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Selective deficits in spatial working memory in the neonatal ventral hippocampal lesion rat model of schizophrenia

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ABSTRACT

The neonatal ventral hippocampal lesion (NVHL) manipulation is a neurodevelopmental animal model of schizophrenia that produces abnormalities in the prefrontal cortex and nucleus accumbens, both efferent targets of the hippocampus, and leads to spatial working memory impairments. To investigate the neuroanatomical basis of spatial working memory in NVHL animals, we assessed performance in two radial arm maze tasks known to be differentially sensitive to the two hippocampal efferent pathways, and measured levels of neuronal activation (Fos immunoreactivity [Fos-IR]) in the prefrontal cortex and nucleus accumbens following task performance. Neonatal rats (postnatal day 6–8) received excitotoxic lesions of the ventral hippocampus (n = 25), or a sham procedure (infusions of artificial cerebrospinal fluid; n = 22). Upon reaching adulthood, animals were trained in either a non-delayed random foraging task or a spatial delayed win-shift task. NVHL animals were impaired on the spatial delayed win-shift task, which depends on communication between hippocampus and prefrontal cortex, but were unimpaired on the non-delayed random foraging task, which requires connections between hippocampus and nucleus accumbens. Fos-IR in the nucleus accumbens was greater in NVHL animals than in shams following the random foraging task, despite similar levels of performance, while no group differences in Fos-IR in either the nucleus accumbens or prefrontal cortex were observed following win-shift performance. These results suggest that although the NVHL manipulation disrupts development of hippocampal efferents to both the prefrontal cortex and the nucleus accumbens, the disruption of hippocampal–prefrontal pathways has the dominant behavioral effect on spatial performance in NVHL rats.

1. Introduction

Schizophrenia is widely believed to have neurodevelopmental origins (Lewis and Levitt, 2002). One of the best characterized neurodevelopmental animal models of schizophrenia is the neonatal ventral hippocampal lesion (NVHL) model, originally developed by Lipska and colleagues (Lipska et al., 1993; Tseng et al., 2009). Early excitotoxic damage to the hippocampus leads to structural and functional abnormalities in the primary efferent targets of the hippocampus, the prefrontal cortex (PFC) and nucleus accumbens (NAcc), in adult animals. Neurons in both regions display reduced dendritic spine density (Flores et al., 2005; Marquis et al., 2008b) and excessive firing in response to dopaminergic input (Goto and O'Donnell, 2002; O'Donnell et al., 2002) in NVHL animals tested as adults. Furthermore, PFC neurons in adult NVHL animals respond abnormally to dopaminergic, glutamatergic, and GABAergic inputs (Tseng et al., 2007, 2008).

As adults, NVHL rats exhibit a variety of abnormal behaviors, some of which reproduce elements of clinical schizophrenia, including hypersensitivity to psychostimulants, reduced social interactions, and impaired prepulse inhibition (Lipska and Weinberger, 2000). Cognitive functions, including working memory (Lipska et al., 2002; Marquis et al., 2008a) and set-shifting (Brady, 2009; Marquis et al., 2008b), are also disrupted in NVHL animals. Such cognitive deficits are also observed in patients with schizophrenia (Pantelis et al., 1999; Silver et al., 2003). In NVHL animals, these non-spatial cognitive impairments are likely to arise from dysregulations of hippocampal–prefrontal and/or hippocampal–accumbens pathways, above and beyond damage to the hippocampus *per se*. Adult NVHL animals display deficits similar to those of animals with primary PFC damage (Brady, 2009; Lipska et al., 2002). Damage to the adult hippocampus does not impair working memory (in a relatively non-spatial task) as it does in NVHL animals (Lipska et al., 2002), and set-shifting deficits in humans are not sensitive to hippocampal damage (Chudasama and Robbins, 2006).

However, the NVHL manipulation also produces impairments on spatial, hippocampal-dependent learning and memory tasks in

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the radial arm maze (Chambers et al., 1996; Levin and Christopher, 2006) and Morris water maze (Le Pen et al., 2000; Silva-Gomez et al., 2003). Such spatial impairments parallel those reported in patients with schizophrenia (Glahn et al., 2003; Hanlon et al., 2006; Lee et al., 2008; Park et al., 1995; Saperstein et al., 2006). In the NVHL animal, it is unclear whether such spatial memory deficits are caused by primary damage to the hippocampus, or by aberrant development of efferent pathways to the PFC and/or the NAcc. Performance in two particular radial arm maze tasks has been linked to these two hippocampal projections, respectively. The non-delayed random foraging task (NDRF) and spatial delayed win-shift (SDWSh) task are both sensitive to hippocampal damage, but the NDRF task depends on the hippocampus–NAcc pathway while the SDWSh task requires an intact hippocampus–PFC connection (Floresco and Phillips, 1999; Floresco et al., 1997; Seamans et al., 1998). Here, we used these tasks to investigate the neuroanatomical basis of spatial working memory deficits in NVHL animals, and measured levels of neuronal activation (Fos immunoreactivity) among cells in the PFC and NAcc following task performance.

2. Methods and materials

2.1. Subjects and surgery

Timed pregnant Sprague-Dawley females were obtained at embryonic day 15–18 from Charles River (Wilmington, MA), and were individually housed with free access to food and water on a 12 h:12 h light/dark cycle (lights on at 7:00 am). Between PD6 and PD8, male pups (15–20 g) received either an excitotoxic lesion of the ventral hippocampus (NVHL; n = 25) or a sham procedure (n = 22), as previously described (Brady, 2009). At approximately PD28, animals were weaned and housed in pairs or groups of three. Upon reaching adulthood (PD56), animals were single-housed, and were handled for 2 days before beginning food restriction and behavioral training. These experiments were conducted in accordance with the US Public Health Service *Guide for the Care and Use of Laboratory Animals*, and all procedures were approved by the Institutional Animal Care and Use Committee at St. Mary's College of Maryland.

2.2. Behavioral testing

All behavioral testing took place in an 8-arm radial arm maze, constructed in-house. The maze had an octagonal center platform 29 cm in diameter, with eight arms (57 cm long, 11 cm wide) extending out radially. Each arm was bounded by 5 cm walls, and contained a sunken food cup (1 cm deep) at the end distal to the center platform. The maze was painted black and placed in the center of a square room (65 cm off the floor) with white drapes covering the walls. Fixed spatial cues (black and white geometrical designs) were located on three of the walls. The position of the experimenter was also fixed and served as an additional spatial cue. Before beginning behavioral testing, animals were restricted to approximately 90% of their free-feeding weight. Sucrose pellets (45 mg Noyes Precision Pellets, Research Diets, Inc., New Brunswick, NJ) were used for reinforcements in the maze. Before beginning training in one of the tasks described below, animals underwent two days of acclimation to the radial arm maze during which they were placed in the maze (without sucrose pellets) for 5 min and allowed to freely explore. Following each acclimation period, rats were given approximately 10 sucrose pellets along with their daily ration of lab chow in the home cage.

2.2.1. Non-delayed random foraging task (NDRF)

The NDRF task requires animals to retrospectively forage for four sucrose pellets located in a randomly chosen set of arms (Floresco et al., 1997). Animals (NVHL n = 9, sham n = 8) were tested once per day. On each day, four arms were baited with one sucrose pellet placed in each food well. A novel set of four arms was chosen each day using a random number generator, and the same set of arms was used for each animal tested on a given day. All arms were unblocked. Animals were placed in the center of the maze, facing a randomly selected arm, chosen by a random number generator and consistent across a day's testing. Animals were then allowed to explore the maze until all four pellets were retrieved or 5 min had elapsed. Errors were defined as re-entries to any arm, and were broken down into baited and unbaited errors. Other measures taken included latency to reach the first food cup (baited or unbaited) and time per arm excluding the first arm visited, calculated as [(Latency to complete task – Latency to reach first cup)/(Number of arms visited – 1)]. The maze was cleaned with an antimicrobial agent in between each rat. Criterion performance in the NDRF task was defined as three consecutive days with one or fewer errors each. Animals were tested for 20 days (one trial per day), and data were analyzed in 5 blocks of 4 days each.

2.2.2. Spatial delayed win-shift task (SDWSh)

The SDWSh task requires animals to prospectively forage for four sucrose pellets by retaining and using knowledge across a variable delay period (Floresco et al., 1997). Animals (NVHL n = 16, sham n = 14) were tested once each day on both a training phase and a test phase. In the training phase, four arms were baited with one sucrose pellet each, and the other four arms were blocked with black plastic boxes (9.5 cm L × 11.5 cm W × 16.5 cm H) weighted with sand. A novel set of four arms to be baited was chosen each day using a random number generator, and the same set of arms was used for each animal tested on a given day. Animals were placed in the center of the maze, facing a randomly selected arm, chosen by a random number generator and consistent across a day's testing. Animals were then allowed to explore the four open arms on the maze until all four pellets were retrieved or 5 min had elapsed. Errors in the training phase were defined as re-entries to an arm. Other measures taken included latency to reach the first food cup, latency to complete the training phase, and time per arm excluding the first arm visited, calculated as above for the NDRF task. Following the training phase, animals were returned to the home cage for a delay period of 5 min until the test phase. In the test phase, the four arms that were previously blocked were baited with one sucrose pellet each. All arms were open in the test phase. Animals were placed in the center of the maze facing the same start arm as in that day's training phase, and were given 5 min to retrieve all four pellets. Errors in the test phase were defined as either across-phase errors (initial entries to unbaited arms) or within-phase errors (re-entries to any arm, baited or unbaited). Other measures taken included latency to reach the first food cup (baited or unbaited) and time per arm, calculated as above. The maze was cleaned with an antimicrobial agent in between each phase, and in between each rat. Criterion performance in the SDWSh task was defined as two consecutive days with one or fewer test phase errors each. Once animals reached criterion performance at the 5 min delay, the delay period was lengthened to 30 min starting with the next day of training. All animals were then tested for 20 days (one trial per day) at the 30 min delay, and data was analyzed in 5 blocks of 4 days each.

2.3. Histological processing

Two hours following completion of the last day of behavioral testing (day 20), animals were transcardially perfused with cold saline (0.9%), followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed and post-fixed in 4% paraformaldehyde for at least 24 h before being transferred to 30% sucrose in 0.1 M phosphate buffer for cryoprotection. Sections (40 μm) through the prefrontal cortex (PFC), nucleus accumbens (NAcc), and hippocampus were taken using a freezing microtome. Sections were stored at 4 °C in 0.1 M phosphate buffer with 0.02% azide until processing, as described below.

2.3.1. Lesion verification

Sections through the hippocampus were mounted on glass slides and Nissl stained using cresyl violet. The hippocampus was examined microscopically for evidence of bilateral damage, which typically included cell loss, thinning, enlarged ventricles, gliosis, and/or cellular disorganization. Each left and right hemisphere was assigned a score of between 0 and 3 based on the amount of observed damage or disorganization (0 = no damage; 1 = minor; 2 = moderate; 3 = severe). Each hemisphere's score was summed to produce a total score ranging from 0 to 6 for each animal (Sams-Dodd et al., 1997). NVHL animals with scores of 0 on either hemisphere, or any animals with significant damage to surrounding areas (e.g. thalamus), were removed from the study.

2.3.2. Immunohistochemistry and Fos cell counting

Sections through the PFC and NAcc were processed for Fos immunoreactivity at room temperature. Briefly, sections were rinsed with Tris-buffered saline (TBS), blocked with normal horse serum, and incubated overnight with polyclonal rabbit anti-Fos (Calbiochem/EMD Biosciences, Gibbstown, NJ) at a dilution of 1:10,000. Subsequently, sections were rinsed with TBS and exposed to biotin-SP-conjugated affini-pure donkey anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA) at a dilution of 1:1000. Following rinses in TBS, sections were incubated in peroxidase-conjugated streptavidin (Jackson Immunoresearch) at a dilution of 1:1600. Sections were developed using diaminobenzidine (DAB), mounted on glass slides, cleared in xylenes, and coverslipped. Cells positive for Fos immunoreactivity (Fos-IR) were viewed through a light microscope and manually counted in the medial prefrontal cortex (PFC) and the nucleus accumbens (NAcc) medial core region. A rectangular counting field of 0.6 mm H by 0.3 mm W was used for the PFC, and a square counting field of 0.3 mm by 0.3 mm was used for the NAcc. All visible stained cells (regardless of staining intensity) were counted. The raw number of Fos-IR cells was normalized to cells per 0.1 mm^2 for each region.

2.4. Statistical analysis

Independent t-tests were used to compare NVHL and sham animals on the number of days to criterion in the NDRF task, and the number of Fos-IR cells counted in the PFC and NAcc following each task. Relationships between lesion score and behavioral performance (in NVHL animals only) were analyzed with separate