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Neonatal hippocampal lesion alters the functional maturation of the prefrontal cortex and the early cognitive development in pre-juvenile rats

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ABSTRACT

Mnemonic and executive performance is encoded into activity patterns of complex neuronal networks. Lesion studies revealed that adult recognition memory critically depends on the activation of the prefrontal cortex (PFC) and hippocampus (HP). However, its developmental profile remains poorly elucidated. We previously showed the rat PFC and HP are functionally coupled in theta- and gamma-band oscillations during neonatal [postnatal day (P) 5–8] and pre-juvenile (P10–15) stages of development. Here, we assess the behavioral readout of this early prefrontal–hippocampal activation by investigating the ontogeny and the mechanisms of novelty detection and recognition memory in relationship to the functional integrity of the PFC and HP. Excitotoxic lesion of the HP at birth led to abnormal oscillatory entrainment of the PFC throughout neonatal and pre-juvenile development. Although the onset of novelty detection correlated rather with the maturation of sensory perception and motor skills than with hippocampal integrity, the pre-juvenile performance in item, spatial and temporal order recognition memory significantly decreased after HP lesion at birth. This poorer performance does result neither from abnormal developmental milestones and locomotion nor from increased anxiety. Thus, novelty recognition in rat emerges during the second postnatal week and requires functional integrity of communication within neuronal networks including the PFC and HP.

1. Introduction

Recognition memory allows the discrimination of novelty vs. familiarity according to previous experience. It involves making judgments about whether an individual item (“what”) or a spatial (“where”) and temporal (“when”) arrangement of items has been encountered before. Correspondingly, the item, spatial and temporal order recognition memory have different anatomical substrates and involve information processing within distinct neuronal networks (Steckler, Drinkenburg, Sahgal, & Aggleton, 1998). Since the pioneering clinical study by Scoville and Milner (1957) that linked the medial temporal lobe amnesia with hippocampal damage, the HP has been considered as main locus for mnemonic abilities in general and for recognition memory in particular. However, studies in rodents were less clear-cut. While the contribution of the HP for item recognition is still controversial (Bussey, Duck, Muir, & Aggleton, 2000; Clark, Zola, & Squire, 2000; Langston & Wood, 2010), rats with lesion of the HP or fornix do not remember whether the stimulus occurred in a particular space or temporal coordinates (Barker & Warburton, 2011; Bussey et al., 2000; Fortin, Agster, & Eichenbaum, 2002; Kesner & Novak, 1982). The anatomical substrate of object recognition appears to be the perirhinal cortex (PRH) (Barker, Bird, Alexander, & Warburton, 2007; Brown, 2008; Brown, Warburton, & Aggleton, 2010). Selective lesion of the PRH left the spatial recognition memory intact, but disrupted the ability to recognize single items (Barker et al., 2007; Brown & Aggleton, 2001; Mumby & Pinel, 1994) and to integrate different senses (Albasser et al., 2011). The third area critically required for recognition memory is the PFC. Its selective damage precluded the judgment of the temporal order of item presentation (Barker et al., 2007; Hannesson, Howland, & Phillips, 2004; Kesner & Holbrook, 1987).

Although these findings highlight the function of various cortical areas, different forms of recognition memory do not have localized representations but are rather encoded in the dynamic entrainment of cortical networks (Barker & Warburton, 2011; Steckler, Sahgal, Aggleton, & Drinkenburg, 1998). While the PFC is reciprocally connected with the PRH, it receives a direct unidirectional drive from the HP (Conde, Maire-Lepoivre, Audinat, & Crepel, 1995; Jay & Witter, 1991; Siapas, Lubenov, & Wilson, 2005; Thierry, Gioanni, Degenetais, & Glowinski, 2000). The

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feed-back prefrontal–hippocampal communication involves the entorhinal cortex or thalamus (Hasselmo, 2005; Vertes, 2006). The interactions within the prefrontal–perirhinal–hippocampal network have been recently shown to account for spatial and temporal recognition memory (Barker & Warburton, 2011).

Novelty detection is not inherited but maturates during early postnatal development as one of the first mnemonic abilities. Since novelty recognition relies on rat' intrinsic exploratory drive to investigate new stimuli and consequently, lacks overt stress components, such as food or water deprivation and forced swimming, assessment of recognition memory is an ideal experimental procedure for cognitive testing of young rodents. However, very few studies focused on the ontogeny of recognition memory (Anderson et al., 2004; Reger, Hovda, & Giza, 2009). Our preliminary data suggest that the ability to distinguish novel items emerges towards the end of the second postnatal week and requires full maturation of sensory (visual and tactile) perception and motor performance (Krüger & Hanganu-Opatz, 2012). During this developmental time-frame the neonatal PFC morphologically and functionally maturates (Van Eden & Uylings, 1985). Its entrainment in oscillatory rhythms emerges during the first postnatal week under the influence of driving hippocampal theta activity. Towards the end of the second postnatal week the communication within prefrontal–hippocampal network switches to mutual interactions (Brockmann, Pöschel, Cichon, & Hanganu-Opatz, 2011). Whether the maturation of recognition memory critically depends on the development of functional communication between the PFC and HP is still unknown. Here, we assess the impact of excitotoxic hippocampal lesion at birth on the pre-juvenile network activity of the PFC and the performance in recognition memory tasks. We provide evidence that early damage of the HP affects the functional integrity of the PFC throughout postnatal development and disrupts the initial ability to recognize novel items as well as their spatial and temporal arrangement.

2. Materials and methods

All experiments were performed in compliance with the German laws and the guidelines of the European Community for the use of animals in research and were approved by the local ethical committee. Pregnant Wistar rats were obtained at 14–17 days of gestation from the animal facility of the University Medical Center Hamburg-Eppendorf, housed individually in breeding cages in a temperature- and humidity-controlled vivarium with a 12 h light/12 h dark cycle and fed *ad libitum*.

2.1. Immunotoxin injection

Anesthetized male P0 pups were placed on a preformed mold and immobilized with tapes. A 26GA straight needle (WPI) attached to a microsyringe pump controller (Micro4, WPI) was used to inject at a slow rate (0.1 μl/min) 20–50 nl of N-methyl D-aspartate (NMDA) (Millipore, Schwalbach, Germany, 20 μg/μl, solved in 0.1 M PBS, pH 7.4) or 20–50 nl of vehicle (0.1 M PBS, pH 7.4) into the intermediate and ventral HP (2 mm posterior to bregma, 2 mm lateral to midline and 2.5–3 mm ventral to pial surface, angle 15°). After injection, the needle was left in place for additional 1–3 min to allow optimal diffusion of the solution. Under anesthesia, the scalp wound was closed with tissue adhesive and the pups were tattooed on the paw or on the tail with animal tattoo ink (Raidex, Dettingen, Germany) using a 27G needle. Pups were returned to the dam only after full recovery of body temperature and activity (30–60 min) to prevent maternal cannibalism. In each investigated litter, PBS- and NMDA-treated pups were observed daily (general and feeding behavior, weight).

2.2. Surgical preparation and recording protocol

Extracellular recordings were performed in the PFC (2.5 mm anterior to bregma and 0.5–0.7 mm from the midline) of postnatal day (P) 17 male rats (Paxinos & Watson, 1998) using experimental protocols as described previously (Brockmann et al., 2011; Janiesch, Krüger, Pöschel, & Hanganu-Opatz, 2011). Briefly, under light urethane-anesthesia (0.5–1 g/kg; Sigma–Aldrich, Taufkirchen, Germany), the head of the pup was mounted into the stereotaxic apparatus (Stoelting, Wood Dale, IL) using two metal bars set on place with dental cement on the nasal and occipital bones, respectively. The bone over the PFC was carefully removed by drilling holes of less than 0.5 mm in diameter. Removal of the underlying dura mater by drilling was avoided, since leakage of cerebrospinal fluid or blood attenuates the cortical activity and single neuronal firing (I.L. Hanganu-Opatz, unpublished observations). The body of the animals was surrounded by cotton and kept at a constant temperature of 37 °C by placing it on a heating blanket. After a 20–60 min recovery period, multielectrode arrays (Silicon Michigan probes, NeuroNexus Technologies, Ann Arbor, MI) were inserted perpendicularly to the skull surface into the PFC until a depth of 3 mm. The electrodes were labeled with DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine, Invitrogen, Darmstadt, Germany) to enable post-mortem the reconstruction of electrode tracks in histological sections (Fig. 1A). One silver wire was inserted into the cerebellum and served as ground and reference electrodes. Miniature earphones placed under the pup's body were sensitive enough to detect the smallest visible movements of the limbs as well as the breathing of pups during recordings. Simultaneous recordings of field potential (FP) and multiple-unit activity (MUA) were performed using one-shank 16-channel Michigan electrodes (0.5–3 MΩ). The recording sites were separated by 100 μm in vertical direction. The recording sites covered in the PFC the anatomical sub-divisions cingulate cortex (Cg) and prelimbic cortex (PL) (Van Eden & Uylings, 1985) (Fig. 1A). Both FP and MUA were recorded for at least 900 s at a sampling rate of 32 kHz using a multi-channel extracellular amplifier (Digital Lynx 4S, Neuralynx, Bozeman, MO) and the corresponding acquisition software (Cheetah).

Data were imported and analyzed off-line using custom-written tools in Matlab software version 7.7 (Mathworks, Natick, MA). To detect the oscillatory events, the raw data were filtered between 4–30 Hz and 30–100 Hz using a Butterworth three-order filter. Continuous slow oscillations in theta-alpha frequency band were detected as FP deflections exceeding five times the baseline standard deviation (SD). Movement-associated epochs were removed. Fast gamma episodes superimposed on slow events were detected either automatically or by eye and confirmed by time–frequency plots. Continuous oscillatory activity was analyzed in its max amplitude (defined as the voltage difference between the maximal positive and negative peaks) and dominant frequency as well as absolute and relative power in delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz) and gamma (30–100 Hz) frequency bands using 5–15 consecutive 60 s-long time windows. Time–frequency plots were calculated by transforming the FP events using Morlet continuous wavelet. Minimal and maximal intensities in power were normalized to values between 0 and 1 and were displayed in dark blue[1] and red, respectively. For spike sorting and analysis the raw signal was firstly high-pas filtered (>407 Hz). The threshold for detection of multiple unit activity (MUA) was individually set depending on the geometry of the recording site. As detailed previously (Nicolelis et al., 2003), the stored signals were

[1] For interpretation of color in Figs. 1–6, the reader is referred to the web version of this article.