ID: 2012p001784 Investigation of Brain Network Dynamics in Depression

Document Date: April 6, 2021

METHODS

Subjects

37 healthy subjects (mean age 23.85 years, S.D. 3.8 years, 26 female) participated in the study. All subjects were screened by MCE (board certified in neurology and psychiatry) to ensure that they had no neurological or psychiatric history, no symptoms of active depression (as assessed by the 17-item Hamilton Depression Rating Scale) and were not taking any psychotropic medications. One subject was excluded because PET data acquisition failed during their post-lateral rTMS PET session. Subjects provided written informed consent in accordance with the guidelines provided by the Partners Human Research Committee of the Massachusetts General Hospital.

Experimental Sessions

The overall experimental design is depicted in Figure 1. All experimental procedures were conducted at the Athinoula Martinos Center for Biomedical Imaging/Massachusetts General Hospital. Subjects participated in three experimental visits on separate days: a baseline visit and two rTMS/FDG-PET/rsMRI visits. At the baseline visit subjects underwent combined FDG-PET/rsMRI imaging. This served to functionally determine, on an individual subject basis, the locations of the medial and lateral rTMS targets. Subjects then returned for two separate experimental sessions, on separate days at least one week apart, during which they underwent pre-rTMS rsMRI imaging, followed by 20Hz rTMS, followed immediately thereafter by post-rTMS FDG-PET/rsMRI imaging. During one of their two experimental sessions, subjects were stimulated at their individualized medial target and during the other

they were stimulated at their lateral target. The order of these sessions was counterbalanced across subjects. This paradigm recapitulates our previous experimental designs (8, 18).

MRI image acquisition

MRI data were acquired using a 3.0-T whole-body scanner (Siemens™), equipped for echo planar imaging with a 12-channel 3-axis gradient head coil. Head movements were restricted using foam cushions. For each MR scanning session, one structural scan (~8 minutes) and three consecutive fMRI BOLD (blood oxygenation level-dependent) resting-state runs (6 minutes each) were performed. In order to limit the time between rTMS and rsMRI/FDG-PET imaging, and thus capture the effects of neuromodulation, the three BOLD runs were acquired first during post-rTMS scans, immediately after the localizer. Mean time from the completion of rTMS to the start of BOLD/FDG-PET imaging was under 6 ½ minutes: 326 seconds for the medial condition and 370 seconds for the lateral condition. This is well within the duration of effects of rTMS, which can last up to one hour (61).

Structural images were acquired via a T1-weighted 3D magnetization prepared rapid gradient echo (MPRAGE) image, acquired with the following parameters: TE = 1.64ms, TR = 2,530ms, TI = 1,200ms, voxel size = 1x1x1mm. fMRI BOLD images were acquired using T2* -weighted sequences (TR=3000ms, TE=30ms, flip angle=90°, voxel size=3.375 x 3.375 x 3.0mm). A fixation dot (a small white dot centered on a black background) was displayed to subjects via a rear projection system. Participants were instructed to stay awake, remain extremely still and to stare at the fixation dot during imaging.

FDG-PET image acquisition

Concurrently to the MRI acquisitions, FDG-PET images were simultaneously acquired on a BrainPET prototype (Siemens Healthineers, Erlangen, Germany). FDG-PET image acquisition procedures were identical across the subjects' three scans (baseline and two post-rTMS sessions). Subjects were injected with 18Flurodeoxyglucose: average 188 MBq (range: 164-212); average 5.1mCi (range 4.4-5.7) of 18F-FDG. rTMS was started approximately 7 minutes (462s for lateral rTMS and 390s after medial rTMS) injection in order to allow some time for the radioligand to circulate in order to best capture the effects of rTMS on FDG uptake. Approximately 34 minutes after injection (mean baseline=33.0 minutes, mean post-medial rTMS=34.3 minutes, mean post-lateral rTMS=34.3 minutes), PET data was collected with the BrainPET prototype for a duration of approximately 36 minutes (up to a timepoint of 70 minutes post-injection). Data was stored in listmode format.

rTMS targeting

Baseline rsMRI data was used to define, based on individualized intrinsic functional connectivity, two dlpfc targets for future sessions: a medial target and a lateral target. To identify the two targets, we used the procedure developed by Fox and colleagues (17). Specifically, we used a seed generated by the sgACC functional connectivity map covering the whole brain, procured from 98 subjects (a subset of the 1000 subjects used in the study by (3). We then measured FC between this seed map and each of 163 DLPFC nodes developed by Fox et al. (17). The node with the highest positive and the highest negative FC *z*-score were used as the medial and lateral targets, respectively, for that subject. The

medial target was embedded in the dorsal prefrontal portion of the default network (BA 9, medial superior frontal gyrus), while the lateral target was situated in the dorsolateral prefrontal node of the salience network (BA46, lateral middle frontal gyrus).

Our rationale for using this targeting paradigm was twofold. First, a sgACC map was chosen because of the importance of the sgACC as a corticolimbic hub and because of multiple studies supporting the clinical importance of this region in MDD. We used a sgACC map, as opposed to an sgACC seed, because negative correlations from this region could be compromised by susceptibility issues driving down signal-to-noise (17). Second, the sgACC map delineated positive and negative correlations which fall along the demarcations of two large-scale, negatively correlated networks. This allowed us to study the differential effects of stimulating two closely approximated prefrontal networks.

rTMS administration

Stimulation was applied using a MagPro X100 Stimulator with a MagPro Cool B-65 coil or a MagPro MCF-65 coil, depending on coil availability. Notably, these coils have identical windings and geometry. Importantly, during rTMS, accuracy and reproducibility of coil location and orientation was ensured with a frameless stereotactic neuronavigation system (Nexstim™ NBS Finland). Immediately prior to each rTMS stimulation, the resting motor threshold (RMT) was obtained by delivering single pulses (with the same coil used for stimulation) delivered to the hand knob in left primary motor cortex (determined through neuronavigation). The RMT was defined as the minimum total machine output required to elicit a motor evoked potential of ≥50 μV in the contralateral (right) first dorsal interosseous muscle 50% of the time. Mean MT across subjects was 52% of the total

machine output for both target sessions and did not differ across the two target sessions (p=0.70). For both stimulation sites rTMS was applied as high frequency (20Hz) stimulation at 110% of RMT, 40 pulses per train, with an intertrain interval of 28 seconds for 45 total trains (1800 total pulses, 22.5 minutes). These parameters are within recommended safety limits for rTMS (62) and were the exact parameters used in our prior protocol (8).

ROI selection

In order to estimate FDG-PET and FC changes in a granular fashion, 114 ROIs from the Yeo et al. 17-network parcellation (3, 63), and covering the entire cortical mantle, were used for data analysis. In addition, in order to better characterize corticolimbic circuits, we added six 8mm spherical cortical ROIs along the anterior cortical midline and three subcortical ROIs, with MNI coordinates derived from relevant prior studies: right and left sgACC (17), as well as right and left dmPFC, right and left vmPFC, right and left amygdala (64).

rsMRI data analysis

Resting-state fMRI data was analyzed with methods described elsewhere (3, 65). Images were first preprocessed using spatial normalization to a standard MNI 152 template brain, as well as motion and slice timing corrected. The following nuisance variables and their temporal derivatives were regressed during preprocessing: whole brain global signal, motion parameters, mean white matter and mean CSF signal. Additionally, data was low-pass filtered to exclude signals > 0.08Hz. Smoothing was performed with a 6mm FWHM Gaussian blur. Following pre-processing, volumetric seed-based FC analyses were conducted by extracting

the BOLD timecourse from an ROI and calculating the *z*-transformed correlation coefficient between this ROI and all other brain voxels.

FDG-PET analysis

PET images were reconstructed using an ordered-subsets expectation maximization (OSEM) algorithm using 6 iterations and 16 subsets and correcting for random coincidences, dead time, isotope decay, detector sensitivity, photon attenuation and scatter. Attenuation correction was provided via the validated and highly reproducible SPM-based method (60, 66, 67)(see FDG-PET repeatability measures section below). Static images were reconstructed 45 to 65 min post injection in order to best capture the glucose metabolism changes induced by rTMS stimulation. The reconstructed PET volume consisted of a 256×256×153 matrix of 1.25 mm isotropic voxels. To avoid potential subject head motion biasing the PET image quantification, motion correction was enabled into the PET reconstruction using a dual-pass image reconstruction method. Finally, the PET images were co-registered back into the MPRAGE images to allow perfect alignment of both image techniques. Images were spatially normalized into the Montreal Neurological Institute (MNI) space using the Dartel toolbox in SPM. This spatial normalization enabled ROI-based analysis using MRI-derived and pre-defined atlas regions. (see above for ROI details). Finally, ROI-based PET values were then normalized using whole-brain as normalization factor.

Quality control for head motion in BOLD data

As head motion has a significant effect on FC (68), we employed a stringent quality control analysis of the BOLD data collected. Each run was evaluated for slice-based SNR (sSNR), mean and maximum relative motion, mean and maximum absolute motion, and movements

greater than 0.mm1 and 0.5mm. A given run was excluded from the analysis if the sSNR for that run was lower than 2 standard deviations below the group mean of all runs, or if there were more than 5 movements greater than 0.5mm in that run (29). By these criteria, only one subject was affected, with 3/9 BOLD runs being unusable, leading to that subject's exclusion from further analysis on the basis of head motion.

FDG-PET repeatability measures

Intrascanner reproducibility for a subset of this PET dataset (13/20 subjects) has been published elsewhere (Izquierdo-Garcia et al., 2019). Briefly, we assessed relative changes, intra-class correlation coefficient, reproducibility coefficient and Bland-Altman limits of agreement to assess repeatability across scans. This revealed minimal, insignificant relative changes across the three PET acquisitions ($p=0.90$).