Supplementary folder: BOLD-CVR and ^{15}O-H_2O PET imaging protocol and analysis method

1 Image Acquisition and Processing

1.1 BOLD-CVR

MRI Data were acquired on a 3 Tesla Skyra VD13 (Siemens Healthcare, Erlangen, Germany) with a 32-channels head coil. Parameters for the BOLD fMRI were: axial two dimensional single-shot echo-planer-imaging sequence planned on the anterior commissure-posterior commissure line plus 20° (on a sagittal image); voxel size 3×3×3 mm^3; acquisition matrix 64x64x35 ascendinginterleaved slice acquisition; slice gap 0.3 mm; GRAPPA factor 2 with 32 ref. lines; repetition time (TR) / echo time (TE) 2000/30 ms, flip angle 85°; bandwidth 2368 Hz/Px, Field of View 192x192 mm^2. In total, 200 volumes were acquired for the BOLD-CVR study. For overlapping purposes and detected of the thalamic region of interest (ROI), a three-dimensional T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo image was acquired with the same orientation as the fMRI scans. Acquisition parameters were: voxel size 0.8×0.8×1.0 mm^3 with a field of view 230x230x176 mm and scan matrix of 288x288x176, TR/TE/TI 2200/5.14/900 ms, flip angle 8°, turbo factor 317. The carbon dioxide stimulus was given with the RespirAct, a computer controlled gas blender with prospective gas targeting algorithms (RespirAct, Thornhill Research Institute, Toronto, Canada).[1] The RespirAct allows for precise targeting of arterial partial pressure of oxygen and carbon dioxide. [2]During the CVR study, for 100 seconds carbon dioxide was initially retained at the subject's own resting carbon dioxide. After, the carbon dioxide was increased ~10 mmHg above the subject's resting carbon dioxide valuefor 80 seconds and rapidly returned to baseline for another 100 seconds. Oxygen was maintained at a level of ~105 mmHg throughout the entire protocol.

Before processing, preprocessing of the raw BOLD fMRI volumes with Statistical Parameter Mapping Software 12 (Wellcome Department of Imaging Neuroscience, University College of London, London, UK) included time and motion correction in six translational and rotational motion estimates of all BOLD volumes. The high-resolution T1-weighted image were then aligned to the BOLD volumes. Then the BOLD volumes were smoothed with a Gaussian Kernel with 6 mm full-width at half-maximum. The BOLD-CVR calculations were done as previously described.[3]In short, after temporal shifting of the carbon dioxide trace for optimal physiological correlation with the BOLD-CVR signal on a voxel-per-voxel basis, CVR was voxel-wise calculated from the slope of a linear least square fit of the BOLD signal time course to the carbon dioxide time trace. CVR was defined as the percentage BOLD signal change per mmHg carbon dioxide change.The extra BOLD volumes were acquired correct potential temporal shift.[3] (see van Niftrik, Piccirelli et al 2017 for more information regarding the analysis pipeline). For ROI specific analysis, the BOLD-CVR image was resliced to match the T1 dimensions.

1.2 (^{15}O-)-H_2O PET study

PET data were acquired on a full ring PET/CT-scanner in 3D mode (PET/CT Discovery STE, GE Healthcare, Chicago, Illinois, USA). After acquisition, the images were corrected for attenuation and scatter by using the manufacturer's own algorithms and a corresponding computed tomography (120 kV/80 mA). The total axial field of view was 15.3 cm. Images were reconstructed using a 3-dimensional Fourier rebinning filtered back projection algorithm giving a 128 x 128 x 47 matrix with 2.34 x 2.34 x 3.27 mm^3 voxel spacing. 300-800 MBq(^{15}O-) $H_2O$was intravenously administeredover a period of ca. 20 seconds using an automatic injection device. The emission data were obtained as a series of 18 times 10-second frames. Diamox(acetazolamide) was injected ~13 minutes before the second PET scan (dose

adjusted according to weight) over a period of 2 minutes.

Images of relative cerebral blood flow before the Diamox challange (PET baseline) and after (PET Diamox) were generated using a method [4 5] in which a standardized arterial input function and image scaling based on the washout rate k2 of (^{15}O-) H_2O are used to determine relative cerebral blood flow. This method was previously described by Treyer et al.[5]and utilizes the fact that k2 is related to the shape and not the scale of the arterial input function and is proportional to cerebral blood flow. A total count-rate threshold was set at 50% of the max. The baseline PET image and PET Diamox image were then co-registered to the mean BOLD and resliced to fit the T1-weighted dimensions.

1. Mark CI, Slessarev M, Ito S, et al. Precise control of end-tidal carbon dioxide and oxygen improves BOLD and ASL cerebrovascular reactivity measures. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine* 2010;64(3):749-56. doi: 10.1002/mrm.22405 [published Online First: 2010/07/22]
2. Slessarev M, Han J, Mardimae A, et al. Prospective targeting and control of end-tidal CO2 and O2 concentrations. *The Journal of physiology* 2007;581(Pt 3):1207-19. doi: 10.1113/jphysiol.2007.129395 [published Online First: 2007/04/21]
3. van Niftrik CHB, Piccirelli M, Bozinov O, et al. Iterative analysis of cerebrovascular reactivity dynamic response by temporal decomposition. *Brain and behavior* 2017:e00705. doi: 10.1002/brb3.705
4. Alpert NM, Eriksson L, Chang JY, et al. Strategy for the measurement of regional cerebral blood flow using short-lived tracers and emission tomography. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 1984;4(1):28-34. doi: 10.1038/jcbfm.1984.4 [published Online First: 1984/03/01]
5. Treyer V, Jobin M, Burger C, et al. Quantitative cerebral H2(15)O perfusion PET without arterial blood sampling, a method based on washout rate. *European journal of nuclear medicine and molecular imaging* 2003;30(4):572-80. doi: 10.1007/s00259-002-1105-x [published Online First: 2003/01/29]