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Altered regional connectivity reflecting effects of different anaesthesia protocols in the mouse brain

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ABSTRACT

Studies in mice using resting-state functional magnetic resonance imaging (rs-fMRI) have provided opportunities to investigate the effects of pharmacological manipulations on brain function and map the phenotypes of mouse models of human brain disorders. Mouse rs-fMRI is typically performed under anaesthesia, which induces both regional suppression of brain activity and disruption of large-scale neural networks. Previous comparative studies using rodents investigating various drug effects on long-distance functional connectivity (FC) have reported agent-specific FC patterns, however, effects of regional suppression are sparsely explored. Here we examined changes in regional connectivity under six different anaesthesia conditions using mouse rs-fMRI with the goal of refining the framework of understanding the brain activation under anaesthesia at a local level. Regional homogeneity (ReHo) was used to map local synchronization in the brain, followed by analysis of several brain areas based on ReHo maps. The results revealed high local coherence in most brain areas. The primary somatosensory cortex and caudate-putamen showed agent-specific properties. Lower local coherence in the cingulate cortex was observed under medetomidine, particularly when compared to the combination of medetomidine and isoflurane. The thalamus was associated with retained local coherence across anaesthetic levels and multiple nuclei. These results show that anaesthesia induced by the investigated anaesthetics through different molecular targets promote agent-specific regional connectivity. In addition, ReHo is a data-driven method with minimum user interaction, easy to use and fast to compute. Given that examination of the brain at a local level is widely applied in human rs-fMRI studies, our results show its sensitivity to extract information on varied neuronal activity under six different regimens relevant to mouse functional imaging. These results, therefore, will inform future rs-fMRI studies on mice and the type of anaesthetic agent used, and will help to bridge observations between this burgeoning research field and ongoing human research across analytical scales.

Introduction

Resting-state functional magnetic resonance imaging (rs-fMRI) is a popular translational fMRI approach to characterize whole brain activity in different species (Biswal, 2012; Pan et al., 2015). Rodent fMRI has been providing an increasing contribution to neuroscience research given the use of murine models in pharmacological studies (Shah et al., 2015; Shah et al., 2016) and the wide availability of transgenic mouse models (Shah et al., 2013; Grandjean et al., 2014b, Zhan et al., 2014; Haberl et al., 2015; Grandjean et al., 2016). These studies further help to translate preclinical findings to research on humans, and may help to link molecular events that are known to occur in these models to specific fMRI signatures found in both the models and human diseases. A major feature of rodent functional imaging has been the use of anaesthesia, which eases the restrain on the subjects, and controls stress levels, while awake mouse fMRI was reported impracticable (Jonckers et al., 2014). Previous studies repeatedly observed several resting-state functional networks (RSNs) in mice

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under different anaesthetic regimen (Jonckers et al., 2011; Guilfoyle et al., 2013; Grandjean et al., 2014a; Mechling et al., 2014; Nasrallah et al., 2014c; Sforazzini et al., 2014; Liska et al., 2015). Comparative studies on rodents reported agent-specific characteristics in functional connectivity (FC) patterns (Williams et al., 2010; Grandjean et al., 2014a; Jonckers et al., 2014). Although there are many points of agreements between these studies, many aspects of the anesthetic effect remain to be uncovered.

Anaesthesia is widely used in the practice of medicine and scientific research. In general, the mechanisms of actions of general anaesthetics are not yet well understood and remain an important question in medicine and neuroscience (Brown et al., 2010). Numerous findings from clinical and research applications suggest that anaesthetics do not affect the brain uniformly and do not all act in the same way. Anaesthetics cause both regionally specific suppression of brain activity and impaired interactions between distributed functional networks (Heinke and Koelsch, 2005). Many studies have examined changes of large-scale functional networks under anaesthesia, supporting the hypothesis that the anaesthetic-induced unconsciousness is a failure of information integration. The more distributed and complex a neural system is, the more vulnerable it may be to accumulated local disruptions. Although the causal relationship between regional suppression and large-scale FC changes remain unclear, alterations of local connectivity within a brain region has been proposed to be equally important for understanding unimodal and multimodal information integration (Hudetz, 2012). These notions suggest that changed functions of the brain as a whole under anaesthesia may at least partially originate from local disturbances of neuronal activity induced by drugs.

Studies investigating anaesthetic effects in human and rodents (Peltier et al., 2005; Lu et al., 2012; MacDonald et al., 2015; Song and Yu, 2015) have mainly adopted independent component analysis (ICA) and/or seed-based analysis (SBA) focusing on synchronous neural activity across the whole brain and/or brain regions that are anatomically distant from each other. However, the effects of anaesthetic agents on local neural activity driven by smaller units of neuronal organization are rarely investigated by rs-fMRI. Regional homogeneity (ReHo) measures the temporal similarity between a given voxel and its closest neighbours (Zang et al., 2004). Whereas ICA and seed-based FC analysis provide information on inter-regional synchronization of spontaneous fMRI signals, ReHo provides information on intra-regional synchronization. It has been used in many studies investigating neurological disorders in humans (Cao et al., 2006; Liu et al., 2006; He et al., 2007; Yuan et al., 2008; Wu et al., 2009; Paakki et al., 2010). In the context of anaesthesia, mapping the regional characteristics of brain activation under different anaesthesia conditions becomes relevant when investigating regional disturbances in neural activity. Furthermore, ReHo analysis presents an additional advantage over the major rs-fMRI analysis schemes, in that it requires minimal user interaction, and is therefore robust to individual bias. This is in contrast to ICA or SBA, where the user interaction is expected to provide either the number of components in the ICA decomposition, or a selection of seeds. Either approach may lead to differences between studies that make it difficult to draw direct comparisons. ReHo is also efficient in computation and very robust against noise (Jiang and Zuo, 2015). Based on voxelwise ReHo maps and motivated by the desire to gain more comprehensive information on the characteristics of regional neural activity, this study further investigated how the distribution of local connectivity among small clusters spreads within brain areas, with a specific focus on the cingulate cortex, primary somatosensory cortex (barrel field), insular cortex, caudate putamen, hippocampus and thalamus.

Anaesthetics and/or sedatives are commonly used in preclinical rodent experiments including alpha-chloralose, isoflurane or halothane, medetomidine, propofol and urethane. Alpha-chloralose is not suitable for longitudinal studies due to its depressive effects on respiration and toxicity (Haensel et al., 2015; Pan et al., 2015; Petrinovic et al., 2016). One recent study reported that this agent led to unstable physiological maintenance of mice for fMRI studies when compared to isoflurane (Low et al., 2016a). It is hence an agent unsuitable for longitudinal mouse rs-fMRI experiments. Halothane and isoflurane are inhalation anaesthetics commonly used in studies involving animals, among which isoflurane is the mostly used anaesthetic in laboratory animals (Tremoleda et al., 2012; Haensel et al., 2015). However, it is a well-known vasodilatory agent that increases the baseline cerebral blood flow in a dose-dependent way, and hence may influence neurovascular interactions that could be detected by fMRI technique. Although increasingly used in rodent studies, medetomidine induces negative effects on cardiovascular functions and causes dose-dependent vasoconstriction (Sinclair, 2003; Jonckers et al., 2015). In addition, animals under medetomidine anaesthesia were reported to show epileptic activities (Fukuda et al., 2013; Grandjean et al., 2014a). Fukuda et al. (2013) proposed a combination of low-dose isoflurane and dexmedetomidine to suppress potential epileptic activity without sacrificing the desired effects of medetomidine. In addition, the vasodilatory effect of isoflurane and the vasoconstrictive effect of medetomidine appeared to compromise each other (Fukuda et al., 2013), showing promising effects of the combination regimen for rodent fMRI experiments. This study compared changes in regional connectivity in mice using rs-fMRI under six anaesthesia regimens relevant to rodent functional imaging studies: isoflurane, two different doses of medetomidine, propofol, urethane, and a combination of medetomidine and isoflurane. After ReHo calculation, several regions of interest (ROIs) were chosen to further investigate how local connectivity distributed within these regions and differed between them. The results shed light on the accumulative and region-specific characteristics of the brain and may help bridge imaging studies in humans and mouse models.

Materials and methods

Data acquisition

Data were collected by (Grandjean et al., 2014a), who provided a detailed protocol in their paper. Briefly, female C57BL/6 mice were mechanically ventilated, paralysed with pancuronium bromide (0.5 mg/kg bolus, 0.5 mg/kg/h continuous infusion), and either anaesthetized with propofol (30 mg/kg bolus, *Propofol30*), isoflurane (1% maintenance, *Isoflurane1*), urethane (1.5 g/kg, *Urethane1.5*), medetomidine (0.1 mg/kg bolus, 0.2 mg/kg/h continuous infusion or 0.05 mg/kg bolus, 0.1 mg/kg/h continuous infusion, *Medetomidine0.1* or *Medetomidine0.05*, respectively) or a medetomidine (0.05 mg/kg bolus, 0.1 mg/kg/h continuous infusion) and isoflurane (0.5%) combination (*Mediso*). Rs-fMRI was acquired on a small animal 9.4 T MR system (Bruker BioSpin MRI, Ettlingen, Germany) equipped with a receiver only 2×2 phased array cryogenic coil. Gradient-echo echo-planar imaging (GE-EPI) data were acquired with repetition time /echo time /flip angle = 1000 ms/10 ms/90°, 360 repetitions, matrix dimension = 90 × 60, in-plane voxel dimension 263 × 233 μm^2. The complete dataset is available on XNAT online repository (http://central.xnat.org, project ID: fMRI_ane_mouse). Animal numbers for each group were 6 in *Propofol30*, 11 in *Isoflurane1*, 13 in *Urethane1*.5, 13 in *Medetomidine0.1*, 6 in *Medetomidine0.05*, 8 in *Mediso*.

Pre-processing

Images were preprocessed using FSL (FMRIB Software Library 5.0.2, http://fsl.fmrib.ox.ac.uk). The image size was multiplied by 10, and the first 10 volumes in each scan were removed to allow for the T_1 relaxation effect, followed by motion correction (MCFLIRT), B0 field correction (FAST), and brain extraction (BET). The EPI images were co-registered to the AMBMC template (Australian Mouse Brain