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Gradual emergence of spontaneous correlated brain activity during fading of general anesthesia in rats: Evidences from fMRI and local field potentials

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ABSTRACT

Intrinsic brain activity is characterized by the presence of highly structured networks of correlated fluctuations between different regions of the brain. Such networks encompass different functions, whose properties are known to be modulated by the ongoing global brain state and are altered in several neurobiological disorders. In the present study, we induced a deep state of anesthesia in rats by means of a ketamine/medetomidine peritoneal injection, and analyzed the time course of the correlation between the brain activity in different areas while anesthesia spontaneously decreased over time. We compared results separately obtained from fMRI and local field potentials (LFPs) under the same anesthesia protocol, finding that while most profound phases of anesthesia can be described by overall sparse connectivity, stereotypical activity and poor functional integration, during lighter states different frequency-specific functional networks emerge, endowing the gradual restoration of structured large-scale activity seen during rest. Noteworthy, our *in vivo* results show that those areas belonging to the same functional network (the default-mode) exhibited sustained correlated oscillations around 10 Hz throughout the protocol, suggesting the presence of a specific functional backbone that is preserved even during deeper phases of anesthesia. Finally, the overall pattern of results obtained from both imaging and *in vivo*-recordings suggests that the progressive emergence from deep anesthesia is reflected by a corresponding gradual increase of organized correlated oscillations across the cortex.

Introduction

The intrinsic complexity of brain organization allows the emergence of a wide range of different activity regimes, referred to as brain states. Ongoing brain activity during waking rest exhibits spontaneous dynamics that are characterized by highly structured patterns of correlated fluctuations known as Resting State Networks (RSN, Biswal et al., 1995; Greicius et al., 2003; Fox et al., 2005; Beckmann et al., 2005; Deco et al., 2011; Cabral et al., 2014a). In recent years, a growing number of studies have indicated the differences in spontaneous dynamics underlying different brain states, as during sleep (Horovitz et al., 2008; Larson-Prior et al., 2009), anesthesia (Kaisti et al., 2002; Boveroux et al., 2010), meditation (Brewer et al., 2011; Hasenkamp and Barsalou, 2012; Tang et al., 2012), psychedelic states (Vollenweider and Kometer, 2010; Carhart-Harris et al., 2012, 2013), and also at different states of brain development (Fransson et al., 2007, 2009). Ongoing activity observed during anesthesia and light sedation (Greicius, 2008; Stamatakis et al., 2010) shows intriguing similarities with slow-wave sleep (Horovitz et al., 2009). Moreover, it has been proposed that many mechanisms underlying anesthesia-induced loss of consciousness are also implicated in the fading of consciousness characterizing the descent to sleep (Franks, 2008; Brown et al., 2010). Many authors have investigated RSN in animals under general anesthesia (Lu et al., 2007; Pawela et al., 2008; Hutchison et al., 2010; Liu et al., 2011; Tu et al., 2011) and during wakefulness (Liang et al., 2011; Zhang et al., 2010), revealing the existence of intrinsic brain networks in primates (Mantini et al., 2011; Dawson et al., 2013) and rodents (Keilholz et al., 2012; Lu et al., 2012). The results obtained so far suggest that deeper stages of anesthesia tend to be characterized by diminished functional connectivity (Lu et al., 2007; Williams et al., 2010; Wang et al., 2011), and that the nature of such decrease is related to the anesthetic agent used (Pawela et al., 2008; Liu et al., 2013). Nonetheless, the modulation of large-scale connectivity during the spontaneous fading from a deep state of anesthesia to a lighter one is still unclear. Investigations of brain states have largely relied on the region-specific metabolic demands related to neural activity, which is at the basis of imaging techniques such as positron-emission tomography (PET, Raichle, 1980) and functional

Abbreviations: BLCs, band-limited correlations; RCNs, robust coupled nodes

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magnetic resonance imaging (fMRI, Ogawa et al., 1992; Ogawa and Sung, 2007), characterized by high spatial accuracy but limited temporal resolution. The application of high temporal resolution techniques such as electroencephalography (EEG), magnetoencephalography (MEG), and electrocorticography (ECoG) and intracortical recordings such as local field potentials (LFPs) has been crucial to elucidate the finer temporal structure of brain activity, revealing that different global states are linked to specific rhythms in humans and animals (Steriade et al., 1996; Buzsáki and Draguhn, 2004; Buzsáki, 2006). A significant portion of brain structural architecture is phylogenetically conserved in vertebrates (Striedter, 2005), with fundamental similarities among mammals (Hofman, 1989). This inter-species similarity in anatomical connectivity gives rise to the emergence of comparable patterns of organized activity, usually referred to as functional networks (for a review see Park and Friston, 2013). The primary objective of this paper is to investigate how different brain states consistently modulate network functionality in the rat, both at the macroscopic (fMRI) and mesoscopic (LFP) scales, and by means of comparing the connectivity between areas pertaining to the same or different network. Our results confirmed that different states of anesthesia are mirrored by broad changes in the underlying functional organization that occur at different spatio-temporal levels, and that the state-related emergence of large-scale functional networks is sustained by inter-areal correlated oscillations at specific frequencies. Additionally, our findings suggest the existence of a frequency-specific association between correlated activity as measured with fMRI and LFP.

Materials and methods

Animal preparation

Animals were deeply anesthetized by intraperitoneal injection of ketamine (60 mg/kg) and medetomidine (0.5 mg/kg). Brain activity was recorded from the deepest phase of anesthesia up to partial recovery. Descent and full recovery were not recorded, as they are experimentally demanding stages that often lead to artifacts. We excluded the possibility of recording full emergence from anesthesia also because the animal was not chronically implanted. The animals were continuously monitored by controlling the respiratory pattern in imaging and the heart rate during *in vivo* experiments. The animals were not paralyzed and the hind paw reflexes were regularly tested during electrophysiological recordings (see below). Atropine (0.05 mg/kg) was injected subcutaneously to prevent secretions. Body temperature was maintained at 37 °C using a water-circulating heating pump (T/Pump, Gaymar, USA). Animal age, sex, weight and body fat are factors known to modify the anesthesia metabolism, thus animals were selected that exhibited similar characteristics (all adult Wistar males, 293 ± 43 g). All the procedures were carried out in compliance with the European Community Council Directive for the care and use of laboratory animals (86/609/ECC) and with the Generalitat de Catalunya's authorization (DOGC 2450 7/8/1997, Comite Ético de Experimentación Animal, Universidad de Barcelona).

fMRI recordings

MRI experiments were conducted on a 7.0 T BioSpec 70/30 horizontal animal scanner (BrukerBioSpin, Ettlingen, Germany), equipped with a 12 cm inner diameter actively shielded gradient system (400 mT/m). The receiver coil was a phased-array surface coil for the rat brain. Each anesthetized animal ($n = 5$) was placed in the prone position in a Plexiglas holder with a nose cone for administering a mixture of 30% O2 and 70% N2, and were fixed using a tooth bar, ear bars and adhesive tape. The animals were not paralyzed during the procedure. Tripilot scans were used to ensure the accurate positioning of the animal's head in the isocenter of the magnet. Echo planar imaging (EPI) sequence started 40 min after anesthesia induction and was continuously acquired over a period of around 2.3 h with the following conditions: echo time (TE) = 50 ms, repetition time (TR) = 3 s, field of view (FOV) = 25.6 × 25.6 × 7 mm, matrix size = 64 × 64 × 7 pixels, resulting in a spatial resolution of 0.4 × 0.4 × 1 mm. Additionally, for recording purposes a T2 weighted anatomical image was acquired by using a RARE (Rapid Acquisition with Refocusing Echoes) sequence and the following parameters: TE = 11 ms, TR = 1.6 s, and same FOV, matrix size and spatial resolution as above. We obtained 7 coronal slices 2 mm thick. The resulting images were then treated in order to obtain the maximum number of isolated brain areas. Consequently, a given number of regions of interest (ROIs) were then obtained from each rat. Images were not treated for motion correction, as they presented stable positioning and alignment along the entire experiment. The selection of ROIs and corresponding spatial normalization was performed by comparing MRI images with a rat-brain atlas (Paxinos and Watson, 2004), taking into account the following criteria: first, the selected areas had to contain at least four voxels per image, but in no case could those in the limit of the area contain borders of brain or cortex or confounding limits between areas; secondly, the area had to be present in at least 80% of the voxels. The reference slices were the ones presenting medial prefrontal cortex area in the rostral side, the one presenting primary visual cortex (V1) in the caudal side, and one central slice where the hippocampal structures were identified. The intermediate slices were treated taking these previous three as a reference and identifying structures such as hippocampal formation, ventricles and corpus callosum as well as different subcortical structures. These two criteria limited the number of ROIs, which in every animal was the maximum number of regions that satisfied these objectives. Those criteria allowed the extraction of BOLD (Blood Oxygen Level Dependent) signal from 14 ROIs from each hemisphere, leading to a total of 28 ROIs in each of the 5 animals. The extracted ROIs were the primary motor cortex (M1), primary and secondary somatosensory cortices (S1, S2), primary and secondary visual cortices (V1, V2M), primary auditory cortex (A1), medial prefrontal cortex (mPF), retrosplenial cortex (Rspl), cingulate cortex (CC), thalamus (Thal), striatum (Str), amygdala (Amy), hippocampus (Hipp) and hypothalamus (Hyp). ROIs and average BOLD signals were extracted with homemade scripts implemented in Matlab (Mathworks, Natick, MA, USA). In order to discard physiological ultra-slow fluctuations of the BOLD signal (Yan et al., 2009), while maintaining those that had been previously shown to be relevant for sampling low-frequency rat brain functional networks (Hutchison et al., 2010), we removed the best-fitting linear trend from the BOLD traces and band-passed them at 0.01–0.1 Hz. Obtained signals were then standardized.

In vivo LFP recordings

Lidocaine was administered at all pressure points and incisions prior to surgery. Approximately 30 min after induction, while the anesthesia was deepest, craniotomies were performed to record from the left medial prefrontal cortex (mPF, 3.2 mm AP, 0.8 mm ML) and left and right cingulate cortex (CC, +1 mm AP, +0.8 mm ML) in 10 rats, and to record from the left primary auditory cortex (A1, −5.2 mm AP, +6.5 mm ML) and the left and right secondary somatosensory cortex (S2, −1.3 mm AP, +5.6 mm ML) in 6 animals. All coordinates are relative to bregma (following Paxinos and Watson, 2004; see Fig. 1D). Extracellular slow-wave recordings were obtained with tungsten electrodes of impedances of 1–2 MΩ (as in Ruiz-Mejias et al., 2011). Electrodes were placed in infragranular layers (3 mm deep in mPF, 2.4 mm in CC, 2.4 mm in A1 and 3.4 mm in S2). Recordings were amplified with a multichannel system (Multichannel Systems, Germany) and the signal was digitized and acquired at 10 KHz with a CED acquisition board and Spike2 software (Cambridge Electronic Design, UK). Local field potentials of the selected cortical areas were simultaneously recorded in the anesthetized rat, using the same anesthesia protocol as in imaging experiments. Extracellular recordings started 49 ± 9 (mean ± SD) minutes after induction (depending on the time needed for surgery) and continued

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