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Oscillatory coupling within neonatal prefrontal–hippocampal networks is independent of selective removal of GABAergic neurons in the hippocampus

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ABSTRACT

GABAergic neurons have been proposed to control oscillatory entrainment and cognitive processing in prefrontal–hippocampal networks. Co-activation of these networks emerges already during neonatal development, with hippocampal theta bursts driving prefrontal oscillations via axonal projections. The cellular substrate of neonatal prefrontal–hippocampal communication and in particular, the role of GABAergic neurons, is still unknown. Here, we used saporin-conjugated anti-vesicular GABA transporter antibodies to cause selective immunotoxic lesion of GABAergic neurons in the CA1 area of the hippocampus during the first postnatal week. Without affecting the somatic development of rat pups, the lesion impaired the generation of hippocampal sharp waves, but not of theta bursts during neonatal development. Moreover, the oscillatory entrainment and firing of neonatal prefrontal cortex as well as the early prefrontal–hippocampal synchrony were largely independent of GABAergic neurotransmission in the hippocampus. Thus, hippocampal interneurons are critical elements for the ontogeny of hippocampal sharp waves, but seem to not control the directed oscillatory coupling between the neonatal prefrontal cortex and hippocampus.

1. Introduction

During cognitive tasks the brain operates in periodic computational episodes that entrain neuronal networks in oscillatory patterns of electrical activity (Buzsaki and Draguhn, 2004). These brain rhythms with different frequencies that range from slow (<1 Hz) to fast (20–100 Hz) and very fast (>100 Hz) not only tightly correlate with the behavioral state but also support information processing within the brain by facilitating the spatiotemporal orchestration of neuronal firing (Womelsdorf et al., 2006; Buzsaki and Wang, 2012). The relevance of information integration by oscillatory entrainment of neuronal networks for higher cognitive abilities is exemplarily illustrated in the case of functional interplay between the prefrontal cortex (PFC) and hippocampus. In line with its structure and connectivity, the PFC has multifaceted functions; it combines inputs from different senses and gates attention, working-memory and decision making (Benchenane et al., 2011; Funahashi et al., 1989; Fuster, 2001; Miller, 2000; Vertes, 2006). Hippocampal theta oscillations modulate the firing of prefrontal neurons and thereby, deliver the temporal coordination, which is mandatory for information storage and transfer (Rutishauser et al., 2010; Siapas et al., 2005; Siapas and Wilson, 1998). At cellular level, the brain rhythms and related cognitive abilities are the result of complex and only partially elucidated interactions (Buzsaki, 2010; Wang, 2010). While hippocampal projections from CA1 area primarily target pyramidal neurons in the deeper layers of the PFC (Jay and Witter, 1991), selective activation of interneurons, especially of parvalbumin- (PV) positive ones, has been reported to be mandatory for the emergence of oscillatory entrainment in the PFC and its behavioral readout (Massi et al., 2012; Peyrache et al., 2011; Rossi et al., 2012). Moreover, selective lesion of GABAergic neurons in the hippocampus triggered network hyperexcitability (Antonucci et al., 2012).

Coupling of neuronal networks in oscillatory rhythms is not a hallmark of the adult brain, but rather emerges early during development. However, the highly discontinuous and fragmented temporal organization of the activity patterns in the immature networks differs remarkably from the adult one (Dreyfus-Brisac, 1962; Vanhatalo and Kaila, 2006; Vecchierini et al., 2007). In the primary sensory cortices, these bursts of activity act as functional

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Abbreviations

CB	Calbindin
Cg	Cingulate cortex
GABA	Gamma aminobutyric acid
DiI	1,1′-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine
DAPI	4′, 6′-diamino-2-phenylindole
LFP	Local field potential
MUA	Multi-unit activity
NG	Nested gamma spindle bursts
P	Postnatal day
PBS	Phosphate buffered saline
PFC	Prefrontal cortex
PL	Prelimbic cortex
PV	Parvalbumin
ROI	Region of interest
RT	Room temperature
SAVA	Saporin-conjugated anti-vesicular GABA transporter antibodies
SB	Spindle bursts
SEM	Standard error of the mean
SPWs	Sharp waves
Str. oriens	Stratum oriens
Str. pyr.	Stratum pyramidale
Str. rad.	Stratum radiatum
TNB	Tris–NaCl–blocking buffer
TNT	Tris–NaCl–Tween buffer
Unc-Ab	Unconjugated antibody

templates of later emerging cortical topography (Dupont et al., 2006; Yang et al., 2009; Hanganu-Opatz, 2010). Using extracellular and patch-clamp recordings *in vivo*, we recently initiated the elucidation of the mechanisms underlying the functional development of neuronal networks involved in cognitive processing. We showed that the neonatal PFC of rodents generate intricate theta-gamma oscillations with superimposed fast gamma episodes, which we classified as spindle bursts (SB) and nested gamma spindle bursts (NG). Discontinuous theta bursts in the hippocampus drive their generation by phase-locking the neuronal firing via synaptic pathways (Brockmann et al., 2011). Remarkably, the early entrainment of prefrontal–hippocampal networks is critical for the ontogeny of recognition memory at juvenile stages of maturation (Kruger et al., 2012).

While these findings give first insights into the communication mechanisms within developing prefrontal–hippocampal networks and highlight their possible role for pre-juvenile behavior, the contribution of distinct cellular elements to the oscillatory entrainment between PFC and hippocampus remain largely unknown. Here, we combined *in vivo* paired recordings from the neonatal PFC and hippocampus with immunohistochemical investigation and selective immunotoxic lesion of GABAergic neurons. We provide evidence that hippocampal GABAergic neurons are critical for the generation of sharp waves (SPWs) in the neonatal hippocampus, but seem to not contribute to the early prefrontal–hippocampal communication.

2. Materials and methods

2.1. Surgical preparation

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 with a 12 h light/12 h dark cycle and fed ad libitum. Extracellular recordings were performed in the PFC (2–2.5 mm anterior to bregma and 0.2–0.5 mm from the midline) and ventral hippocampus (3–5 mm posterior to bregma and 3–5 mm from the midline) of postnatal day (P) 7–8 male rats using experimental protocols as described previously (Brockmann et al., 2011). Under light urethane-anesthesia (0.125–1 g/kg; Sigma–Aldrich, Taufkirchen, Germany), the head of the pup was fixed into the stereotaxic apparatus (Stoelting, Wood Dale, IL, USA) using two metal bars fixed with dental cement on the nasal and occipital bones, respectively. The bone over the PFC and hippocampus 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 damps the cortical activity and single neuronal firing (I. Hanganu-Opatz, personal 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. During recordings, urethane anesthesia (0.1–0.2 times the initial dose) was given when the pups showed any sign of distress ($n = 2$ out of 30 pups). After a 20–60 min recovery period, multi-site recording electrodes (Silicon Michigan probes, NeuroNexus Technologies, Ann Arbor, MI, USA) were inserted perpendicularly to the skull surface into the PFC until a depth of 3 mm, and at 20° from the vertical plane into hippocampus until a depth of 2.5–3.5 mm. The electrodes were labeled with DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine, Invitrogen, Darmstadt, Germany) solved in ethanol (0.1–1 mg/ml) to enable post-mortem in histological 100 μm-thick vibratome sections the reconstruction of electrode tracks in the PFC and hippocampus (Figs. 3A and 4A). Two silver wires were inserted into the cerebellum and served as ground and reference electrodes.

2.2. Recording protocols

Simultaneous recordings of local field potential (LFP) and multi-unit activity (MUA) were performed from the PFC and hippocampus using one-shank 16-channel Michigan electrodes (0.5–3 MΩ). The recording sites were separated by 50 or 100 μm in vertical direction. The recording sites covered the prefrontal sub-divisions cingulate cortex (Cg) and prelimbic cortex (PL) (Van Eden and Uylings, 1985) and the CA1 area in the ventral hippocampus. Both LFP and MUA were recorded for at least 1800 s at a sampling rate of 32 kHz using a multi-channel extracellular amplifier (Digital Lynx 4S with no gain, Neuralynx, Bozeman, MO, USA) and the corresponding acquisition software (Cheetah). During recording the signal was band-pass filtered between 0.1 Hz and 5 kHz.

2.3. Immunotoxin injection

Anesthetized male P0 pups were placed on a preformed mold and immobilized with tapes. A 26G straight needle (World Precision Instruments, Sarasota, FL, USA) attached to a microsyringe pump controller (Micro4, WPI) was used to inject unilaterally at a slow rate (50 nl/min) 280 nl of saporin-conjugated anti-vesicular GABA transporter antibodies (SAVA) [0.9 μg/μl, solved in 0.1 M phosphate buffered saline (PBS), pH 7.4, Synaptic Systems, Göttingen, Germany], 280 nl of unconjugated rabbit anti-VGAT-C IgG (unc-Ab) (0.9 μg/μl, solved in 0.1 M PBS, pH 7.4, Synaptic Systems) or 280 nl of vehicle (0.1 M PBS pH 7.4) into the CA1 area of the ventral hippocampus (1.5 mm posterior to bregma, 3 mm lateral to midline and 2 mm ventral to pial surface at 20° from the vertical plane). 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 warmed up under a filament bulb and returned to the dam only after full recovery of body temperature and motor activity (30–60 min) to prevent maternal infanticide behavior. Rats tolerated well the SAVA dose and no premature death of pups has been observed. In each investigated litter, PBS-, unc-Ab-, and SAVA-treated pups were daily observed (general and feeding behavior, developmental milestones, reflexes). At P7-8, the pups were anesthetized, fixed in the stereotaxic apparatus as described before and investigated for their prefrontal and hippocampal activity patterns.

2.4. Behavioral analysis

Male PBS-, unc-Ab-, and SAVA-treated pups originated from litters culled at birth to max 12 pups. The litters included both males and females to avoid sex-based maternal behavioral biases (Alleva et al., 1989; Hahn and Lavooy, 2005). To minimize the influence of circadian rhythms, all behavioral tests were conducted during the light phase of circadian cycle. One investigator performed the entire testing to prevent inter-observer variability due to different handling of the pups. Pups were tested according to a modified Fox battery (Fox, 1965) for their reflexes and overall