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Detection of functional connectivity in the resting mouse brain ☆

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ABSTRACT

Resting-state functional connectivity, manifested as spontaneous synchronous activity in the brain, has been detected by functional MRI (fMRI) across species such as humans, monkeys, and rats. Yet, most networks, especially the classical bilateral connectivity between hemispheres, have not been reliably found in the mouse brain. This could be due to anesthetic effects on neural activity and difficulty in maintaining proper physiology and neurovascular coupling in anesthetized mouse. For example, α2 adrenoceptor agonist, medetomidine, is a sedative for longitudinal mouse fMRI. However, the higher dosage needed compared to rats may suppress the functional synchrony and lead to unilateral connectivity. In this study, we investigated the influence of medetomidine dosage on neural activation and resting-state networks in mouse brain. We show that mouse can be stabilized with dosage as low as 0.1 mg/kg/h. The stimulation-induced somatosensory activation was unchanged when medetomidine was increased from 0.1 to 6 and 10 folds. Especially, robust bilateral connectivity can be observed in the primary, secondary somatosensory and visual cortices, as well as the hippocampus, caudate putamen, and thalamus at low dose of medetomidine. Significant suppression of inter-hemispheric correlation was seen in the thalamus, where the receptor density is high, under 0.6 mg/kg/h, and in all regions except the caudate, where the receptor density is low, under 1.0 mg/kg/h. Furthermore, in mice whose activation was weaker or took longer time to detect, the bilateral connectivity was lower. This demonstrates that, with proper sedation and conservation of neurovascular coupling, similar bilateral networks like other species can be detected in the mouse brain.

Introduction

Resting-state functional connectivity MRI has emerged as a method for mapping intrinsic brain networks. These networks are based on coherent brain activities that are mostly detected by the blood oxygenation level dependent (BOLD) (Biswal et al., 1995) or perfusion (Chuang et al., 2008) functional MRI (fMRI). Similar and consistent brain networks, especially the bilateral connectivity in sensory and motor related areas, have been identified across species, from humans, monkeys to rats (Fox and Richle, 2007) (R. Hutchison et al., 2010; Vincent et al., 2007). As many transgenic models of diseases are available in mouse, it is enticing to be able to apply the same technique in the mouse brain. Recently, resting-state networks have been reported in mouse using fMRI (Guilfoyle et al., 2013; Jonckers et al., 2011) and optical imaging (Bero et al., 2012) (White et al., 2011). However, despite the structural and anatomical similarities between the mouse and the rat brain, only unilateral connectivity in major cortical and subcortical areas was detected by fMRI (Guilfoyle et al., 2013; Jonckers et al., 2011).

Functional MRI studies in the mouse brain have been challenging due to various technical and physiological issues. It suffers from lower detection sensitivity and more severe susceptibility-induced image distortions and signal losses from larger air-tissue interface in the smaller brain. Strategies such as using cryo-probe (Baltes et al., 2011) or susceptibility matching material (Adamczak et al., 2010) have been explored to overcome these challenges. Since resting-state BOLD signal is only a fraction of the activated signal, the lack of bilateral connectivity in the mouse brain may be due to limited sensitivity.

Besides the technical issues, the need for proper maintenance of physiological conditions to preserve neurovascular coupling is more critical and largely dependent on the choice of anesthetics. So far, a few anesthesias have been demonstrated to allow robust BOLD activation to be measured in the mouse brain. Somatosensory BOLD activations using forepaw or hindpaw stimulation has been reported in isoflurane anesthetized mice with either free-breathing (Nair and Duong, 2004) or mechanical ventilation (Baltes et al., 2011), and in medetomidine sedated mice (Adamczak et al., 2010). In addition, anesthetics have been shown to have different impact on the resting-state networks. For example, we have shown that medetomidine can disrupt synchrony in the brain at high dose (Nasrallah et al., 2012).

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The lack of bilateral connectivity in the mouse brain may be due to the medetomidine dosage used (Jonckers et al., 2011). Another study using high isoflurane level of 1.5% reported functional connectivity in areas related to the default mode network, but no other networks (Guilfoyle et al., 2013). Since isoflurane could cause bursting activity and neural suppression (Liu et al., 2011) and has variable effects on neurovascular coupling (Masamoto et al., 2009), it is crucial to ensure that proper anesthetic level is used and proper neurovascular relationship is maintained.

In this study, we evaluated the influence of medetomidine dosage on neural activation and resting-state functional connectivity in the mouse brain using BOLD fMRI. Similar to what was found in the rat, somatosensory activation was not affected by medetomidine and bilateral connectivity can be detected in all the major brain areas at low dose of medetomidine. The dosage dependent suppression of bilateral connectivity was seen in the very high dose, which indicates different pharmacodynamics in mice compared to rats.

Materials and methods

Experimental design

To investigate the effect of medetomidine on functional connectivity in mice, resting state BOLD was assessed at two time points — 30 and 120 min after bolus injection of medetomidine – and under three dosages of medetomidine sedation – 0.1, 0.6, or 1 mg/kg/h (Fig. 1a). After 2 h of experiment, all mice were very lethargic and therefore atipamezole, an α2 adrenoceptor antagonist, was required for reversal of the effects. For such reason, the dosage effects were investigated in separate sets of animals (n = 11 for 0.1, n = 7 for 0.6, and n = 7 for 1.0 mg/kg/h dosage group) to avoid cumulative effects of the drug. Mice belonging to the 1.0 mg/kg/h dosage group were euthanized after the scan due to the poor recovery even after being antagonized.

To evaluate the hemodynamic response under medetomidine, in between the two resting-state measures, somatosensory activation was studied using electrical stimulations of different currents – 0.5, 0.75, and 1 mA – delivered to the forepaw in different runs in a pseudo-randomized manner among animals. The stimulation was applied to either the left or the right forepaw using a pair of needle electrodes inserted under the skin between digits 2 and 4 and connected to a constant current stimulator (Isostim A320, World Precision Instruments, USA). The stimulus was given by a block design paradigm of 40 s resting and 20 s stimulation alternately repeated for four times and adding 60 s of resting at the end (5 min total time). Another 5 min resting was allowed in between the runs. A pilot study was conducted to determine the optimal stimulation frequency by varying from 3, 6, to 9 Hz in a pseudo-randomized fashion in different fMRI runs with a pulse width of 0.3 ms and 1 mA. It was observed that the 6 Hz stimulus led to the strongest activation in the primary somatosensory (SI) region (data not shown), and was therefore used in the rest of the experiments. Besides, to rule out the effect of stimulation on the resting state network acquired at 120 min, resting-state fMRI was acquired at the same two time points but with no electrical stimulation applied in between in another sets of animals (n = 3 for the 0.1 and 0.6 dosage groups only).

Animal preparation

Animal study was approved by the local Institutional Animal Care and Use Committee (A*STAR, Singapore). C57BL/6 (n = 37 in total; 22 ± 2 g) female mice were used. Before anesthetizing the animals, they were carefully handled to minimize the stress and then quickly anesthetized with isoflurane (3% for induction) in a mixture of air and O_2 gases (40% O_2) and maintained at 2–3% during preparation through a nosecone with spontaneous respiration throughout the entire experiment. The animal was then secured on a MRI-compatible cradle (Rapid Biomedical GmbH, Germany) with ear bars and a bite bar to prevent head motion. A bolus of 0.3 mg/kg medetomidine (Dormitor®, Pfizer, USA) was injected intraperitoneally into the animal. At 15 min following bolus injection, continuous infusion of medetomidine was started through a PE50 catheter, inserted intraperitoneally, and isoflurane was discontinued. Respiration rate (RR), respiration pattern, and rectal temperature were monitored using a MRI-compatible physiological monitoring system (Model 1025, SA Instruments Inc, USA). The rectal temperature was maintained at ~37 °C by a feedback-controlled air-heater (SA Instruments Inc, USA) during the experiments.

Physiology

To understand the physiological variation of mice at different dosages of medetomidine, blood oxygen saturation (*sp*O2), respiration rate, and heart rate (HR) were measured from the thigh using MouseOx (STARR Life Sciences, USA) for up to 200 min on the bench in another sets of mice (n = 2 per dosage group). The same anesthetizing procedure and dosages were used. The room was kept dark and quiet throughout the experiments.

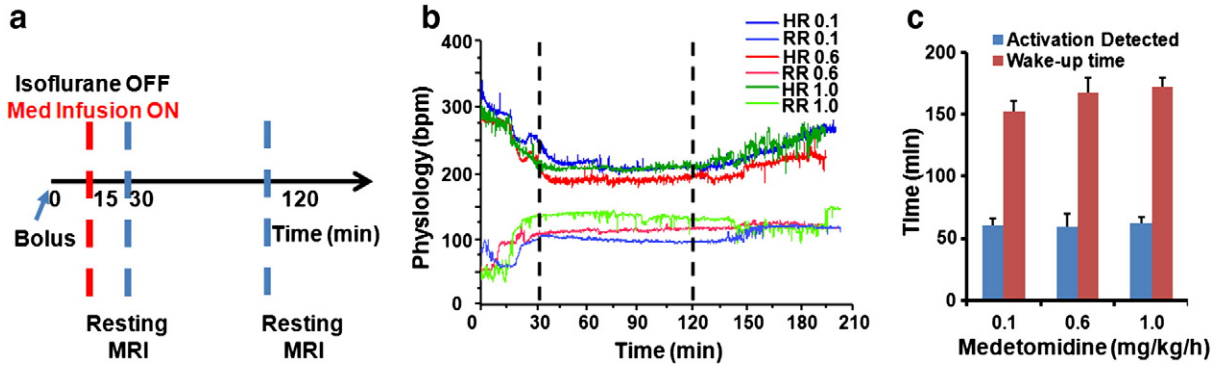

Fig. 1. Experiment design and physiology under different levels of medetomidine. (a) Schematic representation of the time frame of the experiment. At time 0, a bolus of 0.3 mg/kg medetomidine was injected intraperitoneally. At 15 min, isoflurane was switched off and medetomidine infusion started (red dashed line). Resting-state data was acquired at 30 and 120 min (blue dashed lines). FMRI of forepaw stimulation was conducted in between. (b) Traces of RR and HR recorded on the bench from bolus injection (time 0) up to 200 min at different dosages of medetomidine, where light and dark blue traces represent RR and HR at 0.1 mg/kg/h medetomidine (n = 2). Light and dark red traces represent RR and HR at 0.6 mg/kg/h medetomidine (n = 2). Light and dark green traces represent RR and HR at 1.0 mg/kg/h medetomidine (n = 2). The two black dashed lines indicate the timing corresponding to the start of resting-state fMRI scans. (c) The effect of medetomidine on the physiology in MRI. The blue bars indicate the time taken to detect robust and reliable BOLD activation and the red bars indicate the time the animal woke up (n = 8 for 0.1, and n = 6 for 0.6 and 1.0 mg/kg/h). Error bars represent SEM.