Supplementary Methods

Materials & Methods

Subjects

Twelve right-handed subjects between the ages of 22 and 30 were recruited from the Dartmouth community. All subjects were native speakers of English, and were strongly right-handed as measured by the Edinburgh handedness inventory. Subjects reported no significant abnormal neurological history and all had normal or corrected-to-normal visual acuity. Subjects were paid for their participation, and all subjects gave informed consent in accordance with the guidelines set by the Committee for the Protection of Human Subjects at Dartmouth College.

Apparatus and Material

Stimuli were an updated set of Snodgrass and Vanderwart line-drawn objects [S1] that had been colored and shaded [S2]. Stimuli for both fMRI and TMS sessions were presented with an Apple G3 Laptop computer running PsyScope software[S3].

A 1.5-T whole body scanner (General Electric Medical Systems Signa, Milwaukee, WI) with a standard head coil was used to acquire T1 anatomical (SPGR; 124 sagittal slices, TE = 6 msec, TR = 25 msec, flip angle = 25°, voxel size = 1 x 1 x 1.2 mm) and T2* gradient spin-echo, echo planar functional images (EPIs) sensitive to BOLD contrast (TR = 2000 msec, TE= 35 msec, flip angle = 90°, 3.75 x 3.75 mm in-plane resolution). During each functional run, 95 sets of axial images (22 slices; 4.5-mm slice thickness, 1 mm skip

between slices) were acquired allowing complete brain coverage. Stimuli were projected onto a screen positioned at the head end of the bore by an Epson (model ELP-7000) LCD projector. Subjects viewed the screen through a mirror mounted on top of the head coil. Fiber-optic, light-sensitive key presses that interfaced with the PsyScope Button Box (New Micros, Dallas, TX) were used to record subjects' responses. Cushions were used to minimize head movement.

Transcranial magnetic stimulation (TMS) was delivered using a Neotonus PNS stimulator (model# N-0233-A-110V) with an air-cooled iron core butterfly shaped coil. Pulse duration for this stimulator and head coil is 180 μs (at 100% of operating power). Subjects were seated at a work table in front of the laptop computer with their head stabilized in a chin rest. Each subject wore earplugs to reduce any possible discomfort from the clicking noise that accompanied TMS.

Session 1: fMRI

The experimental setup was similar to that used by Buckner et al. [S4]. Functional data were acquired in two runs. Prior to each functional run, subjects were presented with a set of 10 line-drawn, colored objects. Each object was presented individually in the center of the screen for 500 msec, at a rate of one every 2000 msec. During this study phase, the 10 objects were presented 6 times each in random order, for a total of 60 trials. Subjects indicated whether each object was living (e.g. a monkey) or nonliving (e.g. a shoe) by responding with one of two optical key-presses (one in each hand) and were told to make these decisions as quickly as possible, without sacrificing accuracy. Immediately

following the study phase, subjects were imaged using event-related fMRI while viewing the 10 repeated objects and 30 novel objects. During scanning, each of the 10 repeated objects was presented three additional times (repetitions 7-9) while each novel object was presented only once. Presentation parameters and task instructions were identical to the study phase. Repeated and novel objects were pseudorandomly intermixed with trials of fixation such that each trial type followed every other trial type equally often.

fMRI data were analyzed using the general linear model for event-related designs in SPM99 (Wellcome Department of Cognitive Neurology, London, UK). For each functional run, data were preprocessed to remove sources of noise and artifact. Functional data were corrected for differences in acquisition time between slices for each whole-brain volume, realigned within and across runs to correct for head movement, coregistered with each participant's anatomical data, and spatially smoothed (6 mm full-width-at-half-maximum [FWHM]) using a Gaussian kernel. For each participant, a general linear model, incorporating task effects and covariates of no interest (a session mean, a linear trend, and six movement parameters derived from realignment corrections) was used to compute parameter estimates (β) and t-contrast images (containing weighted parameter estimates) for each comparison at each voxel.

To identify regions showing neural priming, individual subject data were normalized to a standard anatomical space (3-mm isotropic voxels) based on the ICBM 152 brain template (Montreal Neurological Institute) which approximates Talairach and Tournoux [S6] atlas space. Individual contrast images comparing novel to repeated objects were then

submitted to a second-level, random effects analysis to create a mean t-image (thresholded at p=0.001, uncorrected).

Session 2: TMS

TMS was performed at least one week after the fMRI session. Each subject's high-resolution anatomical image, overlaid by his or her functional data (Novel objects > Repeated Objects) was displayed as a 3D representation. The focus of frontal TMS was defined functionally by locating subject-specific regions that demonstrated repetition-related reductions within the pars triangularis/pars opercularis of the left hemisphere (Broadman's areas 44/45/47). When multiple foci were observed, the region demonstrating the most significant repetition-related reduction was selected. The position of the coil and the subject's head were monitored using a Polaris Optical Tracking System (Northern Digital, Inc, Waterloo, Ontario). Position data of both rigid bodies were coregistered in real time to a common frame of reference and superimposed on the reconstructed 3D MRI image of the subject via Brainsight frameless stereotaxic software (Rogue-Research, Montreal, Quebec). Thus, the center of the coil (stimulation locus) was continuously monitored to be over the site of site of interest.

The motor threshold of TMS was determined as the intensity required to produce a visible contraction of the intrinsic right-hand muscles 50% of the time with the coil positioned over the hand area of the primary motor cortex. The intensity used during the experiment was 110% of this threshold.

Subjects again made living/nonliving judgments on a new set of colored objects. As before, subjects were instructed to respond as quickly and accurately as possible. For each TMS site, a set of 30 objects was presented. Objects were presented for 500 msec, with a 4500 msec intertrial interval. Each object was presented twice, and TMS was administered to the same site for each presentation of the object. Each stimulation consisted of a 10 Hz train lasting for 500 msec. TMS onset was catered to each subject's individual response times. Median response times were calculated from each subject's performance during fMRI (median response times ranged from 506 to 810 msec). TMS stimulation was time-locked to occur 250-310 msec prior to a subject's median response time. The mean stimulation onset across the group was 334 msec post stimulus-onset; stimulation onsets ranged from 254 to 500 msec post stimulus-onset.

Subjects underwent two blocks of TMS, one for left frontal TMS and one for control-site TMS. Stimulation and timing protocols were identical for both frontal and control-site TMS. Each block lasted approximately 10 minutes. Object-lists and order of stimulation site were counterbalanced across subjects.

Session 3: fMRI

Immediately following TMS, subjects were re-scanned using event-related fMRI. The average time between TMS and this final fMRI scan was 15 minutes.

Functional data were acquired in three runs. Once again, subjects made living/nonliving judgments on colored line-drawn objects using optical key presses (one in each hand). Subjects were presented with novel objects, repeated objects that were previously

accompanied by left frontal TMS, and repeated objects that were previously accompanied by control-site TMS. Trial types were pseudorandomly intermixed with trials of fixation such that each trial type followed every other trial type equally often.

Regions-of-interest were defined based on data from Session 1 (Novel > Repeated). To calculate signal intensities for each of these regions, 8mm-radius spherical regions of interest (ROIs) were created. For each subject, signal intensities from each ROI were calculated separately for each condition in Session 3, and examined statistically using repeated-measures ANOVA's, and paired-samples t-tests for planned comparisons. The ROI approach was performed in normalized atlas space to permit a random effects analysis that would generalize across the population. To ensure that TMS did not produce spurious effects in brain regions that were engaged during task performance but did not exhibit neural priming effects, two additional regions of primary visual cortex (BA 17/18; -30 –90 –18) and primary motor cortex (BA 4; -39 –3 54) of the left hemisphere were also interrogated. Results of the ROI analysis revealed no significant effects of TMS on neural activity in primary visual cortex or primary motor cortex (all p's n.s.). Functional maps and ROI regions were overlaid on top of an inflated cortical rendering of the left hemisphere for visualization purposes[S6].

S1. J. G. Snodgrass, M. Vanderwart, *J Exp Psychol [Hum Learn]* **6**, 174 (Mar, 1980).
S2. B. Rossion, G. Pourtois, *Perception* **33**, 217 (2004).
S3. J. D. Cohen, B. MacWhinney, M. Flatt, J. Provost, *Behavioral Research Methods, Instruments, and Computers* **25**, 257 (1993).
S4. Buckner, R.L. et al. Functional-anatomic correlates of object priming in humans revealed by rapid presentation event-related fMRI. *Neuron* **20**, 285-96 (1998).
S5. J. Talairach, P. Tournoux, *Co-Planar Stereotaxic Atlas of the Human Brain* (Thieme Medical Publishers, Inc., New York, 1988), pp. 122.

S6. D. C. Van Essen *et al*., *Journal of American Medical Informatics Association. (Special issue on the Human Brain Project)* **41**, 1359 (2001).