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THETA FREQUENCY PREFRONTAL–HIPPOCAMPAL DRIVING RELATIONSHIP DURING FREE EXPLORATION IN MICE

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Abstract—Inter-connected brain areas coordinate to process information and synchronized neural activities engage in learning and memory processes. Recent electrophysiological studies in rodents have implicated hippocampal–prefrontal connectivity in anxiety, spatial learning and memory-related tasks. In human patients with schizophrenia and autism, robust reduced connectivity between the hippocampus (HPC) and prefrontal cortex (PFC) has been reported. However little is known about the directionality of these oscillations and their roles during active behaviors remain unclear. Here the directional information processing in mice was measured by Granger causality, a mathematical tool that has been used in neuroscience to quantify the oscillatory driving relationship between the ventral HPC (vHPC) and the PFC in two anxiety tests and between the dorsal HPC (dHPC) and the PFC in social interaction test. In the open field test, stronger vHPC driving to the PFC was found in the center compartment than in the wall area. In the light–dark box test, PFC to vHPC causality was higher than vHPC to PFC causality although no difference was found between the light and dark areas for the causality in both directions. In the social interaction test using *Cx3cr1* knockout mice which model for deficient microglia-dependent synaptic pruning, higher PFC driving to the dHPC was found than driving from the dHPC to the PFC in both knockout mice and wild-type mice. *Cx3cr1* knockout mice showed reduced baseline PFC driving to the dHPC compared to their wild-type littermates. PFC to dHPC causality could predict the actual time spent interacting with the social stimuli. The current findings indicate that directed oscillatory activities between the PFC and the HPC have task-dependent roles during exploration in the anxiety test and in the social interaction test.

Key words: Granger causality, theta oscillation, social interaction, anxiety, local field potential.

INTRODUCTION

The synchronization between the prefrontal cortex (PFC) and hippocampus (HPC) is thought to facilitate communications between two structures. Theta rhythms have been shown to be selectively enhanced between the PFC and HPC during mnemonic processes (Jones and Wilson, 2005; Benchenane et al., 2010). In these memory tasks when the animals acquired the task rules neurons in the PFC and HPC were more correlated and PFC neuron firing was locked to HPC theta phase of local field potentials (LFPs). Such modulations of PFC neuron activities may reflect the inputs of spatial-related information from the HPC, a structure critical for encoding location and navigation (Buzsáki, 2002; Bird and Burgess, 2008), into the PFC which regulates attention and decision making (Miller and Cohen, 2001; Dalley et al., 2004). In anxiety-related behaviors, vHPC activities were correlated with the PFC and the correlation was enhanced in the anxiogenic environments (Adhikari et al., 2010). In the anxiety test using elevated-plus maze, PFC neurons were modulated by ventral HPC theta oscillations and these PFC neurons were inversely correlated with anxiety-related measures (Adhikari et al., 2011).

The underconnectivity theory has proposed that autism is a cognitive disorder marked by underfunctioning integrative circuitry that results in deficient integration of information at the neural and cognitive levels (Courchesne et al., 2005; Just et al., 2012). Similarly, the disconnection hypothesis in schizophrenia also attributes the pathophysiology of the disease to the disrupted synaptic efficacy at circuitry level (Friston, 1999; Pettersson-Yeo et al., 2011). Using a genetic mouse model of schizophrenia which captured chromosomal deficiency to model human chromosome 22 (22q11.2) microdeletion, it was shown that *Df*(*16*)$A^{+/-}$ mice had reduced synchronization between the dHPC and the PFC (Sigurdsson et al., 2010). Theta frequency LFP coherence between the two areas also predicted the learning performance in these mice. In another mouse model of deficient synaptic pruning by microglia, *Cx3cr1* knockout mice showed reduced dHPC–PFC coherence and the coherence was correlated with social behavior (Zhan et al., 2014). Considering the commonly reported connectivity deficits in human brain-

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Abbreviations: ANOVA, analysis of variance; AR, autoregression; HPC, hippocampus; LFPs, local field potentials; PBS, phosphate-buffered saline; PFC, prefrontal cortex.

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imaging studies in schizophrenia (Uhlhaas and Singer, 2010) and autism (Schipul et al., 2011), reduction in synchronized rhythmic activities may contribute to the cognitive dysfunctions and impaired information processing that requires coordination of long-range brain structures.

In this study LFP signals were recorded from the HPC and PFC in free moving mice using a wireless data logging system. Granger causality was used to address the driving relationship between the HPC and the PFC. The Granger causality was modeled as bivariate time series and estimated using autoregression (AR) model. In the open-field test and the light–dark box test, the Granger causality was analyzed between the vHPC and the PFC. In the social interaction test the causality was analyzed between the dHPC and the PFC. It was shown that directed causal influence from the vHPC to the PFC was associated with anxiety-related behavior and PFC causal influence to the dHPC could predict social behavior.

EXPERIMENTAL PROCEDURES

Animals

Two separate cohorts of male mice were used in the anxiety tests and the social interaction test respectively. For the open-field test and the light–dark box test, C57BL/6J mice were purchased from Charles River Laboratories (Calco, Italy) and housed in ventilated cages. For the social interaction test, *Cx3cr1* knockout mice were obtained from internal EMBL breeding colony. The *Cx3cr1* knockout mice also carried a *Thy1*::*GFP* transgene and they were on a C57BL/6J congenic background (Zhan et al., 2014). Animals were kept on a 12-h light, 12-h dark cycle (lights on at 7 a.m.) with ambient temperature (21.5 ± 1 °C) and humidity (55 ± 8%). Food and water were available *ad libitum*. This study was approved by the animal ethics committee of EMBL and the Italian Ministry of Health and experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals.

Surgery

Three-to-six-month-old mice were used for the electrophysiological recording experiments. Mice were anesthetized with a mixture of ketamine and xylazine (100 mg/kg and 10 mg/kg) and placed on a heating pad which maintains the body temperature at 35 °C. The head was fixed on a stereotaxic frame with microscope. Supplemental inhaling isofluorine was provided. An incision above the mouse skull was cut and burr holes were drilled at the locations of dHPC (using bregma as reference and the depth is relative to the brain surface, 1.9 mm posterior, 1.4 mm lateral and 1.35 mm depth), vHPC (3.1 mm posterior, 3.2 mm lateral, and 3.9 mm depth) and PFC (1.8 mm anterior, 0.5 mm lateral and 1.5 mm depth). Tungsten wire electrodes (Advent Research Materials, Oxford, UK) were advanced into the brain at the above locations and these coordinates aimed at the dorsal CA1 region of HPC, the ventral part of HPC and the deep layer of medial PFC. Two additional micro screws were anchored on the posterior and anterior portions of the skull as ground and reference, respectively. The electrode wires were inserted into a 7-pin connector which serves as an interface for Neurologger recording and dental cement was carefully applied over the skull to form a headstage that protected the electrodes and wiring. After surgery, animals were housed individually and allowed at least 1 week to recover.

Open-field test

Before the test, the animals were habituated to the handling of putting on the Neurologger for three consecutive days. A dummy Neurologger with the similar shape and weight was fitted to the headstage and remained on the animal's head for at least 10 min each day. The open-field was a round arena with diameter 40 cm and the wall 20 cm. The 5-min test was started by placing the mice in the center and behavior was recorded and tracked by Viewer2 video-tracking systems (Biobserve, St. Augustin, Germany).

Light–dark box test

The light–dark box consisted of a 40 cm by 40 cm Plexiglas box in which half of the chamber contained the dark compartment. The same group of mice from the open-field test were used and the light–dark test was performed 1 week after the open-field test. The dummy Neurologger was habituated to the animal before the test. The 10-min test was started by placing the mice in the center of the light area and the mice were tracked by Viewer2 video-tracking systems.

Social interaction test

Similar habituation handling was also done before the social interaction test. The test apparatus consisted of a three-compartment box with separating plates that had opening doors for the animals to go through the compartments. Metal wire mesh tubes were placed into the outside compartments away from the door, and a same-sex juvenile (P21–P24) mouse was placed into one of the two tubes. The test started with a 5-min free exploration of the test apparatus and followed by a 10-min social interaction period. The behavior of the mice was video-tracked by Viewer2 software.

Data acquisition

Electrophysiological recordings were acquired via the wireless Neurologger system (Vyssotski et al., 2009). The LFP data were recorded wirelessly and logged onto the memory card simultaneously on the Neurologger and this ensured stable and good quality recordings. After the experiments the data were downloaded to a computer offline. The Neurologger 2A device (Brankačk et al., 2010; Zhan et al., 2014) was small and light with the weight about 2 g and the additional animal headstage was only about 1 g. The Neurologger had four recording channels and only LFP recording options were available