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Optical imaging of disrupted functional connectivity following ischemic stroke in mice

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ABSTRACT

Recent human neuroimaging studies indicate that spontaneous fluctuations in neural activity, as measured by functional connectivity magnetic resonance imaging (fcMRI), are significantly affected following stroke. Disrupted functional connectivity is associated with behavioral deficits and has been linked to long-term recovery potential. FcMRI studies of stroke in rats have generally produced similar findings, although subacute cortical reorganization following focal ischemia appears to be more rapid than in humans. Similar studies in mice have not been published, most likely because fMRI in the small mouse brain is technically challenging. Extending functional connectivity methods to mouse models of stroke could provide a valuable tool for understanding the link between molecular mechanisms of stroke repair and human fcMRI findings at the system level. We applied functional connectivity optical intrinsic signal imaging (fcOIS) to mice before and 72 h after transient middle cerebral artery occlusion (tMCAO) to examine how graded ischemic injury affects the relationship between functional connectivity and infarct volume, stimulus-induced response, and behavior. Regional changes in functional connectivity within the MCA territory were largely proportional to infarct volume. However, subcortical damage affected functional connectivity in the somatosensory cortex as much as larger infarcts of cortex and subcortex. The extent of injury correlated with cortical activations following electrical stimulation of the affected forelimb and with functional connectivity in the somatosensory cortex. Regional homotopic functional connectivity in motor cortex correlated with behavioral deficits measured using an adhesive patch removal test. Spontaneous hemodynamic activity within the infarct exhibited altered temporal and spectral features in comparison to intact tissue; failing to account for these regional differences significantly affected apparent post-stroke functional connectivity measures. Thus, several results were strongly dependent on how the resting-state data were processed. Specifically, global signal regression alone resulted in apparently distorted functional connectivity measures in the intact hemisphere. These distortions were corrected by regressing out multiple sources of variance, as performed in human fcMRI. We conclude that fcOIS provides a sensitive imaging modality in the murine stroke model; however, it is necessary to properly account for altered hemodynamics in injured brain to obtain accurate measures of functional connectivity.

Introduction

Stroke is a major health concern in the United States, where it is the fourth leading cause of death and the leading cause of adult disability (Anon). Although tissue death from ischemic injury is often well localized, it is becoming increasingly clear that focal injuries affect distributed patterns of synchronized neural activity throughout the brain. Recent studies using resting-state functional connectivity magnetic resonance imaging (fcMRI) have demonstrated that intra- and inter-hemispheric connections are altered shortly after stroke in humans and predict performance in tasks related to the injury (Carter et al., 2010; Corbetta, 2010). In particular, disruption of functional connectivity between

Abbreviations: MRI, magnetic resonance imaging; fMRI, functional magnetic resonance imaging; fcMRI, functional connectivity magnetic resonance imaging; OIS, optical intrinsic signal imaging; fcOIS, functional connectivity optical intrinsic signal imaging; GSR, global signal regression; MSR, multiple signal regression; EMCCD, electron multiplying charge coupled device.

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homotopic cortical regions appears to be a strong predictor of poor performance after injury in domains of both attention and motor tasks (Carter et al., 2010, 2012; Corbetta, 2010; He et al., 2007), findings which underscore studies reporting altered evoked responses in the affected brain regions of stroke patients (Calautti and Baron, 2003; Corbetta et al., 2005; Cramer and Bastings, 2000).

FcMRI studies of stroke in rats have generally produced similar results to those in humans. Both stimulus-induced cortical responses (Corbetta et al., 2005; Dijkhuizen et al., 2001, 2003) and functional connectivity (van Meer et al., 2010a) are reduced following focal ischemia, and correlate with behavioral deficits and subsequent recovery. However, interhemispheric homotopic connectivity and contralesional ipsilateral connectivity in somatosensory and motor regions in rats have been reported to subacutely increase (van Meer et al., 2010a). These two latter results might suggest more rapid system level reorganization in rats following focal ischemia than has been otherwise observed in humans (Rehme et al., 2011) or at the cellular level in other animal models of stroke recovery (Johnston et al., 2013; Mostany and Portera-Cailliau, 2011; Mostany et al., 2010).

Because the size of the mouse brain has presented a more significant challenge than rats for fcMRI, to date, there have not been analogous hemodynamic-based studies of functional connectivity in mice subjected to ischemic injury. Establishing analogous functional imaging in both mouse and humans is one of the most promising strategies to providing clinical translation. It is important to extend functional connectivity methods to mouse models of stroke so that molecular studies in mice (Clarkson et al., 2010, 2011; Li and Carmichael, 2006; Lu, 2003) can be related to human stroke fcMRI findings. To address this need, we have developed functional connectivity optical intrinsic signal imaging (fcOIS) in mouse models of healthy (White et al., 2011) and diseased (Bero et al., 2012) brain. The observed functional connectivity patterns are robust and reproducible across mice and reveal cross-species homologies with humans (e.g. compare Fig. 3 in (White et al., 2011) with Fig. 1 in (Zhang and Raichle, 2010)).

To establish fcOIS in the context of an acute ischemic stroke model, we performed fcOIS before and 72 h after transient middle cerebral artery occlusion (tMCAO). Functional status of the mice was evaluated in a manner akin to human stroke studies. Mice were separated into three groups based on infarct size and location to determine if graded ischemic injury incrementally impacts the relationship between functional connectivity and infarct volume, stimulus-related activations, and behavior. Determining how these relationships are affected after stroke will provide a more complete understanding of acute system-level damage, but in a model capable of facilitating targeted studies of stroke recovery mechanisms using genetic and molecular approaches.

Because functional connectivity measures depend on a preprocessing strategy, as a secondary goal, we examined how alternative regression approaches affect observed functional connectivity measures. These investigations indicated that global signal regression (GSR) alone can lead to distorted functional connectivity measures, and that multiple regression of nuisance variables is necessary to obtain accurate results. Overall, we found that fcOIS is a useful tool for understanding functional disruption in a mouse model of focal ischemia, and for bringing a robust and efficient functional assay into mouse studies of stroke recovery.

Methods

Animal preparation

Male ND4 Swiss Webster mice, aged to 6–10 weeks (22–32 g), were used for experimentation. Mice were given *ad libitum* access to food and water. All experimental protocols were approved by the Animal Studies Committee at Washington University.

In accord with our previously published animal preparation protocol for fcOIS imaging (White et al., 2011), anesthesia was initiated via i.p. injection with a bolus of ketamine-xylazine (1× dose: 86.9 mg/kg ketamine, 13.4 mg/kg xylazine) and animals were allowed 15 min for anesthetic transition. After induction, the animal was placed on a heating pad maintained at 37 °C via feedback from a rectal probe (mTCII, Cell Microcontrols) and its head was secured in a stereotactic frame. The head was shaved and cleaned, a midline incision was made along the top of the head to reflect the scalp and the skull was kept intact. To facilitate longer imaging times, after the initial bolus, mice were infused (i.p.) with a saline-ketamine cocktail (34.8 mg/kg/h ketamine) during the imaging sessions.

Imaging system

Sequential illumination was provided at four wavelengths by a ring of light emitting diodes (LEDs) placed approximately 10 cm above the mouse's head. Our field of view included most of the cerebral cortex (approximately 1 cm^2). Diffuse reflected light was detected by a cooled, frame-transfer EMCCD camera (iXon 897, Andor Technologies); the LED ring and the camera were time-synchronized and controlled via a computer using custom-written software (MATLAB, Mathworks) at a full frame rate of 30 Hz.

Imaging

Mice were imaged 7–14 days prior to and 3 days after tMCAO. Thirty minutes of activation data (15 min each paw, 18 stimulus presentations per paw) and up to 45 min of resting state data were collected for each mouse in 5 minute data sets (75 min of data total per mouse). The skull was kept moist with mineral oil during imaging.

Forepaw stimulation

Needle electrodes were inserted into the dorsal and ventral sides of the left and right forepaws between digits 2 and 3. The stimulation paradigm consisted of 5 s of rest, followed by a 10 second stimulus train, then 35 s of rest administered in a block design. Electrical stimuli were 0.3 ms pulses delivered at 3 Hz at an amplitude of 1.5 mA driven by a constant current stimulus isolation unit (World Precision Instruments). Fifteen minutes of data was collected for each paw (18 trials per paw total).

Image processing

Data from all mice were subject to an initial quality check prior to spectroscopic analysis. Data runs (5 min) in which reflected light level intensity (mean value over the brain) varied as a function of time by greater than 1% for any wavelength were excluded from further analysis. This preliminary quality control yielded 45–75 min of data per mouse. For subsequent analysis, image light intensity at each wavelength was interpreted using the Modified Beer–Lambert Law, usually expressed as: $\Phi(\mathbf{r},t) = \Phi_0 \times \exp(-\Delta\mu_a(\mathbf{r},t) \times L)$. Here, $\Phi(\mathbf{r},t)$ is the measured light intensity, Φ_0 is the baseline light intensity, $\Delta\mu_a(\mathbf{r}, t)$ is the change in absorption coefficient due to hemodynamic changes, and L is the optical path length factor for photons in the tissue (Arridge et al., 1992). As there is no pre-stimulus baseline in resting-state experimentation, we normalized relative to the average light intensity at each pixel, resulting in differential measures of absorption at each wavelength at each pixel: $\Delta\mu_{a,\lambda}(\mathbf{r},t) = -\ln(\Phi_\lambda(\mathbf{r},t)/\langle\Phi_{0\lambda}(\mathbf{r},t)\rangle)/L_\lambda$. Absorption coefficient data were converted to hemoglobin (Hb) concentration changes by inverting the system of equations, $\Delta\mu_{a,\lambda}(\mathbf{r},t) = E_{\lambda,i}\,\Delta[Hb_i](\mathbf{r},t)$ (where E is the extinction coefficient matrix (Prahl, 2002), and *i* runs over hemoglobin species). This inversion was performed using least-squares methods, yielding changes in oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (HbR) at each pixel at each time point. Differential changes in hemoglobin concentration were filtered to retain the functional connectivity band

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