Neuroscience 207 (2012) 110–123

INTACT NEUROBEHAVIORAL DEVELOPMENT AND DRAMATIC IMPAIRMENTS OF PROCEDURAL-LIKE MEMORY FOLLOWING NEONATAL VENTRAL HIPPOCAMPAL LESION IN RATS

L. LECOURTIER,[a] M.-C. ANTAL,[b,c] B. COSQUER,[a] A. SCHUMACHER,[d] B. SAMAMA,[b,c] M.-J. ANGST,[b] A. FERRANDON,[b] E. KONING,[b] J.-C. CASSEL[a] AND A. NEHLIG[b]*

[a]*Laboratoire d'Imagerie et de Neurosciences Cognitives, UMR, 7237 Université de Strasbourg/CNRS, Strasbourg, France*

[b]*INSERM U666, Université de Strasbourg, Faculté de Médecine, Strasbourg, France*

[c]*Université de Strasbourg, Faculté de Médecine, Institut d'Histologie, Strasbourg, France*

[d]*Neurochemical Research Group, Department of Neurology, University of Lübeck, Germany*

Abstract—Neonatal ventral hippocampal lesions (NVHL) in rats are considered a potent developmental model of schizophrenia. After NVHL, rats appear normal during their preadolescent time, whereas in early adulthood, they develop behavioral deficits paralleling symptomatic aspects of schizophrenia, including hyperactivity, hypersensitivity to amphetamine (AMPH), prepulse and latent inhibition deficits, reduced social interactions, and spatial working and reference memory alterations. Surprisingly, the question of the consequences of NVHL on postnatal neurobehavioral development has not been addressed. This is of particular importance, as a defective neurobehavioral development could contribute to impairments seen in adult rats. Therefore, at several time points of the early postsurgical life of NVHL rats, we assessed behaviors accounting for neurobehavioral development, including negative geotaxis and grip strength (PD11), locomotor coordination (PD21), and open-field (PD25). At adulthood, the rats were tested for anxiety levels, locomotor activity, as well as spatial reference memory performance. Using a novel task, we also investigated the consequences of the lesions on procedural-like memory, which had never been tested following NVHL. Our results point to preserved neurobehavioral development. They also confirm the already documented locomotor hyperactivity, spatial reference memory impairment, and hyperresponsiveness to AMPH. Finally, our rseults show for the first time that NVHL disabled the development of behavioral routines, suggesting dramatic procedural memory deficits. The presence of procedural memory deficits in adult rats subjected to NHVL suggests that the lesions lead to a wider range of cognitive deficits than previously shown. Interestingly, procedural or implicit memory impairments have also been reported in schizophrenic patients.

Key words: schizophrenia, rodent model, neurobehavioral development, procedural memory.

*Corresponding author. Tel: +33-03 68 85 32 43; fax: +33-03 68 85 32 56.
E-mail address: nehliga@unistra.fr (A. Nehlig).
Abbreviations: AMPH, amphetamine; ANOVA, analyses of variance; APO, apomorphine; NVHL, neonatal ventral hippocampal lesions; PD, postnatal day.

0306-4522/12 $36.00 © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2012.01.040

The bilateral neonatal ventral hippocampal lesion (NVHL) in rats is accepted as a neurodevelopmental model of schizophrenia. NVHL induces deficits occurring after puberty, therefore, closely modeling the time of onset of the pathology in humans, that is, during late adolescence. The deficits in rats resemble many of the symptoms seen in schizophrenic patients, including social withdrawal, sensory gating deficits, hyperresponsiveness to dopaminergic drugs such as amphetamine (AMPH), hypersensitivity to stress, alterations of working and reference memory in spatial tasks (see Tseng et al., 2009 for review), and set-shifting deficits (Brady, 2009).

Despite the large amount of data gathered during the past 20 years, some questions remain open, including the effects of NVHL on prepubertal neurobehavioral development of the rats. Indeed, many of the impairments observed at adulthood might be provoked or precipitated by defective development through early ages, leading to both motoric dysfunctions and impaired behavioral coordination. A second question that still needs to be addressed is the level of performance of NVHL rats in a paradigm assessing procedural memory. Indeed, the presence of procedural memory deficits in schizophrenic patients is still a matter of debate. Although some authors reported clear-cut procedural (or implicit) memory impairments (Bigelow et al., 2006; Exner et al., 2006; Foerde et al., 2008; Green et al., 1997; Horan et al., 2008; Kumari et al., 2002; Schwartz et al., 1996; Weickert et al., 2002), others did not find such deficits in schizophrenic patients (Danion et al., 2001; Gomar et al., 2011; Gras-Vincendon et al., 1994; Kern et al., 1997, 2010; Siegert et al., 2008; Takano et al., 2002; see also for review Gold et al., 2009) or evidenced a slower but still efficient processing (Perry et al., 2000; Schérer et al., 2003).

Therefore, in the present study, NVHL were performed as classically described at postnatal day (PD) 7, and three main goals were pursued. First, besides weight gain, we explored the consequences of NVHL on classical parameters of neurobehavioral maturation, as previously validated (Bouayed et al., 2009; Raffo et al., 2009; Schroeder et al., 1995); evaluations were made from PD11 to PD25 and included negative geotaxis, grip strength, locomotor coordination, and open-field testing. Our second goal was to replicate previous findings to validate our lesion approach. Therefore, we assessed anxiety levels in the elevated plus-maze and spatial reference memory in the water maze. We also measured locomotor activity in reaction to a novel environment, and subsequently, over the diurnal and the nocturnal periods, a comparison never performed

to date. Furthermore, locomotor activity was also quantified after apomorphine (APO) or AMPH treatment. Finally, our last goal was to assess procedural memory capabilities in NVHL rats using a novel water maze task, namely the double-H maze (Pol-Bodetto et al., 2011), which has been designed to train rats to quick acquisition and retention of a behavioral routine.

EXPERIMENTAL PROCEDURES

Subjects

Female Sprague–Dawley rats with eight male pups provided by Charles River Breeding Center (L'Arbresle, France) were housed under controlled, uncrowded conditions at 20–22 °C (light/dark cycle, 7:00 AM–7:00 PM lights on). Food and water were available *ad libitum*. All animal experimentation was performed in accordance with the rules of the European Communities Council Directive of November 24, 1986 (86/609/EEC), and the French Department of Agriculture (License N° 67–97 to A.N. and N° 67–215 to J.C.C.). The protocol was approved by the ethical Animal Research Committee Board of Louis Pasteur University (CREMEAS # AL/01/19/10/07).

Neonatal hippocampal lesion

Surgical procedures have been described previously (François et al., 2009, 2010). PD7 pups, anesthetized by isoflurane inhalation, were placed in the stereotaxic apparatus over a heating pad (37 °C) and received bilateral infusions of either ibotenic acid (0.3 μl; 10 μg/μL in artificial cerebrospinal fluid [aCSF], pH 7.4; NVHL group, $n=25$) or aCSF (0.3 μL; sham group, $n=8$) into the ventral hippocampus (AP: −3.0 mm; ML: ±3.5 mm; DV: −5.0 mm from bregma). Rats were allowed to recover on a heating pad before being returned to their home cage with the dam. After completion of the open-field testing, around PD25/PD26, rats were weaned and housed in cages of four. Subsequently, at adulthood, around PD56, rats were single-housed, moved to another facility to be tested on a battery of behavioral tasks. All rats were left unhandled for 10 days to permit their habituation to the new facility, after which they were handled for 5 min/d for a couple of days and subsequently tested. The different tests used and their chronological order are indicated on a time scale in Fig. 1. All tests were performed by experimenters blind to the surgical conditions.

Negative geotaxis

This reflex was tested at PD11. Pups were positioned on an inclined plane with a 20% slope covered with antislippery material, their head facing downward. The time (limited to 120 s) needed by the pups to turn completely and reach a position with the head facing upward was measured.

Grip strength

Pups were suspended with their forepaws grasping a metal rod (1 mm diameter), stretched between two poles of a frame at about 20 cm above the table covered with a thick layer of cotton. A plexiglas sheet was placed in front of the rat to prevent turning around the rod. The latency to fall off the wire was recorded. There was no time limit for this test.

Locomotor coordination

This test, adapted from Altman and Sudarshan (1975), was performed at PD21. Rats were placed in a round container (15 cm in diameter and 23 cm in height) half-filled with water. They had to swim until finding a metal rod (8 mm in diameter) located deep enough inside the water so that they necessarily encountered it. Then they had to climb along the rod (35 cm) to escape from the water, until reaching and landing onto a horizontal platform located almost at the top of the metal rod. The time taken by the rats to reach a final quadruped posture on the platform was recorded.

Open field

The open-field testing was performed at PD25. The setting consisted in a circular arena (48 cm in diameter) subdivided into three distinct areas: periphery (36 sections of identical size), intermediate (12 sections of identical size), and central (four sections of identical size). Each animal was gently placed in the arena facing the wall of the maze. The testing lasted 5 minutes and was videotaped. The number of entries within the three different areas, as well as the number of rearings were counted and subsequently pooled.

Elevated plus maze

This test was performed at PD64. The elevated plus-maze was made of black Plexiglas and consisted of four arms (50 cm long×10 cm wide) fixed to a central platform (10×10 cm^2): two with 40-cm high sidewalls (closed arms) and two with 1.5-cm high borders (open arms). The closed and open arms crossed at a right angle. The maze was elevated to a height of 73 cm above the floor. The testing room was equipped with four halogen lamps positioned on the "diagonals" of the plus maze (45° of each arm), in each corner of the room, at an identical distance from the central platform. Using a luxmeter, light adjustments were made to have the same intensity on both open arms (i.e. 10 Lux; e.g. Koenig et al., 2008). Rats were placed individually in the center of the maze, their head facing a closed arm, and the test lasted for 5 min. Each rat was followed on a monitor connected to a camera (fixed on the roof, 180 cm above the floor level). An entry in a given arm was counted when the rat had all four paws in the arm. Between two rats, the apparatus was cleaned with 100% ethanol. Data analyses considered the total number of entries and the total amount of time spent in open vs. closed arms.

Morris water maze test

This test was performed from PD69 to PD74.

Apparatus. The Morris water maze consisted of a circular white plastic pool, 160 cm in diameter and 60 cm in depth, filled with water at room temperature (23±1 °C). The water was made

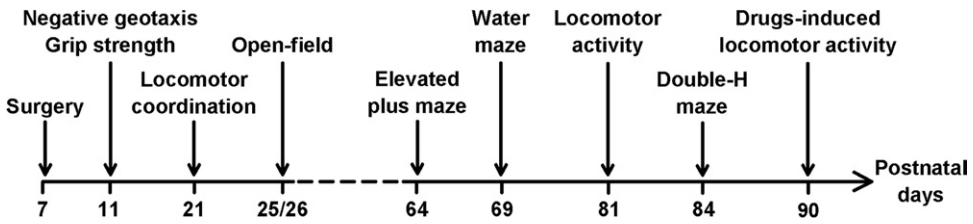

Fig. 1. Schematic representation of the time course of the different behavioral tests performed during the present study.