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Research Report

Isolation rearing in rats: Effect on expression of synaptic, myelin and GABA-related immunoreactivity and its utility for drug screening via the subchronic parenteral route

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ABSTRACT

Depriving weaned rats of social contact by rearing them in isolation brings about a spectrum of behavioural and neuropathological changes in adulthood which resemble some of the characteristics observed in schizophrenia. Hence, isolation rearing provides a non-pharmacological means to induce in an animal model certain aspects of schizophrenia with a neurodevelopmental origin. We compared the prepulse inhibition and locomotor activity behaviours in group-reared and isolation-reared rats in the context of determining the robustness of any behavioural changes following a subchronic parenteral drug administration protocol. The expression of synaptic, myelin and GABA-related proteins was also assessed in the brains of these rats using semi-quantitative fluorescence immunohistochemistry. Compared to their group-reared counterparts, isolation-reared rats displayed disruption in prepulse inhibition which was lost after repeated testing and subchronic vehicle administration. However, isolation-reared rats showed open-field hyperlocomotion post-subchronic vehicle treatment compared to group-reared rats. Isolation rearing resulted in reduced expression of synaptophysin, synapsin I, myelin basic protein and $GABA_{B1}$ receptor proteins, along with an increase in 2′,3′-cyclic nucleotide 3′-phosphodiesterase. Of the brain areas examined these observed changes were localised to the hippocampal regions and the substantia nigra. These results suggest an alteration in the synaptic, myelin and GABA-related functions in the brains of isolation-reared rats that displayed behavioural anomalies. Since dysfunction in these systems has also been implicated in schizophrenia, our findings provide additional evidence to support the use of isolation rearing for schizophrenia research; however, its use in the screening of putative antipsychotics following subchronic administration needs to be undertaken warily.

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Abbreviations: AcbC, Nucleus accumbens core; AcbSh, Nucleus accumbens shell; Amy, Amygdala; CA1, Hippocampal CA1 subfield; CA2, Hippocampal CA2 subfield; CA3, Hippocampal CA3 subfield; CNPase, 2′,3′-Cyclic nucleotide 3′-phosphodiesterase; CPu, Caudate putamen; DG, Dentate gyrus; GABA, γ-Aminobutyric acid; $GABA_BR1$, $GABA_{B1}$ receptor; GAD65, Glutamic acid decarboxylase 65; i.p., Intraperitoneal; MBP, Myelin basic protein; mPFC, Medial prefrontal cortex; PND, Postnatal day; PPI, Prepulse inhibition; SN, Substantia nigra

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1. Introduction

Isolation rearing involves housing rats individually for 8 weeks post-weaning, and has been demonstrated to bring about sensorimotor gating anomalies in adulthood, as reflected by disruption of prepulse inhibition (PPI) of the startle response (Cilia et al., 2005; Geyer et al., 1993; Varty and Geyer, 1998). PPI denotes lowering of the startle magnitude when a prepulse is presented 30–500 ms in advance of a startling pulse (Swerdlow et al., 1992). Whilst the disruptive effect of isolation rearing on PPI is generally reliable, there are factors which can influence the PPI outcome resulting in the deficits not being observed. The success of the model in eliciting PPI disruptions is sensitive to factors such as the type of home cages (Weiss et al., 1999), strain of rats (Varty and Geyer, 1998), range of prepulse intensities (Geyer et al., 1993), prior exposure to behavioural tests (Domeney and Feldon, 1998) and the amount of handling during the isolation period (Krebs-Thomson et al., 2001; Rosa et al., 2005).

In comparison to their group-reared peers, isolation-reared rats display spontaneous hyperlocomotion in an unfamiliar environment (Domeney and Feldon, 1998; Einon and Morgan, 1978; Hall et al., 1998a; Phillips et al., 1994; Weiss et al., 2000). In addition, differences in brain anatomy and neurochemistry have been reported as consequences of isolation rearing, for example, a decrease in the volume of the medial prefrontal cortex (Day-Wilson et al., 2006; Schubert et al., 2009), a reduction in neuronal dendritic arborization (Pascual et al., 2006; Silva-Gómez et al., 2003) as well as changes in the dopamine (Hall et al., 1998b; Heidbreder et al., 2000), γ-aminobutyric acid (GABA) (Harte et al., 2007), glutamate (Melendez et al., 2004), serotonin (Jones et al., 1992; Muchimapura et al., 2003) and endocannabinoid (Malone et al., 2008; Robinson et al., 2010) systems (see Fone and Porkess (2008) for review). Since these changes mimic some of the characteristics of schizophrenia in humans, isolation rearing provides a non-drug means of inducing in an animal model certain aspects of this disorder (Geyer et al., 1993; Lapiz et al., 2003; Stevens et al., 1997; Weiss and Feldon, 2001). As this model involves a young age manipulation to bring about changes in adulthood, it is consistent with the neurodevelopmental hypothesis of schizophrenia (Ellenbroek and Cools, 1998; Weinberger and Marenco, 2003; Weinberger, 1995).

Specifically relating to a neurodevelopmental pathology, schizophrenia has been linked to an alteration in synaptic function, said to be a result of aberrations in synaptic pruning during the juvenile period (Feinberg, 1982–1983; Keshavan et al., 1994). Synaptic density is highest in childhood, decreases by up to 40% in adolescence and then remains relatively constant in adulthood (Huttenlocher, 1979). Myelinogenesis is another aspect of brain development, and has been reported to occur from the gestational period up to adulthood depending on which brain region was investigated (Arnold, 1999). Evidence suggests a disruption of myelination in the central nervous system of patients with schizophrenia (Chambers and Perrone-Bizzozero, 2004; Flynn et al., 2003; McInnes and Lauriat, 2006). It is also well established that the GABA neurotransmitter system is dysfunctional in schizophrenia (see Blum and Mann (2002) for review).

In this study we further investigated PPI and locomotor activity in the isolation rearing model. Although these parameters have previously been characterized, the focus in the past has been mostly on behavioural changes alone (Geyer et al., 1993; Varty and Geyer, 1998; Weiss et al., 2000) or in conjunction with acute administration of antipsychotics in attempts to normalise the anomalies (Bakshi et al., 1998; Varty and Higgins, 1995). Insight into the suitability of this animal model for chronic administration of putative or clinically used antipsychotics especially via the parenteral route is lacking. Thus, in the present study, the value of this model for subchronic drug administration was assessed. In addition, we sought to determine if there are differences in the relative expression of synaptic, myelin and GABAergic markers in the brains of these isolation-reared rats measured against their group-reared counterparts. These markers represent certain aspects of the central nervous system which have been implicated in schizophrenia. If similar perturbations were to be found in the brains of these animals, the validity of this model in schizophrenia research will be further strengthened, and may also offer an avenue in which drugs which target some of the aforementioned aspects could be screened for potential antipsychotic properties.

2. Results

2.1. *Startle reactivity*

In the first (postnatal day (PND) 77) and second (PND 84) PPI sessions, there was no effect of rearing condition on the startle reactivity of the rats (Fig. 1B and D). However, in the third session (PND 91), isolation-reared rats had a significantly higher startle reactivity compared to that of the group-reared rats ($F_{1,16}=5.352$, $p<0.05$; Fig. 1F).

2.2. *Prepulse inhibition (PPI)*

PPI values obtained using a 300 ms interstimulus interval were relatively low for rats from both rearing conditions (13–50%) and therefore not used for analysis (data not shown) as any reduction of PPI would most likely not be observed. Only PPI data from the 100 ms interstimulus interval are presented and discussed.

In all three PPI sessions, there was a significant effect of prepulse intensity, indicating prepulses were more effective in eliciting higher PPI as they increase in magnitude ($F_{2,32}=34.632$, $p<0.001$ on PND 77; $F_{2,32}=16.085$, $p<0.001$ on PND 84; $F_{2,32}=46.972$, $p<0.001$ on PND 91).

No prepulse intensity × housing interaction was detected in any of the sessions. During the first PPI testing (PND 77), there was a significant effect of rearing condition on PPI ($F_{1,16}=4.660$, $p<0.05$; Fig. 1A), in that the isolation-reared rats demonstrated a lower PPI. A post-hoc test revealed this significance to be at the prepulse intensity of 3 dB above background. However, during the next PPI session a week later (PND 84), there was no significant effect of rearing condition on PPI (Fig. 1C). In the third PPI session (PND 91), there was again no effect of rearing condition on PPI detected (Fig. 1E).