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Neonatal hypoxia–ischemia impairs juvenile recognition memory by disrupting the maturation of prefrontal–hippocampal networks

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ABSTRACT

High-prevalence/low-severity cognitive deficits represent the life-long burden of a perinatal hypoxic–ischemic (HI) insult. They have been proposed to result from dysmaturation of prelimbic-hippocampal networks, which account for mnemonic and executive performance. Already at neonatal age the communication within these networks is largely reduced after an early HI insult with mild/moderate structural outcome. However, the long-lasting consequences of the neonatal network dysfunction remain unknown. Here, we combine MRI and electrophysiology in vivo with behavioral testing to assess the effects of an early HI insult on the structure and function of prelimbic-hippocampal networks and on related cognitive abilities of juvenile rats. Despite the absence of lesions over the prelimbic cortex (PL) and hippocampus (HP), juvenile rats experiencing an early HI have lower performance in item and temporal order recognition memory. These cognitive deficits do not result from delayed somatic development or increased locomotion or anxiety. More likely, abnormal activity patterns and interactions within prelimbic-hippocampal networks account for behavioral impairment. The early HI insult causes power reduction of the fast (12–48 Hz) network activity and diminishment of neuronal firing in the PL and HP. This weaker entrainment of local circuits at juvenile age emerges in the absence of sufficiently strong directed interactions within neonatal prelimbic-hippocampal networks. Similar developmental mechanisms may account for poorer academic achievements of HI-injured infants.

1. Introduction

Complex interactions within large-scale neuronal networks account for information storage and transfer during cognitive tasks. Especially the encoding of memories relies on functional communication between the prefrontal cortex (PFC) and hippocampus (HP) (Buzsaki, 2004; Colgin, 2011; Schwindel and McNaughton, 2011; Harris and Gordon, 2015). The co-entrainment of both areas in oscillatory rhythms via monosynaptic projections from HP to PFC (Swanson, 1981; Thierry et al., 2000) enables temporal coordination of neuronal firing that is mandatory for organizing and consolidating the engrams (Fujisawa and Buzsaki, 2011; Jones and Wilson, 2005). Moreover, these directed interactions within prefrontal–hippocampal networks emerge early in life and seem to control the development of long-range circuitry. We have previously shown that in neonatal rodents the prefrontal firing and entrainment of local networks in gamma-band activity critically depend on the hippocampal oscillatory activity (Brockmann et al., 2011). The absence of the hippocampal drive during the neonatal period disturbs the emergence of mnemonic abilities at juvenile age (Kruger et al., 2012).

In the light of their crucial function for circuit maturation, it is not surprising that the directed interactions between developing PFC and HP are highly sensitive to early injury. In particular, a hypoxic–ischemic (HI) episode shortly after birth, a developmental period corresponding to the third gestational trimester in humans (Clancy et al., 2001), caused a mild/moderate structural impairment, but profoundly perturbed the prefrontal–hippocampal interactions by decreasing the synchrony and the hippocampal drive to the prefrontal subdivision PL (Brockmann et al., 2013). These data might provide the first mechanistic insights

Abbreviations: ASL, arterial spin labeling; CCL, common carotid artery ligation; EPI, echo planar imaging; FOV, field of view; HI, hypoxic–ischemic; HIE, hypoxic–ischemic encephalopathy; HP, hippocampus; LFP, local field potential; LIA, large irregular activity; MRI, magnetic resonance imaging; MUA, multiple unit activity; P, postnatal day; PFA, paraformaldehyde; PFC, prefrontal cortex; PL, prelimbic cortex; ROIs, regions of interest; S1, primary somatosensory cortex; SEM, standard error of the mean; SO, slow oscillation; SPW, sharp wave; TE, echo time; TOF, time-of-flight; TR, repetition time; TSE, T2-weighted turbo spin echo.

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into the ontogeny of functional deficits and resulting behavioral abnormalities in hypoxic–ischemic encephalopathy (HIE) with mild/moderate outcome (Douglas-Escobar and Weiss, 2015). Due to advance in neonatal care over the last decades and decrease of mortality/morbidity after HI, this form of HIE with high-prevalence/low-severity dysfunctions became more and more frequent and thus, moved into the focus of research (de Vries and Jongmans, 2010; Robertson and Finer, 1993). A large number of longitudinal cohort studies of neurobehavioral outcome after HI injury identified subtle learning difficulties and poorer mnemonic performance, which are more pronounced as the children grow and face heavier cognitive demands (Aylward et al., 2003; Bass et al., 2004; Lindstrom et al., 2006; Marlow et al., 2005; Van Handel et al., 2007). They might result from abnormal architectural patterns in gray matter, and subtle disruption of interregional connectivity (Allin et al., 2004; Deng, 2010; Inder et al., 1999). The decreased white matter density in the frontal gyrus, miswired neural circuitry between the PFC and limbic brain areas as well as hippocampal atrophy are of particular interest in the context of HIE-related cognitive disabilities through life (Aylward, 2005; Rutherford et al., 1995).

These HIE-characteristic deficits have been proposed to reflect the dysmaturation of neuronal networks in injured infants (Back and Miller, 2014; Salmaso et al., 2014). The identified diminishment of neonatal prefrontal–hippocampal communication in a rat model of HIE supports this hypothesis, yet the mechanisms of impairment at later stages of development remain fully unknown. Here, we experimentally tested the hypothesis that weaker prefrontal–hippocampal communication at neonatal age hinders the wiring of local circuitry in the PFC and HP during development and lowers the cognitive performance. For this, we used a rat model of early HI injury with mild/moderate outcome, which we previously investigated at neonatal age (Brockmann et al., 2013), because it reliably mimics the HI-induced injury in a third trimester human fetus or preterm child with high prevalence of asphyxia and circulatory collapse (Sizonenko et al., 2003). In these rats, we combined imaging and electrophysiology in vivo with histology and behavioral investigation to assess the degree of injury, characterize the activity patterns and communication within prelimbic-hippocampal networks and test the recognition memory at juvenile age.

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 of the city Hamburg (94/08, 111/12, 132/12). All efforts were made to minimize animal suffering and the number of animals used. 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 with a 12 h light/12 h dark cycle and fed ad libitum.

2.1. Hypoxia–ischemia model

HI episodes with different outcome were induced using experimental protocols previously described (Brockmann et al., 2013). Since rodents are not prone per se to hypoxic–ischemic injury during pregnancy or at birth, the HI episode was mimicked by unilateral ligation of the common carotid artery (CCL) followed by exposure to hypoxic environment (Fig. 1). We aimed at monitoring the impact of HI on the entire developmental period of directed communication from HP to PFC during the first postnatal week. For this, the Rice–Vannucci model (Rice et al., 1981) was modified by performing the manipulation as soon as possible after birth instead of at postnatal day (P) 7. All pups considered for this study received the HI episode at P2, because the mortality rate is lower for pups manipulated at P2 than at P1 and the injury patterns are similar at both ages (McQuillen et al., 2003). The mortality rate after a HI episode at P2 was 13.9%. The investigation was performed on a total of 141 rats from 29 litters. Each litter was culled at birth to 12 pups. Due to sex-dependent differences in the outcome of neonatal HI, all experiments were conducted in male pups, which, similar to male human infants, have stronger behavioral and cognitive deficits after a HI episode (Hill and Fitch, 2012; Raz et al., 2012). The pups were anesthetized with isoflurane (5% in O_2 for induction, 2–3% in O_2 for maintenance), which does not induce ischemic tolerance, because of its short time of action (<12 min) (Sasaoka et al., 2009). A midline incision was made in the neck and the right common carotid artery was dissected, isolated from the jugular vein and vagus nerve and permanently ligated. After analgetic treatment with bupivacaine, pups were returned to the dam for 2–2.5 h for recovery. Subsequently, rats with unilateral CCL were exposed to preheated 5% O_2/95% N_2 mixture delivered at 5.3 l/min by placing them for 120–200 min into chambers floating in a water bath at 37 °C. The body temperature of the pups was kept constant at 35 °C. Sham-operated animals received the same operation without CCL or hypoxia and were used as controls. The outcome of the protocol for inducing HI was variable and assessed for each rat. Only rats classified as having a mild/moderate HI outcome (n = 24) (Supplementary Fig. 1) have been considered for further investigations.

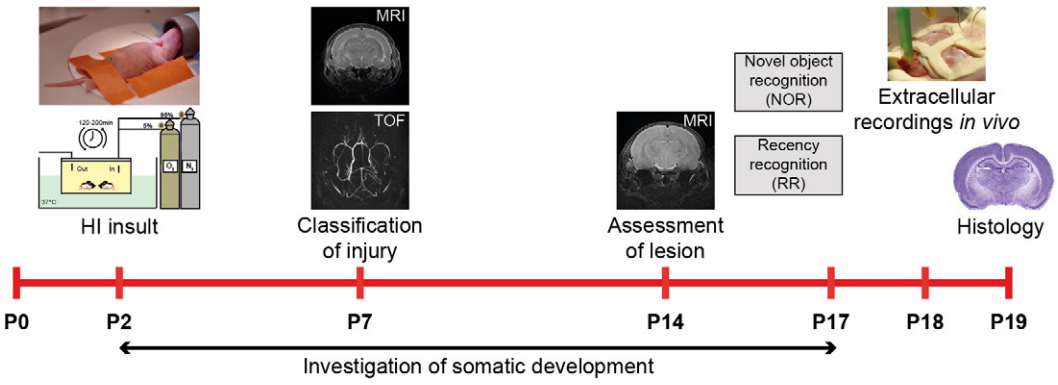

Fig. 1. Schematic diagram of the experimental protocol for inducing and investigating an early HI insult. At postnatal day (P) 2 ligation of the right common carotid artery in isoflurane-anesthetized pups was followed by maintenance into the hypoxic chamber (5% O_2/95% N_2) at 37 °C for a variable period of time (120–200 min) (modified Rice–Vannucci model). The injury was confirmed by TOF and classified as severe or mild/moderate by MRI at P6–8 using previously set criteria (Brockmann et al., 2013). The HI-induced structural changes were assessed once again at P13–14. Testing of cognitive abilities in NOR and RR tasks at P14–17 was followed by recordings of LFP and MUA in vivo at P18–19. Slices including PL, HP and S1 were Nissl-stained post-mortem. The day of birth was P0.