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Research report

Sensorimotor gating deficits are inheritable in an isolation-rearing paradigm in rats

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HIGHLIGHTS

- Environmentally induced abnormalities can be translated to the next generation.
- Social isolation impaired prepulse inhibition is inheritable.
- Social isolation-induced alterations to central monoaminergic functions are inheritable.
- Social isolation-induced alterations to central *Slc1a2* gene is inheritable in males.

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ABSTRACT

Early life experience is a key etiological factor of neuropsychiatric dysfunctions and is associated with developmental origins. Impaired prepulse inhibition (PPI) following an acoustic startle response is acknowledged as a cardinal characteristic in socially deprived weanling rats, which has been employed to investigate the underlying mechanisms of sensorimotor gating abnormalities in certain mental disorders, including schizophrenia. Because impaired PPI is a postnatal malfunction, it is interesting to examine whether it can be passed to the next generation. Isolation-rearing (IR) rats had been socially deprived since weaning, which mated with social rearing rats. Next, the offspring of IR rats were reared in a normal social environment. Locomotion, PPI, monoamines, and genes in schizophrenia-relevant brain areas [medial prefrontal cortex (mPFC) and hippocampus] were later measured. To this end, we observed that the next generation of IR offspring rats appeared with impaired PPI in which the PPI deficit can be observed as early as three weeks after birth. The third generation also exhibited lower levels of dopamine and serotonin in the mPFC and hippocampus; however, higher levels of both monoamines were measured in the striatum. Finally, *Slc1a2* was more highly expressed in the mPFC of the third generation male rats. The present study demonstrates a transgenerational inheritance of IR-induced character and may help to elucidate the underlying pathoetiology of schizophrenia.

1. Introduction

Gene-environment interactions are involved in the pathogenesis that underlies many psychiatric disorders [1,2], including schizophrenia, which is a debilitating neuropsychiatric disorder with heterogeneous symptoms and etiology that remains unclear [3]. Among the pathoetiologies of schizophrenia, impairment of prepulse inhibition (PPI) can be employed in modeling the gating dysfunction of this disease in both genetic [4,5] and non-genetic [6,7] approaches.

PPI refers to the diminution of a startle reflex when a low-intensity non-startling stimulus immediately precedes the startling stimulus [8]. The impairment of PPI may destabilize cortico-striatal and hippocampal monoamines, such as dopamine (DA) and serotonin (5-HT) [6,9,10], as well as their relevant genes, such as *Comt*, *Grid2*, *Ncam1*, *Nrg*1, and *Slc1a2* [11–14]. As an operational measurement of sensorimotor gating, PPI operates across species [15,16], and its dysfunction can be observed in various neuropsychiatric disorders [17,18].

Among the hypotheses of the etiology of schizophrenia, genetic hereditability, such as single nucleotide polymorphisms in cer-

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tain schizophrenia-related genes, including *Erbb4*, *Reln*, *Grid2*, and *Nrg*1, is of particular interest [11]. These genetic mutations should be interpreted together with the non-genetic factors, including environmental distress, such as early-life, adverse experiences [9,10]. Previously, Swerdlow and colleagues demonstrated that the gene-environment interactions or epigenetic impacts are crucial in determining PPI function, as it can be sabotaged by amphetamine or neonatal ventral hippocampal lesions [14,19]. In the present study, we employed a non-pharmacological schizophrenia animal model, an isolation-rearing (IR) paradigm, to examine the epigenetic hypothesis.

Because the etiology of schizophrenia can be attributed to both genotypic and phenotypic disturbances [13,14], and possibly does not follow the Mendelian model of inheritance [20], non-genetic impact is thus important for the psychopathologies of the disease in terms of epigenetic inheritance [21]. This finding is particularly relevant for the role of environmental impact in schizophrenia, given that environmental information can be inherited to offspring [21,22]. Accordingly, environmental impact during prenatal or postnatal period sabotages the normality of brain development and is responsible for many pathoetiologies underlying schizophrenia, including gene expression, neurotransmissions, social cognition, and sensorimotor gating [1,5,21].

In certain circumstances, environmental influence may even exceed genetic influences in causing the disease, considering the relative risk of the disease [23,24]. In terms of PPI, evidence obtained from genetic and epidemiological studies [1] supports the existence of gene-environment interaction in that the performance of PPI is highly heritable, for example, in Huntington's disease [25,26]. Nevertheless, there is a lack of direct evidence regarding whether environmentally induced phenotypic abnormalities can be imbued with a transgeneration nature.

The present study sought to investigate the degree to which IR impaired PPI performance and whether it can be translated to the next generation. IR is appropriately employed in this regard because it represents a developmental origin of pathogenesis [4–6], particularly in its effects on altering brain development and behavioral deficits around puberty, thereby exhibiting a robust validity in modeling the development-associated sensorimotor gating dysfunction of schizophrenia [5,16]. The present study is the first to examine whether the acquired schizophrenia-related abnormalities can be preserved to the next generation, including PPI and other schizophrenia-relevant genetic expression and central neurotransmissions. The results of the present study may help to elucidate the developmental hypothesis in terms of transgenerational inheritance.

2. Materials and methods

2.1. Animals

Two male Sprague-Dawley (SD; BioLASCO Taiwan Co., Ltd.) IR rats (singly housed since weaning, aged from 21 to 23 days) were mated with two female SD social rearing (SOC; housed in 2–3) rats, referred to as ancestors (F0). All offspring were group reared and the multiplication procedures (i.e., for generations F1–F3) were not executed until they were sexually mature (8 weeks old) and were based on the principle of random selection with no inbreeding. For controls of F1–F3, both male and female SD rats were used. The rats were subsequently divided randomly into two groups [SOC and IR; SOC1, SOC2, SOC3, IR1, IR2, and IR3], with each group containing 5–6 males and 5–6 females (see for Fig. 1). All animals were kept in a temperature- ($22 \pm 4\,^{\circ}C$) and humidity-controlled ($50 \pm 20\%$) room under a 12-h light/dark cycle (lights on from 0700 to 1900) and given *ad libitum* access to a standard laboratory chow diet (Ralston Purina, St. Louis, MO, USA) and sterile water. The Institutional Animal Care and Use Committee of the National Defense Medical Center (NDMC) approved the present study, and the rats were raised, handled, and euthanized according to the guidelines issued by Laboratory Animal Center (LAC) of NDMC with a full accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

2.2. Locomotor activity

Rat's locomotor activity was monitored with a computerized automated activity monitoring system (MED Associates, Inc., St. Albans, VT, USA). Total traveled distance were individually recorded every 5 min and expressed cumulatively for 30-min [27].

3. Prepulse inhibition measurement

The acoustic startle response and PPI tests were performed in four startle chambers (SR-LAB; San Diego Instrument, San Diego, CA) consisting of a transparent Plexiglas cylinder (9-cm diameter). The speaker was controlled by a SR-LAB and was mounted 24 cm above the animal to provide background noise, startle stimuli, and prepulse stimuli. Startle responses were induced by the speaker, and transduced and measured by a piezoelectric accelerometer mounted below the cylinder and processed by SR-LAB. Rats were allowed to habituate to the background noise of 65 dB for 5 min after being placed into the chambers. Movement was measured for 100 ms after the startle stimulus onset. In total, 60 trials were conducted during each test session with an average inter-trial interval of 15 s. The first and last six trials consisted of single 40-ms 120-dB white-noise startle stimuli. The middle 48 trials consisted of random startle stimuli: 12 no-stimuli trials, 12 trails of startle stimuli alone, and 24 prepulse trials including a prepulse and startle stimulus. Prepulse trials consisted of a single 120-dB pulse preceded by a 20-ms non-startling prepulse stimulus with intensities of 71, 73, or 75 dB. PPI was calculated according to the formula: [1 − (startle response for prepulse + pulse pair)/(startle response for startle stimuli alone)] × 100% [27]. The PPI test was conducted when rats were seven weeks of age. For SOC3 and F3 rats, the PPI test was also conducted when they were three weeks of age. This was to evaluate how early the hypothesized transgenerational inheritance can be effective.

3.1. Neurochemical analysis

SOC3, IR3, and F3 rats were sacrificed by decapitation, and their brains were rapidly removed. Various brain areas, including the medial prefrontal cortex (mPFC), striatum, and hippocampus, were rapidly dissected on an icy cold plate, then weighed and stored at −70 °C until homogenization with ultrasonication in 0.2 mL of 7 N perchloric acid (Sigma Chemical Industries, Ltd., Saint Louis, MO, USA). Homogenates were centrifuged at 12,000 × *g* for 30 min at 4 °C [28]. The concentrations of DA and 5-HT were determined by high performance liquid chromatography (HPLC) equipped with an electrochemical detector (ECD, LC-4C, BAS, West Lafayette, IN, USA; 10 nA, filter 2.0 Hz, AppE cell 0.750 V) and autosampler (Shimadzu SIL-10ADvp autosampler, Shimadzu, Japan) based upon the method of Xu et al. [29]. The supernatant was filtered through a 0.22-μm filter and was analyzed by HPLC equipped with an Alltima™ reversed-phase C18 column (4.6 × 150 mm, 5 μm). The injection volume was 20 μL. The mobile phase contained 100 mM $NaH_2PO_4 \cdot H_2O$, 0.74 mM sodium octane sulfonate (SOS), 0.02 mM EDTA, and 20% methanol adjusted to pH 3.0 by using H_3PO_4 and with a flow rate of 1.0 mL/min. The standard coefficient of determination (r^2) was greater than 0.995.