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Effects of repeated dizocilpine treatment on adult rat behavior after neonatal lesions of the entorhinal cortex

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Abstract

Disturbed cortical development is implicated in some psychiatric diseases, *e.g.* in schizophrenia. Additionally, *N*-methyl-D-aspartate (NMDA) receptor antagonists like ketamine or phencyclidine have been reported to exacerbate schizophrenic symptoms. We here investigated the effects of neonatal entorhinal cortex (EC) lesions on adult rat behavior before and after repeated high-dose treatment with the NMDA antagonist dizocilpine, in order to combine these etiopathogenetical factors in an animal model.

Bilateral neonatal (postnatal day 7) lesions were induced by microinjection of ibotenic acid (1.3 μg/0.2 μl PBS) into the EC. Naive and sham-lesioned rats served as controls. Adult rats were tested for behavioral flexibility on a cross maze, for locomotor activity in the open field and for sensorimotor gating using prepulse inhibition (PPI) of startle. Rats were then treated with dizocilpine (0.5 mg/kg b.i.d. for 7 days) and retested 1 week after withdrawal using the same behavioral tests as before. PPI was additionally measured after acute low-dose challenge with dizocilpine (0.15 mg/kg).

EC lesions reduced behavioral flexibility as shown by impaired switching between spatial (allocentric) and non-spatial (egocentric) maze strategies. High-dose dizocilpine treatment disturbed switching to the egocentric strategy in all groups, which added to the effect of EC lesions. Neonatal EC lesions did not alter locomotor activity or PPI, but high-dose dizocilpine treatment reduced motor activity of all groups without changing PPI.

The combination of neonatal EC lesions and adult dizocilpine treatment does not lead to super-additive effects on behavior. However, both treatments may serve to model certain aspects of psychiatric symptoms.

Keywords: Behavioral flexibility; Locomotor activity; Neurodevelopment; NMDA receptor antagonist; Sensorimotor gating

1. Introduction

Psychiatric disorders are increasingly related to neurodevelopmental disturbances of cortico-limbic brain regions (Beckmann, 1999; Duncan et al., 1999; Weinberger, 1995). Neonatal lesions deteriorate neuronal network function and related behavior mainly by disturbing development and maturation of the brain. Rats with neonatal excitotoxic lesions are therefore considered useful to model the etiopathogenesis of certain psychiatric disorders (Lipska and Weinberger, 2000). For example neonatal lesions of the ventral hippocampus (HIPP) have been shown to induce behavioral deficits reminiscent of cortico-limbic dysconnection in schizophrenia (Chambers et al., 1996; Le Pen et al., 2000; Lipska et al., 1993, 1995, 2002).

We recently showed that neonatal lesions of the entorhinal cortex (EC), the major input to the HIPP (Dolorfo and Amaral, 1998; Witter et al., 1989), disturbed the rats ability to hold information during delays, *i.e.*, behavior presumably related to the

Abbreviations: ANOVA, analysis of variance; ASR, acoustic startle reflex; b.i.d., bis in die; EC, entorhinal cortex; HIPP, hippocampus; ITI, intertrial interval; NMDA, *N*-methyl-D-aspartate; PFC, prefrontal cortex; PND, postnatal day; PPI, prepulse inhibition; SPL, sound pressure level.

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EC itself, but also lead to behavioral impairments related to dysfunction of distal brain circuits (Schmadel et al., 2004). Additionally, adult rats with neonatal EC lesions showed enhanced perseveration in spatial allocentric and non-spatial egocentric learning tasks (Harich et al., under revision), *i.e.*, behavior that is thought to be mediated by the HIPP and striatum, respectively (Kesner et al., 1993). Notably, enhanced perseveration is one of the most robust findings in schizophrenia patients (Lysaker et al., 1998; Perry and Braff, 1998). However, although the EC has strong connections with the limbic-striatal system (Totterdell and Meredith, 1997; Witter et al., 1989), locomotor activity and sensorimotor gating were not disturbed (Schmadel et al., 2004).

Glutamate *N*-methyl-D-aspartate (NMDA) receptor antagonists, such as ketamine or phencyclidine (PCP), induce psychotic states in humans (Javitt and Zukin, 1991; Krystal et al., 1994) and exacerbate symptoms in schizophrenic patients (Goff and Coyle, 2001; Vollenweider et al., 2000). In rats, NMDA antagonists induce behavioral abnormalities after acute and chronic high-dose treatment (for review see Jentsch and Roth, 1999), and repeated application of the non-competitive NMDA antagonist dizocilpine has been reported to induce behavioral sensitization (Breese et al., 2002; Schulz et al., 2001; Xu and Domino, 1994). Also interesting in this regard is the observation that rats with neonatal lesions of the ventral HIPP show hypersensitivity to the locomotor stimulating effects of NMDA receptor antagonists when injected in lower doses that do not influence behavior in normal rats (Al Amin et al., 2001; Kato et al., 2000). Since the EC is strongly interconnected with the HIPP, rats with neonatal lesions of the EC may also show hypersensitivity to behavioral effects of NMDA receptor antagonists.

We here investigated the effects of neonatal EC lesions on behavioral flexibility measured by switching between a spatial allocentric and a non-spatial egocentric task. Additionally, locomotor activity in an open field and sensorimotor gating measured as prepulse inhibition (PPI) of the acoustic startle reflex (ASR), were assessed in these rats. Rats were then treated with a high dose of dizocilpine for 7 days. One week after withdrawal from the drug they were retested for behavioral flexibility, locomotor activity and PPI. Finally, we tested the effects of an additional acute low-dose challenge of dizocilpine on PPI in order to examine possible sensitizing effects of previous subchronic high-dose treatment with dizocilpine. The overall aim of the present study was to investigate the separate and combined effects of treatments that may be appropriate to model etiopathogenetic aspects of psychosis. The rationale for focusing on the EC was twofold: (1) A variety of researchers studied the role of different brain regions in the pathophysiology and symptoms of schizophrenia by inducing adult lesions of, *e.g.*, the prefrontal cortex (PFC), HIPP and also the EC (*e.g.*, Eijkenboom et al., 2000; Goto et al., 2002; Pouzet et al., 2004; Risterucci et al., 2003). However, increased interest lies on the developmental aspects of this disorder. Neurodevelopmental deficits may be induced by neonatal lesions of different brain regions. Effects of neonatal lesions of the PFC and HIPP have been extensively studied (Al Amin et al., 2000; Becker et al., 1999; Grecksch et al., 1999; Le Pen et al., 2000; Lipska et al., 1993, 1995, 1998; Lipska and Weinberger, 1993, 1994, 2000; Schwabe et al., 2004, 2006), while only a limited number of animal studies assessed effects of neonatal lesions of the EC on behavior later in life (Schmadel et al., 2004; Uehara et al., 2000); (2) the EC has been shown to be affected in schizophrenic patients. Cytoarchitectural anomalies suggesting abnormal development of the EC have been found (Arnold et al., 1991; Falkai et al., 2000; Jakob and Beckmann, 1986, 1994; Kovalenko et al., 2003; but see also Akil and Lewis, 1997; Krimer et al., 1997). Additionally, reduced volume of the EC (Falkai et al., 1988), decrease of synaptic proteins and abnormalities relative to specific neurotransmitter systems have also been detected in the EC of schizophrenic patients (Bachus et al., 1997; Eastwood et al., 1995; Hemby et al., 2002; Mizukami et al., 2002; Wolf et al., 1995). Furthermore, the EC is strongly interconnected with a variety of other brain regions. Thus, neonatal lesions of the EC may influence normal development and maturation of these neuronal circuits and thereby disturb functioning of connected structures.

2. Methods

2.1. Experimental design

Bilateral neonatal lesions were induced by microinjection of ibotenic acid into the EC on postnatal day (PND) 7. Adult rats (PND 70) were first tested for behavioral flexibility using a cross maze where rats had to switch between spatial allocentric and non-spatial egocentric tasks. Thereafter, spontaneous locomotor activity in an open field and subsequently PPI of ASR were measured. Starting 3 days after measuring PPI, rats were treated with 0.5 mg/kg dizocilpine twice daily for 7 days without behavioral testing. One week after the last injection rats were retested for behavioral flexibility starting with an allocentric task. Thereafter (*i.e.*, 30 days after the last injection), locomotor activity and subsequently PPI of ASR were measured, the latter with one additional low-dose challenge of dizocilpine (0.15 mg/kg). A diagram of all the treatments and their temporal relationships is shown in Fig. 1. Body weight of all rats was assessed before dizocilpine treatment and directly after, 1 week as well as 2 weeks after dizocilpine challenge.

2.2. Animals

A total of 59 male Wistar rats (offspring of 12 mothers from Harlan-Winkelmann, Borchen, Germany) were used in this study. The litters were culled to eight male pups directly after birth. In case of less than eight male pups, females were used to fill up the litter. The day of birth was designated as PND 0. Male pups were weaned on PND 21 and then housed in groups of four to seven in macrolon cages (type IV) under controlled environmental conditions (ambient temperature 22–24 °C, 12 hours light–dark cycle, lights on at 7:00 a.m.). The rats had free access to tap water and were fed *ad libitum* until reaching a body weight of approximately 180 g. Thereafter, a restricted diet of 12 g standard laboratory chow per day was given to each rat keeping the animals' body weight on approximately 85% of the free-feeding weight throughout the whole testing period. Behavioral studies began when rats were at least 70 days old. All tests were conducted during the light period by an experimenter who was blind to the animals' treatment. During the light cycle a softly playing radio was used to provide a