharmacological agents, like for instance the anti-mitotic agent methylazoxymethanol acetate (MAM) during gestation (embryonic day 17), with the aim to alter a specific period of fetal brain development [16]. The treatment blocks cell proliferation and induces neuro-anatomic modifications that are particularly significant in hippocampus and frontal cortex. On a behavioral point of view, several deficits are present in adulthood in social interactions, sensory gating, learning, and a hypersensitivity to amphetamine [17].

3.2. Models based on obstetric complications

Epidemiological data have revealed that complications that can occur during birth constitute a risk factor for several mental disorders. Obstetric complications that have been related to schizophrenia are hemorrhage, prematurity, growth retardation, and asphyxia [18]. Some of

these factors have been there after used to develop new murine models, and for instance, one of them consists in immersing the whole uterus containing full term fetuses during 20 minutes at the temperature of 37°C [19]. Such a procedure induces an asphyxia that is expected to mimic a lack in blood oxygenation and therefore asphyxia. After the immersion, pups are manually extracted from the uterus, and adopted by another female. In this model, alteration of synaptic ultrastructure of the prelimbic cortex is observed, but also a decrease in brain levels of neurotrophic factors, a strong astrocyte reaction, a decrease in long-term potentiation in the hippocampus, together with behavioral troubles (spatial and non-spatial memory) [19,20]. Although the procedure takes into account an important issue originating from epidemiological data, the model has to be used with prudence given that rodents are born with a high degree of brain immaturity, even more than human babies, therefore their brain at the date of birth and consequently at the date the procedure is applied is not at the same degree of development than the human baby.

3.3. Models based on environmental events

Many hypotheses suggested that stress, and more precisely hyper-reactivity to stressors, is the central element that can explain the appearance of psychosis. It is proposed that in an individual displaying a high sensitivity to stress (from a potentially genetic origin or even after a brain lesion for instance), the occurrence of another source of stress will induce a hyperfunctioning of the hypothalamic-pituitary-adrenal (HPA) axis, that will induce a high level of cortisol release. One of the consequence of such an increase is HPA functioning is an alteration of dopamine transmission, one of the landmark of brain modification in schizophrenia [21]. This environmental hypothesis gives explanations about the increase in psychosis cases in populations that have suffered early in life from wars, migrations, etc. The effects of stress on brain networks could constitute one of the factors rendering the brain more fragile to further events, and even represent vulnerability markers [5,22]. The models based on the stress vulnerability consist in submitting animals to stressing situations in the early life (pre- peri- or post-natal period). The most frequently used models involve stress of gestating mothers, by contention, isolation, or unpredictable changes of the environment or stress of the pups by maternal separation. After a period of latent fragility running till adolescence, these stressing manipulations induce behavioral alteration such as sensory gating deficit, depressive and anxiety-like behavior, and increase in impulsivity, classically described as mimicking symptoms of psychosis.

4. Genetic Models

The influence of genes, and therefore heritability, in schizophrenia has been pointed out several years ago according to families and particularly twins' studies, and has been more deeply assessed thanks to advances in molecular biology. Genome-wide association studies have then revealed a large number of risk loci ("Biological insights from 108 schizophrenia-

associated [23]). Some of these loci are associated to dopamine and glutamate dysfunction (justifying the validity of pharmacological models cited above), other underlining the involvement of the major histocompatibility complex. Even if these observations are quite recent, genetic manipulations in the mouse, who share 99% homologous genes with Human, have been initiated about 20 years ago with the aim to model several pathology, and in particular, psychiatric diseases. Such manipulations of the genome can be achieved either by inserting genes sequences (that will be then transmitted to the offspring) or by mating animals that spontaneously express a particular gene (mutation). Concerning schizophrenia, around 100 mice models are now available [24]. One of the first genes which has been involved in schizophrenia is DISC for *Disrupted in Schizophrenia*. Discovered in a Scottish family comprising several individuals displaying psychiatric troubles (schizophrenia, bipolar disorders, depression), this gene codes for the protein DISC, involved in several cellular functions such as proliferation, migration, neuronal and axonal growth etc.). Based on these observations, several animal models have been generated (mutation, deletion or truncation of the DISC gene), and their characterization permitted to point out similarities with pathophysiological aspects of the human disease (behavioral deficits, brain alterations). Currently, the models which are the most often used for animal research on schizophrenia through a genetic approach are those targeting the dopaminergic system [25], the glutamatergic system, and those affecting neuregulin, reelin, dysbindin, etc [2,26].

5. Combination of Factors

Experimental manipulations described in this chapter mainly concerns a sole factor, either pharmacological, environment, or genetic. The huge complexity of schizophrenia etiology, even if still unknown, suggests that a sole factor cannot be responsible of the diversity of clinical symptomatology encountered between patients. Consequently, and given that the pathology is undoubtedly multifactorial, the animal modeling has to take into account several combined factors. It is indeed more and more difficult to consider that new treatments will be discovered in models engaging a unique factor. From about 10 years, efforts have been made in this direction and literature reports several studies using combined models: genetic + pharmacological agents, genetic + environment, etc. [20,27]. As an example, Giovanoli et al. showed that the combination of maternal infection with a stress procedure during puberty induced synergistic effects of behavioral and neurochemical deficits [28]. In the sense that the initial maternal infection rendered the pups more vulnerable to a later stressing event, the latter being applied during a very precise period to observe the synergy. On another point of view, it has been shown that particular genetic modifications, known to be involved in schizophrenia could induce a higher sensitivity to maternal infection (DISC-1 [29,30] and *Neuregulin* 1 [31]).

6. A New Field of Research in Psychiatry: The Microbiota

From 10 years ago, the role of communication between brain and gut has been emphasized (brain-gut axis). The initial idea was to put in relation psychosis with dysfunction of the immune system. There after, focus has been redirected to the gastrointestinal system. It is postulated that a defective gastrointestinal barrier would let microbes, bacteria and toxins pass into the blood circulation. This intrusion would trigger not only a global inflammatory reaction, but would also alter developing and functioning of the brain [32]. This field of research opens new doors in the development of new therapeutic strategies but also gives rises to the understanding of resistance phenomena to medication (10 to 30% of patients). In animals, it has been shown that the composition of microbiota could influence behaviors like memory, exploration, anxiety, but also brain levels of BDNF, dopamine, noradrenaline, serotonin and corticotropin hormone [33]. It has also been reported that the development of HPA axis is closely influenced by the early exposure to microbes [34]. Although it is still the early beginning of investigations on brain-gut axis role in psychiatric disease, this field of research could bring in the next future new animal models of schizophrenia, and possibly new treatments.

7. Conclusion

Even if important advances have been performed in the improvement of antipsychotic treatments, still none of them can reverse all the symptoms found in the disease and even less totally cure the patients. Efforts must be pursued to create animal models which mimic the disease with the best validity criteria namely: face, construct, and predictive validity. The existence of a perfect isomorphic model is illusory so far, not only because of the variability of the symptomatology encountered in clinics, but also because of the interspecies differences. To decrease the length of this latter gap, researchers have developed methods (or applied those from rodents) to induce schizotypic-like behaviors in non-human primates. They are indeed closer to humans from a phylogenetic point of view, and therefore display more similarities in brain anatomy and functioning, together with a broader behavioral repertoire, that gives access to more complex brain functions than in rodents. However, given costs and ethical reasons of using such animal models, there are very scarce in literature. Conversely, and to allow a large screening of new molecules within a short time, but also genetic manipulations, other species have been used to model psychiatric disease, and now a days zebra fish models are increasingly used.

Because they require simplification, animal models play an essential role in the understanding of several components of schizophrenia. In turn, their construction can evolve gradually with the knowledge acquired from day to day. Finally, these models are absolutely essential from an ethical point of view if we want to develop new therapeutic strategies. However, the predictive validity that must display an animal model can appear as one of the most

puzzling given that still, there is no efficient treatment for negative and cognitive symptoms.

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