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Review

Gene–environment interaction during early development in the heterozygous *reeler* mouse: Clues for modelling of major neurobehavioral syndromes

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ABSTRACT

Autism and schizophrenia are multifactorial disorders with increasing prevalence in the young population. Among candidate molecules, reelin (RELN) is a protein of the extracellular matrix playing a key role in brain development and synaptic plasticity. The heterozygous (HZ) reeler mouse provides a model for studying the role of reelin deficiency for the onset of these syndromes. We investigated whether early indices of neurobehavioral disorders can be identified in the infant reeler, and whether the consequences of ontogenetic adverse experiences may question or support the suitability of this model. A first study focused on the link between early exposure to Chlorpyryfos and its enduring neurobehavioral consequences. Our data are interesting in view of recently discovered cholinergic abnormalities in autism and schizophrenia, and may suggest new avenues for early pharmacological intervention. In a second study, we analyzed the consequences of repeated maternal separation early in ontogeny. The results provide evidence of how unusual stress early in development are converted into altered behavior in some, but not all, individuals depending on gender and genetic background. A third study aimed to verify the reliability of the model at critical age windows. Data suggest reduced anxiety, increased impulsivity and disinhibition, and altered pain threshold in response to morphine for HZ, supporting a differential organization of brain dopaminergic, serotonergic and opioid systems in this genotype.

In conclusion, HZ exhibited a complex behavioral and psycho-pharmacological phenotype, and differential responsivity to ontogenetic adverse conditions. HZ may be used to disentangle interactions between genetic vulnerability and environmental factors. Such an approach could help to model the pathogenesis of neurodevelopmental psychiatric diseases.

Contents

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1. Introduction

Recent studies have emphasized the importance of gene–environment interaction in the etiology of psychiatric disorders (Tsuang et al., 2000; Vanyukov and Tarter, 2000). "Environment" should be understood in the broadest possible sense: we can speak of a "genetic environment", meaning the whole genome where a particular mutation or genetic polymorphism is present. As an example of the weight of this genetic environment in modulating the expression of a given gene, the same genetic mutation in different subjects may lead to a full-blown autism phenotype or have no behavioral consequences at all (Yan et al., 2005). However, "environment" is usually understood as everything else besides the genome, interacting with the genome and modulating the expression of genes. In the real world, it is often impossible to distinguish these two types of gene–environment interactions. A genetic vulnerability can be predictive of later-onset disorders, but it is also clear that environmental conditions can either mitigate or exacerbate its consequences (Jobe and Harrow, 2005). Gottesman and Hanson (2005) systematically formulated the "double-hit" hypothesis to give account of gene–environment interactions in the etiology of neuropsychiatric disorders. This hypothesis, coming from concepts belonging to oncogenesis, indicates the need for a convergence between genetic vulnerability and secondary external agents for the development of a given disease.

Autism and schizophrenia are complex and multifactorial psychiatric disorders, with an increasing prevalence in the young population (Agid et al., 1999; Hofer, 1994). It is well established that the first symptoms of autism can be detected during the first three years of life. Some early-onset psychotic states seem to be related to schizophrenia, which is frequently diagnosed in humans between 13 and 18 years of age (Jobe and Harrow, 2005). One of the molecules that are under examination as a risk factor, playing a role in autism and schizophrenia, is Reelin (RELN). This is a protein of the extracellular matrix, with a key role in migration and positioning of neurons (Costa et al., 2001, 2002; Tissir and Goffinet, 2003; Andersen et al., 2002; Keller and Persico, 2003; Keller et al., in press). Reelin messenger-RNA (mRNA) and protein are down-regulated in cortical GABAergic neurons of patients suffering of schizophrenia, psychotic bipolar disorder, and autism (Persico et al., 2001; Fatemi, 2001, 2002). A down-regulation of glutamic acid decarboxylase 67 (GAD67) mRNA is associated with the reelin deficiency (Guidotti et al., 2000; Impagnatiello et al., 1998). Indeed, a recent study demonstrated that hyper-methylation of the CpG islands in the *RELN* promoter is present at significantly higher levels in post-mortem brains of schizophrenic patients, thus providing a mechanism for the observed decrease in gene expression (Abdolmaleky et al., 2005; Grayson et al., 2005).

Some autistic patients, indeed, show a marked hypoplasia of the cerebellum (Courchesne et al., 1988). At a microscopic level, a reduced number of Purkinje' cells are evidenced, in the absence of gliosis (Kemper and Bauman, 1993). All these evidences are in agreement with the hypothesis that reelin could be studied as a marker of severe neuropsychiatric disorders. The gene coding for reelin is highly conserved between the mouse (symbol *Reln*) and the human (symbol *RELN*) (De Silva et al., 1997). Because of reelin (haplo)insufficiency, the heterozygous reeler mouse (HZ) became of immediate interest as a possible animal model for psychosis (Tueting et al., 1999). The reeler mutation arose spontaneously, showing autosomic recessive transmission. The original mutation is characterized by a deletion of 150 kb that removes a wide portion of the *Reln* gene. The homozygous reeler mouse (RL) completely lacks the protein, presenting an impaired phenotype characterized by striking neurological signs (dystonia, ataxia, tremor) and severe alterations in the architecture of laminar structures like the cerebral cortex, the cerebellum and the hippocampus (Caviness and Rakic, 1978; Goffinet, 1984, 1990). Recent studies in hippocampal cultures demonstrated that reelin promotes dendrite maturation and may contribute to the establishment of synaptic contacts (Niu et al., 2004). Reelin seems to also act after embryonic development, particularly in the process of synaptogenesis and synaptic plasticity (Rice and Curran, 2001).

Levels of Reelin are reduced by 50% in HZ compared to wild-type (WT). HZ do not show lamination defects in the SNC nor the classical reeler phenotype. It has been documented that, in the first weeks of life, HZ mice show progressive loss of Purkine cells of the cerebellum (Marrone et al., 2006). The HZ phenotype, however, shows subtle neuro-anatomical and behavioral abnormalities (Liu et al., 2001; Salinger et al., 2003; Tueting et al., 1999; Laviola et al., 2006; Ognibene et al., 2007a,b). Brain abnormalities in HZ are partly similar to those found in post-mortem brain tissue from psychotic patients. The distribution and density of interstitial white matter neurons in the temporal cortex is altered in schizophrenia (Eastwood and Harrison, 2003). A similar abnormal distribution of neurons in white matter is also present in male HZ (Tueting et al., 1999), together with a decrease in age-dependent pre-pulse inhibition (Tremolizzo et al., 2002; Tueting et al., 1999).

The present review provides a report on a series of recent behavioral and neurobiological studies carried out in the HZ–RL model. These studies were aimed to substantiate a psycho-biological basis for the neuro-developmental hypothesis of autism and schizophrenia. HZ have been previously investigated in several behavioral paradigms by the group of Salinger et al. (2003) and by other investigators, mainly in adult subjects (Qiu et al., 2006; Podhorna and Didriksen, 2004). In our group, a first attempt consisted in the characterization of the behavioral profile during early ontogenesis in this model, including the RL. Indeed, the inclusion of RL subjects seemed to be useful for the assessment of any dose-dependency in genetic vulnerability. Specifically, we assessed the interplay between genetic background and some environmental insults, represented by (1) prenatal exposure to a widespread organophosphorous pesticide like Chlorpyrifos; (2) repeated episodes of early maternal separation, a source of unusual stress during the first week of life. A second attempt was to evaluate the enduring consequences of these early-life adverse experiences, as a function of genotype. The third approach consisted in a battery of investigations, aimed to verify the reliability of the model at critical age windows.

2. Behavioral characterization of the model early in development, and assessment of gene–environment interactions using a cholinergic neurotoxin

2.1. Ontogenesis of neuro-behavioral functions in the reeler mouse model following prenatal exposure to Chlorpyrifos

Previous studies addressing the effects of the reeler mutation on anxiety-related behavior, pain perception and cognitive impairments, as well as the response to drug stimulated behavioral stereotypy, were mainly focused on adult subjects (Costa et al., 2001; Laviola et al., 2006; Qiu et al., 2006; Podhorna and Didriksen, 2004; Salinger et al., 2003). However, these mutant mice are proposed as a model for human behavioral disorders which are characterized by an early onset. It is thus surprising that, whereas the body of evidence gathered in adult subjects has grown rapidly, near-to-nothing was known about the developmental behavioral outcomes of this spontaneous genetic mutation. Indeed, a deeper investigation of the normative developmental aspects of this mouse model may disclose new avenues in the refinement of potential therapeutic strategies.