Cerebral Vascular Reactivity Impairment Contributes to Functional Connectivity Loss in the Transient MCAO Rat Brain

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Target Audience: MRI scientists and researchers interested in resting-state fMRI of brain physiology, pathology and pre-clinical disease model studies.

Purpose: Resting-state fMRI (rs-fMRI) is an ever-growing and powerful function imaging tool for studying brain functional organization and connectivity in the absence of task performance. It has been employed to study many clinical cerebral vascular diseases[1-2] and psychiatric disorders[3-5]. It is generally accepted that the spontaneous cerebral blood flow (CBF) / blood oxygen level dependent signal (BOLD) fluctuations originate mainly from underlying neural activity, however, the loss of spontaneous coherence of brain activity may not exclusively result from the neuron origin due to the complicated nature of BOLD signal under the diseased condition. In this study, we aim to examine the influence of the state of vascular reactivity to the rs-fMRI signal by performing rs-fMRI, CBF and cerebral vascular reactivity (CVR) imaging in the transient Middle Cerebral Artery Occlusion (MCAO) rat brain.

Materials and Methods: Six MCAO rats[6] under 1.8% isoflurane anesthesia were scanned on day 1 and three of them were repeated on day 7 after a 1-hour MCA occlusion. MRI measurements were performed using a 9.4T/31cm magnet interfaced with VNMRJ consoles (Varian) and a ^{1}H surface coil. T_2-weighted images were acquired with a fast spin echo sequence (TE=10ms; TR=4sec; FOV=3.2×3.2cm; matrix=256×256; thickness=1 mm; 8 echo train length). The continuous arterial spin labeling (CASL) CBF calculation follows: $CBF=[\lambda\times R_1\times(S_C-S_L)]/[S_L+(2\times\alpha-1)\times S_C]$ (Eq. 1), where S_C and S_L are signal intensity of the image without and with the RF spin labeling respectively, α is the effective efficiency of the arterial spin labeling. The CVR calculation based on ASL follows Eq. 2, where CBF_{normo} and $PetCO_{2\text{-}normo}$ are the CBF and end-tidal CO_2 concentration under normocapnia condition respectively; CBF_{hyper} and $PetCO_{2\text{-}hyper}$ are those values under hypercapnia condition. Mild hypercapnia was induced with 6% CO_2 inhalation. Gradient-echo (GE)-EPI was used to acquire the rs-fMRI data (TE=17ms; TR=612ms; FOV=3.2×3.2cm; matrix=64×64; thickness=1 mm). For each rs-fMRI run, all GE-EPI images were spatially smoothed with a Gaussian filter and the initial 20 image volumes were discarded to avoid the transient BOLD signals at the beginning of the acquisition. The time course of each image pixel was normalized by its mean and then band-pass (0.005--0.1 Hz) filtered to remove the DC component, linear drift and high frequency noise. A 2x2 pixel region located in the left/right S1FL region was selected as the reference region and the BOLD time courses of all image pixels were then cross correlated (Pearson's correlation) with the reference time course extracted from the reference region to generate a correlation coefficient (CC) map for each rs-fMRI run.

$$CVR_{ASL}=\frac{100}{CBF_{normo}}\cdot\frac{CBF_{hyper}-CBF_{normo}}{PetCO_{2\text{-}hyper}-PetCO_{2\text{-}normo}}\quad(Eq.2)$$

Results: The lesion area shows hyper-intensity in the T_2-weighted images, loss of synchronization with both cortex and the contralateral sub-cortex in the CC maps, hyper-perfused blood flow in CBF images (see an example in Fig.1) and impaired CVR. The BOLD time courses obtained from the intact cortex (black square) show well-synchronized bilateral triangular-shaped BOLD signal bumps, which is similar to the previous study under identical isoflurane anesthetic level[7]. Interestingly, the BOLD time course extracted from the sub-cortex (white square in Fig. 1) at the lesion side (red line) shows flattened fluctuation in contrast to that obtained from the contralateral reference region (blue line) presenting the comparable bumpy shape to the intact brain reference region. Note that the baseline CBF in the reference region at the lesion side is higher while the CVR is significantly lower than that at the control side. Figure 2 shows the BOLD correlation maps and CVR images acquired on day 1 and day 7 after a 1-hour MCA occlusion in another representative rat. The coherent BOLD fluctuation between the two brain hemispheres is weakened and impaired on day 1 of the occlusion, especially in the slice on the left. Moreover, the day 1 BOLD correlation maps appear more scattered than day 7 maps, which show a nice generalized synchronization between the two hemispheres. However, consistent deficit of CVR areas associated with coherence loss are seen in the right side of the caudate putamen (hyperintensity on the T_2-weighted images) on both day 1 and day 7 of post-occlusion, indicating the persistent vascular dysfunction and potential neuron death in these areas. In contrast, the regions indicated by the white arrows show improved CVR and enhanced brain functional coherence on day 7 of the post-occlusion.

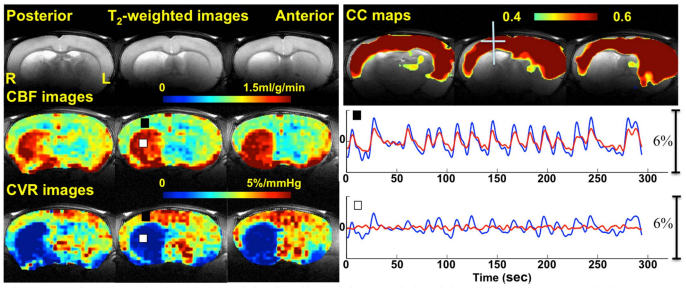

Figure 1 Three continuous coronal brain slices of T_2-weighted images, BOLD correlation maps, CBF and CVR images on day 1 after 1-hour MCA occlusion in a representative rat. The BOLD time courses were extracted from two different reference regions at the lesion side (black and white squares, their time courses were indicated by red lines) displayed in the middle slice of CBF and CVR image, as well as that from the corresponding contralateral reference regions (time courses were indicated by blue lines).

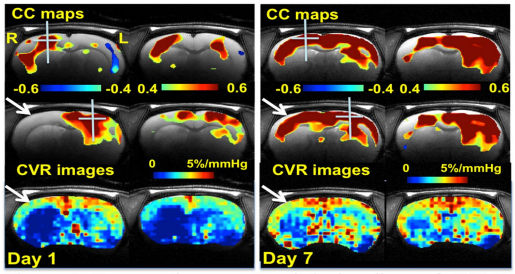

Figure 2 Two continuous coronal brain slices of BOLD correlation maps and the CVR images on day 1 and day 7 after 1-hour MCA occlusion in a representative rat. The reference region for the correlation maps was located at S1FL. The white arrows point to the loss of spontaneous coherence in the right cortex coincides with the impaired CVR on day 1 and the regaining of the spontaneous coherence correlates with the recovery of CVR on day 7 of the post-occlusion.

Discussion: Depending on the severity of the ischemic attack, interruption of blood flow may lead to the dysfunction of cerebral vascular reactivity, alteration of the neurovascular coupling, metabolic energy depletion and even to neuron death. Since the spontaneous hemodynamic fluctuations of the rs-fMRI reflect the underlying neuron activity, and the BOLD signal is determined by the interplay among CBF, cerebral blood volume (CBV) and cerebral metabolic rate of oxygen ($CMRO_2$), then, any of the above mentioned lesion-induced disorders could potentially affect the rs-fMRI signal. Our data show significant spontaneous coherence loss in the lesion areas of the transient MCAO rat brain, and these areas are coincidence with the regions showing impaired response to the vascular dilator CO_2 although the baseline CBF is even higher than the control side, indicating the impaired intrinsic hemodynamic condition. Interestingly, the spontaneous coherence in the cortex resumes when the CVR recovers on day 7, suggesting the existence of the survival neurons in these regions (Fig. 2). Our overall results demonstrate CVR deficit is one of the causes of unsynchronized functional connection, however, other causes such as impaired neuron activity and the neurovascular decoupling cannot be completely excluded. It would be important to further explore the status of oxidative metabolism[8] and adenosine triphosphate (ATP) metabolic rate[9] of the lesion areas, and reveal their correlation to the severity of damage, the viability of the tissue and impairment of resting-state connectivity. In addition, caution needs to be exercised when interpreting the absence or lack of spontaneous coherence using rs-fMRI, which does not necessarily mean the death of neuron, especially under diseased or pathological conditions in which the neurovascular coupling relationship can alter.

Conclusion: In this study, we have demonstrated that the cerebral vascular reactivity deficit could lead to the loss of rs-fMRI spontaneous coherence and the baseline CBF might not be a good indicator of the state of hemodynamic response. Caution needs to be exercised when interpreting the absence or lack of spontaneous coherence using rs-fMRI, the conclusion that neuron death is the main cause can only be reached when the extent of vascular reactivity impairment and the neurovascular coupling relationship are determined under diseased condition.

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