

Supplemental material online

Methods

Subjects

Eleven patients (8 male), mean age 60 years, who had a right fronto-parietal lesion and initially demonstrated neglect, participated in this study after providing their informed consent in agreement with procedures established by the Washington University Institutional Research Board. All patients underwent standard rehabilitation for at least 3 months after stroke. The patients were tested twice; once in the acute stage, i.e., 3-4 weeks after the onset of the stroke and second at the chronic stage, i.e., longer than 6 months after the onset of the stroke.

Inclusion criteria: 1. Age 18 or greater. No upper age limit applied. 2. Single right hemisphere lesion, ischemic or hemorrhagic in etiology. 3. Clinical evidence of neglect upon clinical screening. 4. Awake, alert, and capable of understanding and participating in research. 5. Able to tolerate the scanner environment for about 2 hours within the first 3-4 weeks after the stroke. Exclusion Criteria: 1. Evidence by CT or MRI of other strokes, although up to 2 lacunes were allowed in the subcortical white matter. 2. Inability to maintain wakefulness. 3. Presence of other neurological, psychiatric or medical conditions that precluded active participation in research and/or altered the interpretation of the behavioral/imaging studies (e.g. dementia, schizophrenia), or limited life expectancy to less than 1 year (e.g. cancer or congestive heart failure class IV). 4. Carotid Stenosis greater than 50% by Doppler studies or angiogram for fMRI studies as the BOLD response in the hemisphere ipsilateral to a carotid stenosis may not reliably track neuronal activity. 5. Report of claustrophobia excluded patients from the fMRI study.

To generate independent ROIs to test hypotheses in the patient population, we used a young adult group (N=13, age range 18-38) previously studied on the same paradigm¹.

Apparatus and stimuli

Stimuli were generated by an Apple Power Macintosh computer and projected onto a screen at the head of the magnet bore by a Sharp LCD projector. Participants viewed the stimuli through a mirror attached to the head coil. Stimuli were white on a black background.

Task and procedure

The display contained two boxes (unfilled squares) each 1° on a side, plotted with their centers 3.3° to the right and to the left of the central fixation point. Each trial started with the fixation point changing color from red to green. After 800 ms an arrow cue pointing left or right was presented at fixation for 2360 ms. Following a delay ranging from 1500 to 3000 ms, a target (an asterisk) was presented for 100 ms within one of the two boxes with equal probability (left, right). On 75% of the trials the target was presented at the location indicated by the cue (valid), while on 25% of the trials it was presented at the opposite location (invalid). The subject was asked to detect the target as quickly as possible with a right hand key-press, and RTs were measured in milliseconds from the appearance of the target until the subject's key-press. The next trial began after an intertrial interval that was randomized between 4760-9440 ms. The standard session involved about eight scans of 5 minutes, where each scan contained about 20 trials. At the acute stage, we obtained as few as 5 scans and as many as 11 scans (mean=8.2). At the chronic stage, the number of scans ranged from 7 to 12 (mean=9.8).

fMRI scan acquisition and data analysis

A Siemens whole-body 1.5 T Siemens Vision MRI scanner and asymmetric spin-echo, echoplanar sequence were used to measure blood oxygenation level-dependent (BOLD) contrast over the entire brain [repetition time (TR), 2.36 sec; echo time (TE), 37 msec; flip angle, 90°; 16 contiguous 8mm axial slices, 3.75x3.75mm in-plane resolution]. Anatomical images were acquired using a sagittal magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence (TR, 97 msec; TE, 4 msec; flip angle, 12°; inversion time, 300 msec). Functional data were realigned within and across scanning runs to correct for head motion using an eight parameter (rigid body plus in-plane stretch) crossmodal registration similar to the method described by ². A whole-brain normalization factor was applied to each scan to correct for changes in signal intensity between scans. Differences in the time of acquisition of each slice within a frame were compensated by sinc interpolation. For each subject, an atlas transformation ³ was computed on the basis of an average of the first frame of each functional run and MP-RAGE structural images to the atlas representative target using a 12 parameter general affine transformation. Functional data were interpolated to 2 mm cubic voxels in atlas space. The atlas representative MP-RAGE target brain was produced by mutual coregistration (12 parameter affine transformations) of images obtained in 12 normal subjects. The BOLD signal in each subject was analyzed with a “between-trial” linear regression model that estimated separate time courses for each trial type (left and right valid, left and right invalid), without assuming a shape for the hemodynamic response ⁴. The model included terms on each scanning run for an intercept, linear trend, and temporal high-pass filter with a cutoff frequency of 0.009 Hz. Time courses from the model were put into atlas space using the atlas transformation. Group analyses were conducted using voxel-wise random-effect ANOVAs. Subjects were treated as a random effect so that all results generalized across the population. Correlations across time points were corrected by adjusting the degrees of freedom ⁵. Statistical images for the

young adult group, acute and chronic neglect patients were corrected for multiple comparisons over the entire brain ($p < 0.05$), using a magnitude threshold derived from Monte-Carlo simulations that takes into account the number of contiguous activated voxels ⁶. To identify regions that were sensitive to recovery a within-subject ANOVA was run with MR frame (1–8), stage (acute, chronic), visual field (left, right), and target validity (valid, invalid) as factors. This image was arbitrarily thresholded at $z=2.5$, $p=0.01$ uncorrected for multiple comparisons. Coordinates for each cluster of activation were identified by an automated algorithm that searched for local maxima and minima. A regional ANOVA was then run using the same factors (MR frame, stage, visual field, validity) to obtain descriptive statistical values of their reliability (Table 2). In a different analysis ROIs from left and right posterior parietal cortex and FEF sites from the young adult group were applied onto the neglect group, and regional ANOVA with the same factors were run. For the retinotopic analysis ROIs were selected in the young adult group by projecting target-related activity onto a flattened atlas representation ⁷, and identifying active voxels within the borders of retinotopic areas in the atlas. A dorsal ROI (areas V1d, V2d, V3, V3A) and a ventral ROI (areas V1v, V2v, VP, V4) were selected.

Anatomical imaging and lesion segmentation

Structural images were acquired using multiple sagittal T1-weighted (MP-RAGE), optimized for contrast-to-noise ratio and resolution (TE=4ms, TR=9ms, TI=300ms, flip angle=12 degrees, 128 slices, 1x1x1.25 mm voxels) ⁸⁵ and T2-weighted (fast turbo spin echo) sequences. These images were realigned and averaged to increase signal-to-noise and white-gray matter contrast. Lesion boundaries were determined with the aid of an unsupervised fuzzy class means (FCM) based segmentation procedure. We automatically correct for image intensity inhomogeneity using a variant of a previously published method ⁸, and the apriori information that the objects imaged in the Siemens

circularly polarized head coil exhibit a parabolic 3D gain field (10 free parameters). Voxels are classified into one of 4 tissue types: air, CSF, gray matter and white matter. Expert judgment is required to definitively determine the lesion boundaries, e.g., in distinguishing the cystic cavity from ventricle. Semi-automated segmentation significantly decreases the variability associated with manual tracing. Structural images were transformed to atlas space for lesion averaging across subjects. We have measured the error associated with the atlas transformation of lesions by running the transformation with and without masking of the lesion, and found that the error is less than 2 mm in any axis even for large lesions.

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