



BRAZILIAN JOURNAL
OF MEDICAL AND BIOLOGICAL RESEARCH

www.bjournal.com.br

ISSN 0100-879X
Volume 45 (3) 179-290 March 2012

BIOMEDICAL SCIENCES
AND
CLINICAL INVESTIGATION

Braz J Med Biol Res, March 2012, Volume 45(3) 179-186

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The Brazilian Journal of Medical and Biological Research is partially financed by



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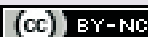
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Animal models of prenatal immune challenge and their contribution to the study of schizophrenia: a systematic review

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Abstract

Prenatal immune challenge (PIC) in pregnant rodents produces offspring with abnormalities in behavior, histology, and gene expression that are reminiscent of schizophrenia and autism. Based on this, the goal of this article was to review the main contributions of PIC models, especially the one using the viral-mimetic particle polyribonucleosinic-polyribocytidylic acid (poly-I:C), to the understanding of the etiology, biological basis and treatment of schizophrenia. This systematic review consisted of a search of available web databases (PubMed, SciELO, LILACS, PsycINFO, and ISI Web of Knowledge) for original studies published in the last 10 years (May 2001 to October 2011) concerning animal models of PIC, focusing on those using poly-I:C. The results showed that the PIC model with poly-I:C is able to mimic the prodrome and both the positive and negative/cognitive dimensions of schizophrenia, depending on the specific gestation time window of the immune challenge. The model resembles the neurobiology and etiology of schizophrenia and has good predictive value. In conclusion, this model is a robust tool for the identification of novel molecular targets during prenatal life, adolescence and adulthood that might contribute to the development of preventive and/or treatment strategies (targeting specific symptoms, i.e., positive or negative/cognitive) for this devastating mental disorder, also presenting biosafety as compared to viral infection models. One limitation of this model is the incapacity to model the full spectrum of immune responses normally induced by viral exposure.

Key words: Schizophrenia; Animal models; Neurodevelopment; Prenatal immune activation; Poly-I:C

Introduction

Schizophrenia is a devastating lifetime mental illness that affects ~0.5-1.0% of the population. The disorder has a heterogeneous course and consists of several symptom dimensions, namely, positive (hallucinations and delusions), negative (e.g., avolition), affective (e.g., depression), and cognitive (e.g., memory deficits) (1,2). Schizophrenia is responsible for 15% of first medical appointments in psychiatric clinics, as well as 30% of the occupation of psychiatric wards. The disorder generates high financial costs to health services and to society (3-5).

Epidemiological studies provide compelling evidence

that perinatal factors, such as exposure to environmental insults, including maternal exposure to stress, infections and/or immune activation, maternal malnutrition, as well as obstetric complications (e.g., hypoxia), confer a high attributable risk to schizophrenia (6-8).

According to the classic neurodevelopment hypothesis of schizophrenia, relevant genes involved in brain development would be susceptible to gene-environment interactions - mainly during the second gestational trimester - that would lead to disruption of brain development trajectories, limbic disorganization and neurochemical (e.g., monoaminergic)

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Received October 8, 2011. Accepted February 10, 2012. Available online March 9, 2012. Published March 19, 2012.

imbalances (9,10).

The neurodevelopment theory of schizophrenia receives further support from several brain autopsy and neuroimaging studies (11-14). Minimal neuronal cytoarchitecture abnormalities are observed in the brain of schizophrenia patients, with the absence of massive glial proliferation (gliosis), suggesting that damage during brain development contributes to the pathophysiology of this particular disorder (15,16). Several insults that occur during early neurodevelopment phases may impair late postnatal cerebral maturation, ultimately leading to an increase in vulnerability to schizophrenia. According to Nestler and Hyman (17), at least four elements can play an important role in the postnatal brain maturation of schizophrenic individuals: i) maturation of GABAergic interneurons; ii) pruning of glutamatergic synapses; iii) maturation of dopaminergic projections (mainly mesocortical), and iv) differentiation/myelination of oligodendrocytes. All of these elements are associated with the action of novel proteins such as Neuregulin-1 (NRG1) and Disrupted-In-Schizophrenia-1 (DISC-1) (17).

Several experimental models are currently available to test hypotheses about the pathophysiology of schizophrenia (17,18). However, this is a remarkably multifaceted disorder with complex clinical presentations that pose significant challenges to the development of reliable animal models. Therefore, efforts to replicate the schizophrenic syndrome in animals are far from complete, leading to a certain degree of skepticism in the scientific community (19).

The animal models developed for the study of schizophrenia are: i) drug-induced models (e.g., NMDA receptor antagonists - ketamine and apomorphine); ii) lesion-induced preparations (e.g., ventral hippocampal lesion); iii) genetic (e.g., alpha-CaMKII knockout mice), and iv) neurodevelopment approaches (e.g., human influenza virus and rat maternal malnutrition).

Recently, research efforts have been directed at the development of the so-called neurodevelopment models of schizophrenia. Particularly, the prenatal immune challenge (PIC) has generated substantial advances in the field, as it might longitudinally mimic several behavioral and neurobiological aspects of the disorder (20,21).

The objective of the present study was to perform a systematic literature review of relevant articles about PIC schizophrenia models and to discuss their main contributions to the elucidation of the pathophysiology and treatment of schizophrenia. Since there is currently a diversity of PIC models, we decided to focus on the *in vivo* model of PIC induced by polyriboinosinic-polyribocytidylic acid (poly-I:C), investigating its contributions to the understanding of the biological bases and treatments of schizophrenia.

Methods

Articles published in English from May 2001 to October 2011 were searched in relevant web datasets (PubMed,

SciELO, LILACS, PsycINFO, and ISI Web of Knowledge). Search terms included combinations of the following: 'schizophrenia and prenatal immune challenge'; 'prenatal immune challenge and animal model'; 'poly-I:C and schizophrenia', and 'schizophrenia and animal model'.

The search covered original articles. However, reference lists of reviews and original articles were hand-searched for additional references. Conference abstracts were not included. Two authors performed the search independently (DSM and DPA) and relevant articles were selected by three authors (AFC, DSM, and DPA). To prevent biases, inclusion criteria were pre-specified as the following: i) development of schizophrenia by the administration of poly-I:C injection during pregnancy in rodents; ii) experimental data of at least one endophenotype for schizophrenia (for more details, see Gottesman and Gould (22) and Amann et al. (23), and iii) inclusion of a control group in the study design. The search retrieved 45 articles, 30 of which contemplated the inclusion criteria.

To facilitate the discussion of the main findings, this article was organized according to the following topics: 1) general presentation of the models and their contributions to the understanding of the etiology of schizophrenia; 2) contributions of the poly-I:C model to the understanding of the biological bases of schizophrenia, and 3) contributions of the poly-I:C model to the treatment of schizophrenia.

Discussion

General presentation of the models and their contributions to the understanding of the etiology of schizophrenia

The animal PIC models developed to date are: experimental models of virus exposure, such as human influenza (10) and Borna virus (24), as well as those that employ cytokine-releasing agents, such as lipopolysaccharide (LPS) bacterial endotoxin (25) or the viral mimetic particle poly-I:C (26).

The prenatal influenza infection model seems to be useful in the investigation of known epidemiological relationships between exposure to prenatal viral infection and the heightened risk of schizophrenia-related psychoses in the offspring (27). This model mimics the behavioral and morphological changes that follow schizophrenia, since it relies on one of the most well-known environmental risk factors implicated in its etiology (8,28). The influenza infection model has good face and construct validity for schizophrenia-like brain and behavioral pathology.

The cytokine-release models were developed to measure how maternal and/or fetal cytokine imbalance would act as core mediators of the association between maternal infection and the development of postnatal behavioral and cerebral pathology (29). These models are induced through the prenatal exposure of animal rodents (female rats or mice) in specific gestational stages to diverse immunologic

stimuli, such as viruses (24), poly-I:C (30), or LPS (31). Such models are having a great impact on the search for possible neurodevelopmental and neuroimmunologic causes of complex human disorders like schizophrenia and autism (32). Therefore, PIC models might guide the identification of underlying neurobiological mechanisms enrolled in gene-environment and environment-environment interactions relevant to the etiology of schizophrenia (33,34).

Contributions of the poly-I:C model to the understanding of the biological bases of schizophrenia

Poly-I:C is a synthetic analogue of the viral double-stranded RNA. The injection of this compound leads to the activation of pro-inflammatory cytokines for a limited time by the activation of Toll-like receptors 3 (TLR3) (35). Thus, poly-I:C is a strong interferon (IFN) inducer, mostly of the IFN- α and IFN- β types (36,37). The injection of poly-I:C mimics the acute phase response to a viral infection

Animal studies suggest that the prenatal injection of poly-I:C elicits subcortical dopaminergic hyperfunction and cognitive impairment in the offspring, representing a neurodevelopmental animal model of schizophrenia (38).

Within the spectrum of behavioral changes, prenatal poly-I:C injections have been reported to induce severe changes in various schizophrenia-related endophenotypes, such as: i) sensorimotor gating deficits in adult rat offspring (39-41); ii) latent inhibition disruption only in adulthood (42); iii) postpubertal emergence of enhanced sensitivity to MK-801 and amphetamine-induced hyperlocomotion (43,44), and iv) morphofunctional alterations in the hippocampus and the entorhinal cortex in adult offspring, consistent with the well-documented mesolimbic dopaminergic and temporolimbic neuropathology of schizophrenia (43). Rats injected with poly-I:C develop anxiety-related behaviors, as well as impaired object recognition memory (45) and social behavior dysfunction, when compared to controls (40). The occurrence of behavioral endophenotypes mainly in adulthood is consistent with the later onset of schizophrenia symptoms seen in clinical settings (46).

Prenatal poly-I:C immune challenges in the middle and late gestational phases lead to a quantitative increase in mesencephalic dopaminergic neurons in the fetal brain (30). This effect is followed by several gene expression changes related to the developmental course of dopaminergic neurons, including the sonic hedgehog (ShhN) and fibroblastic growth factor 8 (FGF-8), as well as transcription factors like nuclear receptor related 1 protein (Nurr1) and pituitary homeobox 3 (Pitx3) (30,47). The ShhN and FGF-8 genes, as well as the transcription factors Nurr1 and Pitx3, are essential for the generation, differentiation, and maintenance of midbrain dopamine (DA) cells during embryonic development (48).

Recently, following prenatal immune activation, a decrease in NMDA receptor function and elevated basal extracellular glutamate in the prefrontal cortex was demon-

strated. Together, these are two key features of the NMDA glutamate receptor hypofunction model of schizophrenia, occurring during late adolescence (49).

Maternal exposure to poly-I:C significantly increased tumor necrosis factor- α (TNF- α) protein levels in the maternal plasma and placenta, with no alterations in fetal brain, while brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) levels decreased in the placenta and fetal liver/spleen. In the neonatal whole brain, TNF- α was decreased while NGF and BDNF were unchanged at 1 and 7 days after birth (50). These changes represent potential mechanisms by which maternal infection can alter neuron development and increase the risk for neurodevelopment disorders.

It is important to state that this model allows the recognition of critical gestational periods of exposure to PIC that specifically contribute to the development of postnatal changes related to the onset of schizophrenia. The gestational period of rodents is 21 days on average. The major deficits in pre-pulse inhibition (a behavioral test to evaluate sensorimotor gating, one of the most replicated schizophrenia endophenotypes) were found mainly in animals submitted to PIC on the 9th gestational day (GD9) (30,51-53), which is equivalent to an early/middle gestational exposure.

According to Meyer et al. (20), the early/middle gestational exposure of mice (GD9) impaired sensorimotor gating and decreased dopaminergic D₁ receptors located in the pre-frontal cortex (PFC) of the offspring, whereas the exposure in the late gestational phase (GD17) impaired working memory and enhanced locomotor changes induced by the NMDA receptor antagonist dizocilpine. Furthermore, a decrease in the expression of subunit 1 of NMDA hippocampal receptors was found. However, the same study has shown that the potentiation of amphetamine-induced hyperlocomotion and a reduction of PFC neurons that expressed reelin and parvalbumin (GABAergic neurons) occurred independently of the prenatal exposure period. Such exposure periods correspond to the end of the first trimester (GD9) and middle/late phase of the second trimester (GD17) in humans (54). Thus, these data demonstrate that the exposure on GD9 generates phenotypes predominantly related to positive symptoms, whereas exposure on GD15 or GD17 is more suited for the study of behavioral correlates of negative/cognitive symptoms, since adult offspring also presented histopathological abnormalities in the parahippocampal consistent with evidence that this is a point at which the parahippocampal region undergoes neurogenesis (42,43) and also in the cerebellum (26). A retarded myelination and axonal abnormalities in early postnatal stages caused by maternal immune activation were also detected (55) (Figure 1).

Available evidence suggests, therefore, that the PIC in early/middle gestational phases can lead to dysfunctions over multiple neuropsychological domains (e.g., sensorimo-

tor gating and spatial working memory) (20). This symptomatic heterogeneity may be associated with the impairment of fundamental neurodevelopmental processes, such as cellular proliferation and differentiation triggered by early inflammation in the fetal brain, leading to abnormalities in subsequent cellular migration, synaptogenesis and maturation of synapses (52,56). Indeed, inflammatory events occurring in the initial phase of fetal brain development can result in even more severe damage when compared to exposure that occurs later in gestation, culminating with multiple postnatal dysfunctions and symptomatic heterogeneity, as observed in schizophrenia and autism (57).

In addition to the findings of deleterious effects of direct inflammatory challenges, results exist showing that healthy control animals reared by foster immune-challenged mothers are associated with the occurrence of learning disabilities of early and late onset (58). This suggests that maternal stress during pregnancy somehow affects postpartum maternal behavior and underscores the importance of future research along this line.

These data suggest that the precise periods of prenatal immune challenges influence the susceptibility to distinct behavioral and/or cognitive symptoms that emerge in adult life and draw attention to the temporal link between PIC and the etiology of these disorders (see Table 1).

Contributions of the poly-I:C model to the treatment of schizophrenia

Prenatal poly-I:C injection elicits changes similar to those observed in schizophrenia that are only fully manifested

in adult offspring. In rodents, the prepubertal stage - also known as periadolescence - can be assessed on postnatal days 28-35 (PND28-35), whereas on PND56 the postpubertal period begins (67). Based on the fact that schizophrenia symptoms commonly start in late adolescence and early adult life, this model might provide a valuable means to assess the effects of preventive strategies by the administration of drugs during the prepubertal period (i.e., prodrome), as well as after the complete development of schizophrenia symptoms in adult life. In other words, it enables the study of preventive and therapeutic approaches to schizophrenia. Another highlight of this model is the possibility of studying drugs treating the main dimension of schizophrenia, since the exposure to the viral particle on GD9 is associated with the development of endophenotypes related to positive symptoms in the offspring, while the exposure on GD17 can be useful to study the negative and cognitive symptoms of schizophrenia (20) (Figure 1).

A recently published article has shown that chronic injection of antipsychotics (haloperidol and clozapine) or the antidepressant fluoxetine during periadolescence blocked the onset of psychotic symptoms in animals predisposed to schizophrenia by PIC exposure on GD9. The same study revealed several negative influences of early pharmacological intervention on normal behavioral development of control animals (68). These data suggest that the careful selection of at-risk individuals (psychopathologically and genetically) is necessary, since preventive treatment of individuals without real liability to develop the disorder might result in long-lasting deleterious behavioral effects. The admin-

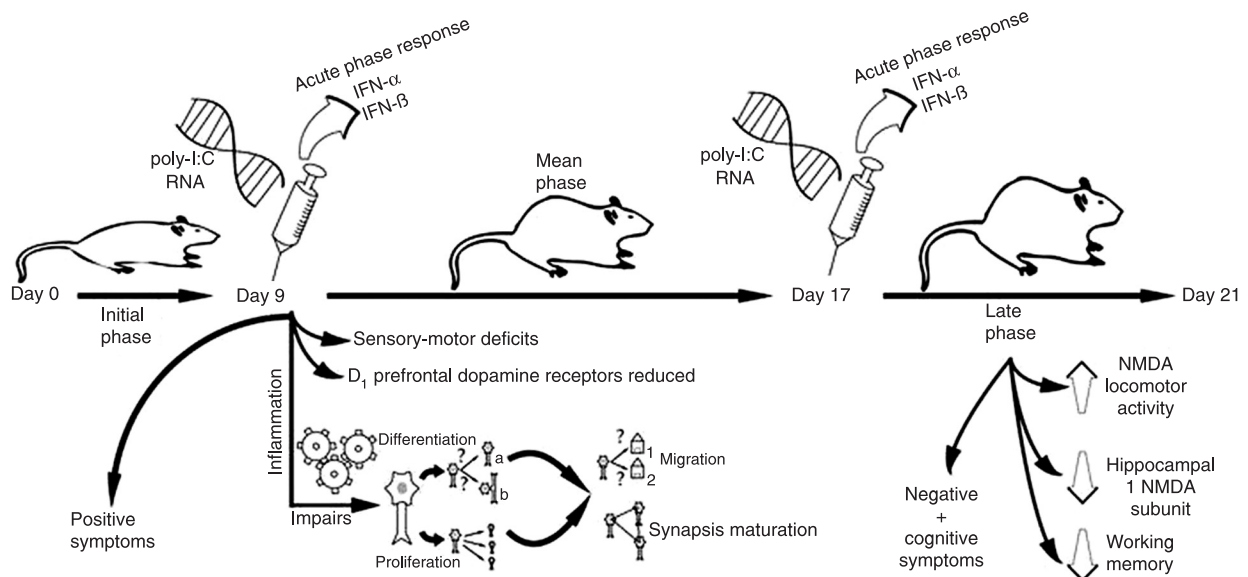


Figure 1. Prenatal exposure to polyriboinosinic-polyribocytidylic acid (poly-I:C) during the early/middle gestational period (gestational day 9 - GD9) generates phenotypes related to positive symptoms. The exposure on GD17 (late gestational period) is related to the occurrence of behavioral correlates of negative/cognitive symptoms of the disorder.

Table 1. Main contributions of the poly-I:C model to the study of the pathophysiology of schizophrenia.

Reference	Gestational day challenge	Main behavioral findings	Main neurobiological findings
Meyer et al., 2005 (51)	9	Behavioral deficits in adulthood (PPI, LI, spatial working memory, enhanced behavioral response to amphetamine)	Increase of IL-10 at 1 and 6 h post-injection, increase of IL-1 β at 12 h post-injection
Meyer et al., 2006 (57)	6, 9, 13, or 17	GD6, GD9 or GD13 LI deficiency	GD17 - elevation of the IL-10/TNF ratio
Meyer et al., 2006 (58)	9	Postpubertal LI disruption, pre- and postpubertal loss of LI in control animals adopted by poly-I:C-treated surrogate mothers	-
Fortier et al., 2007 (31)	10-11, 15-16 or 18-19	No significant effect on PPI	-
Smith et al., 2007 (59)	12.5	Co-administration of anti-IL-6 antibody prevents the PPI, LI, and exploratory and social deficits and normalizes the associated changes in gene expression in the brains of adult offspring	-
Meyer et al., 2008 (20)	9 or 17	GD9 - impairment of sensorimotor gating; GD17 - impairment of working memory, potentiation of locomotor reaction to NMDA antagonists; time-independent locomotor reaction to a dopamine agonist	GD9 - reduction of prefrontal D1 receptors in adulthood; GD17 - reduced hippocampal NMDA-receptor subunit expression; time independent reduction in reelin and parvalbumin-expressing prefrontal neurons
Meyer et al., 2008 (30)	9	-	Increase in the number of mesencephalic dopamine neurons in the fetal brain at middle/late gestation; changes in fetal expression of genes involved in dopamine neuron generation, differentiation and maintenance (ShhN, FGF-8) and transcription factors Nurr1 and Pitx3
Winter et al., 2009 (53)	9	-	Increased levels of DA and its major metabolites in the lateral globus pallidus and prefrontal cortex in adult brain; decreased serotonin in the hippocampus, nucleus accumbens and lateral globus pallidus; reduction of taurine in the hippocampus
Li et al., 2009 (34)	9 or 17	GD9 - disruption in PPI	GD9 - enlargement of lateral ventricles in adulthood; GD17 - expansion of 4th ventricle volume
Li et al., 2010 (60)	9 or 17	-	GD9 - alterations in neuronal myelination
Bitanirwe et al., 2010 (61)	17	Male and female offspring: deficits in social interaction, anhedonic behavior, alterations in locomotor response to apomorphine. Male offspring only: enhanced LI	Sex-specific changes in neurotransmitter levels
Cardon et al., 2010 (62)	-	Abnormal PPI with delayed appearance found in congenitally immune-deficient mice (severe combined immune deficient, SCID), reverted by immune reconstitution	Impairment of both hippocampal neurogenesis and expression of the gene encoding kisspeptin (Kiss1)* manifested in adulthood
Dickerson et al., 2010 (63)	-	Significant reductions in mPFC-HPC EEG coherence correlated with decreased prepulse inhibition of startle	-
Oh-Nishi et al., 2010 (64)	15 and 17	-	Synaptic dysfunction without neuronal loss in the hippocampus of juvenile offspring; decreased synaptophysin expression
De Miranda et al., 2010 (65)	16	Impaired neonatal locomotor development and abnormal sensorimotor gating responses in adult offspring	Inhibited embryonic neuronal stem cell replication and population of the superficial layers of the neocortex by neurons; effects were dependent on Toll-like receptor 3 (TLR3)
Hsiao and Patterson, 2011 (66)	12.5	-	Increased IL-6 mRNA as well as maternally derived IL-6 protein in the placenta

The table summarizes the main findings using polyribinosinic-polyribocytidylic acid (poly-I:C) on different gestational days (GD) in rodents and the behavioral and neurochemical pre- and post-natal alterations. PPI = prepulse inhibition of the acoustic startle reflex (i.e., reduction of startle reaction to a startle-eliciting stimulus [pulse] when it is shortly preceded by a weak stimulus [prepulse], a measure of sensorimotor gating); LI = latent inhibition (i.e., a form of selective associative learning considered to index an organism's capacity to ignore irrelevant stimulus); IL-10 = interleukin-10; IL-1 β = interleukin 1-beta; TNF = tumor necrosis factor; ShhN = sonic hedgehog gene; FGF-8 = fibroblast growth factor 8; DA = dopamine; Nurr1 = nuclear receptor-related 1 protein; Pitx3 = pituitary homeobox 3; HPC = hippocampus; mPFC = medial prefrontal cortex; NMDA = N-methyl-D-aspartate. *Kisspeptin is a protein that triggers the cascade of biochemical changes leading to puberty.

istration of risperidone during the periadolescence period of animals prevented both ventricular enlargement and reduction of hippocampal size in adult offspring, two of the most common brain abnormalities observed in schizophrenia (69). Risperidone, paliperidone (70), haloperidol (43),

and clozapine (43,71) prevented the behavioral changes observed in adult animals submitted to PIC between GD14 and GD15, showing that this model predicts responsiveness to antipsychotic drugs.

Risperidone and paliperidone treatment beginning

at the periadolescent period lowered basal glutamate in offspring of poly-I:C-treated dams to levels similar to the offspring of saline-treated dams (49), also stabilizing the response to amphetamine exposure persisting into early adulthood (70).

Although more studies are needed to establish the real predictive validity of the model, these data raise the possibility that, in the near future, the PIC model might be a valuable tool to test antipsychotic drugs, as well as compounds that might prevent the onset of schizophrenia throughout various developmental stages.

Concluding comments

The PIC models - especially the poly-I:C model discussed in further detail in this article - are capable of mimicking several aspects of the schizophrenia syndrome in a neurodevelopmentally oriented approach, reproducing behavioral abnormalities as well as some genetic and neurochemical alterations present in schizophrenia, which may contribute to 1) the study of prenatal and postnatal brain dysfunction after

exposure to immune challenges across different gestational stages; 2) provide evidence for the neurodevelopment and neuroimmunologic basis of this disorder; 3) enable the study of antipsychotic drugs in a preventive (prenatal life and prodrome) and therapeutic approach; 4) permit the study of medications targeting specific symptoms (i.e., positive or negative/cognitive). One advantage of this model is its biosafety as compared to viral infection models. The main disadvantages of this model are the limited duration of the immune response as compared to viral infection models and the non-mimicking of the precise immunological insults occurring in the human environment. Put differently, it fails to model the full spectrum of immune responses normally induced by viral exposure.

Thus, the poly-I:C model might open new avenues for the identification of novel molecular targets and the eventual preclinical development of new treatments for the disorder. In particular, the search for effective prevention strategies (e.g., at the prodrome) might benefit from the use of this model.

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