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Dynamic reorganization of intrinsic functional networks in the mouse brain

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ABSTRACT

Functional connectivity (FC) derived from resting-state functional magnetic resonance imaging (rs-fMRI) allows for the integrative study of neuronal processes at a macroscopic level. The majority of studies to date have assumed stationary interactions between brain regions, without considering the dynamic aspects of network organization. Only recently has the latter received increased attention, predominantly in human studies. Applying dynamic FC (dFC) analysis to mice is attractive given the relative simplicity of the mouse brain and the possibility to explore mechanisms underlying network dynamics using pharmacological, environmental or genetic interventions. Therefore, we have evaluated the feasibility and research potential of mouse dFC using the interventions of social stress or anesthesia duration as two case-study examples. By combining a sliding-window correlation approach with dictionary learning, several dynamic functional states (dFS) with a complex organization were identified, exhibiting highly dynamic inter- and intra-modular interactions. Each dFS displayed a high degree of reproducibility upon changes in analytical parameters and across datasets. They fluctuated at different degrees as a function of anesthetic depth, and were sensitive indicators of pathology as shown for the chronic psychosocial stress mouse model of depression. Dynamic functional states are proposed to make a major contribution to information integration and processing in the healthy and diseased brain.

Introduction

Functional connectivity (FC) is a measure of statistical interdependence of the activity traces of two brain regions, providing insight into interactions between brain areas and how they jointly support information processing (Power et al., 2014). FC analysis has gained in importance over the past decade, shedding light on large-scale brain organization by identifying a set of autonomous network modules such as the default mode network (DMN), and contributing to improved understanding of brain function and the changes underlying several brain disorders (Greicius, 2008). While information processing by the brain is a highly dynamic process requiring exquisitely orchestrated regional interactions, the majority of FC studies on spontaneous brain activity as assessed by functional magnetic resonance imaging (fMRI) assume stationarity, i.e. constant interactions throughout the duration of a resting-state scanning session. However, rapid changes in EEG microstate, i.e. coherent activation at a sub-second time scale within global functional brain networks, have been described (Van de Ville et al., 2010). Dynamic functional connectivity (dFC) aims to capture aspects of time-varying coupling patterns between regions and therefore to reveal the dynamic features of network organization. Interestingly, dynamic EEG microstates have been shown to correlate significantly with activity in fMRI resting-state networks despite the pronounced temporal filtering imposed by the hemodynamic response function (Van de Ville et al., 2010).

A wide range of approaches has been used to analyze the dynamic characteristics of the fMRI signal (Calhoun et al., 2014). Changes in FC across time can be estimated by applying a so-called *sliding-window* approach, in which the resting-state brain signals are subdivided into time-shifted segments of short duration, each of which then undergoes correlation analysis. For studies considering more than a few pairwise interactions or aiming at group-level analysis, dimensionality reduction is commonly achieved by applying multivariate techniques to the large set of FC time courses. Allen and colleagues proposed the application of

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k-means clustering to identify dynamic functional states (dFS) (Allen et al., 2014), while previous work by Leonardi et al. introduced eigenconnectivities by the application of principal component analysis (PCA) (Leonardi et al., 2013), and dictionary learning (Leonardi et al., 2014).

The dynamic nature of FC raises questions concerning the neuronal basis underlying this phenomenon, both in the normal and diseased brain. It has been suggested that dynamic FC patterns in awake humans at rest might be driven by both conscious and unconscious brain processes, which may vary across subjects (Hutchison et al., 2013), rendering the elucidation of mechanistic aspects difficult. Analogous studies in anesthetized animals serve as a powerful complementary approach to gain mechanistic insight. Hutchison et al. demonstrated the non-stationary behaviour of FC in anesthetized monkeys, proving the existence of dFS in the unconscious brain and in the absence of potential confounds due to head motion (Hutchison et al., 2013). Analysis of the dynamic properties of FC in anesthetized rats revealed similarities with dFC patterns in awake humans and monkeys (Majeed et al., 2011), and furthermore demonstrated a correlation between dFS derived from resting-state fMRI and the dynamics of electrophysiological recordings (Thompson et al., 2013). Studies in mice offer additional opportunities to examine factors regulating dFC aspects. Optogenetics (Lee et al., 2010) and pharmacological interventions (Razoux et al., 2013) may be used to modulate specific neuronal populations in order to analyze their involvement in wide-range neural networks and in dynamic network interactions. In addition, models of human pathology might indicate disease-specific alterations in dFC, which could be relevant from a mechanistic point of view or serve as early disease indicators (Grandjean et al., 2014b).

We have evaluated whether dFC analysis of anesthetized mice resting-state fMRI data enables the identification of dFS that is sufficiently reproducible to study potential alterations due to changes in physiological conditions or in response to pathological stress. The results are organized into four sections: i) quality control with regard to reproducibility of brain parcellation and stationary FC analysis, ii) estimation of reference dFS based on rs-fMRI data of healthy mice, iii) a test of dFC on surrogate data, as well as of the reproducibility and generalization of dFS in an independent dataset, and iv) analysis of the sensitivity of the approach in identifying dFC changes induced by pathology (murine model of chronic psychosocial stress) or alterations in physiological state (prolonged anaesthesia).

Stationary FC analysis revealed a segregated organization into distinct modules such as the sensory-motor cortical networks, subcortical networks, and DMN. To establish meaningful group-level components of fluctuations of FC, we applied dictionary learning to the dFC time courses obtained with sliding-window correlation. This method allows for generalization upon conventional subspace methods such as PCA and ICA by adding constraints such as temporal sparsity, bounded values, and positivity (Leonardi et al., 2014). The positivity constraint allows for discrimination of increases and decreases in connectivity, so that these are not forced to have the same temporal occurrence for the whole duration of data acquisition. Furthermore, positivity enables the capturing of strongly anti-correlated patterns as two different building blocks with anti-correlated time courses. Inclusion of temporal sparsity can be justified in view of a recent report providing evidence that the various networks are acting together, but not the whole repertoire at once (Karahanoglu and Van De Ville, 2015). This indicates that connectivity states exist economically, with only a subset being active together at a given time point.

The dFC analysis revealed dynamic interactions between and within the modules derived by stationary FC analysis. Furthermore, we show that the patterns identified by dictionary learning could be reproduced in an independent dataset using a different preprocessing pipeline, and that they are largely independent of parameter choices throughout the analytical procedure. Finally, we demonstrate that dFS might constitute sensitive indicators of: abnormal processing, as illustrated in a mouse model of psychosocial stress-induced depression-like brain and behaviour (Azzinnari et al., 2014; Grandjean et al., 2016a); and physiological adaptations, as observed during prolonged anaesthesia. The data show that dFC analysis identifies rich information on functional brain organization that remains hidden under conventional stationary FC approaches. Overall, we demonstrate that dFC analysis constitutes a number of promising research avenues with practical guidelines that can lead to better and more sensitive imaging-based biomarkers.

Materials and methods

Animals and preparation

All experiments were conducted following the Swiss federal ordinance for animal experimentation, and were licensed by the Zürich cantonal veterinary office. A total of 92 C57BL/6 mice bred in-house, 14 females and 78 males aged 8–12 weeks, were studied. Animals were kept in standard housing, with 12 h day/night cycle, and food and water provided *ad libitum*. Anesthesia was induced with isoflurane 3.5% in 1:4 O_2 to air mix. Mice were endotracheally intubated, and positioned onto an animal MRI-compatible support, equipped with a hot water-flowing bed, and ear-bars to maintain the animal stable. Mice were ventilated mechanically with a small animal ventilator (CWE, Ardmore, USA) at 80 breaths per minute, with 1.8 ml/min flow. The tail vein was cannulated to administer anaesthetic and muscle relaxant. A bolus injection of medetomidine 0.05 mg/kg and pancuronium bromide 0.2 mg/kg was administered, and isoflurane was reduced to 1.5%. After 5 min, an infusion of medetomidine 0.1 mg/kg/h and pancuronium bromide 0.4 mg/kg/h was administered, and isoflurane was further reduced to 0.5%. The temperature was monitored using a rectal thermometer probe, and maintained at 36.5 ± 0.5 °C throughout measurement. Physiological parameters during anaesthesia were acquired in 5 mice outside the magnet using a mouse pulse oximeter placed on the left hind paw (MouseOX Plus, STARR Life Sciences). During physiological testing, pancuronium was omitted to allow testing of the reflex response to forelimb pinches.

Dataset description

Functional imaging data were acquired in three separate runs, and then grouped into different datasets for the analysis. Run 1: 14 female mice imaged at baseline (ME1 dataset). Run 2: 25 male mice imaged first at baseline, during which two fMRI scans separated by 30 min were acquired. The animals then underwent a chronic psychosocial stress (CPS, see below) paradigm and were imaged post-treatment. Data from this run were included in two datasets: ME2 consisting of only the baseline session and CPS2 including both baseline and post-treatment session. Run 3: 53 male mice imaged at baseline followed by CPS paradigm and imaged post-treatment. Data from this run, for which stationary FC analysis has been reported previously (Grandjean et al., 2016a), were divided into the FIX dataset consisting of only the baseline session and CPS1 dataset including baseline and post-CPS session. These datasets were further grouped as ME(all), encompassing datasets processed with mutli-echo pipeline (ME1 and ME2), and CPS(all), encompassing all datasets from the CPS paradigm (CPS1 and CPS2). Datasets and respective acquisition procedures are detailed in Table 1.

Chronic psychosocial stress

Chronic psychosocial stress (CPS) was conducted as described previously (Azzinnari et al., 2014; Fuertig et al., 2016). Briefly, each C57BL/6 CPS mouse was placed singly in the home cage of an aggressive CD-1 mouse, separated by a transparent, perforated divider. Across 15 days, the CPS mouse was placed daily in the same compartment as the CD-1 mouse