SI Methods

Participants. All participants were right-handed, except for one ambidextrous EM, as assessed by Edinburgh Handedness Inventory (1), and all but three were male (one EM, one NM, and one INM were female). Nine EMs were Tibetan, and five were Caucasian (four were native English speakers). Two LHEMs were Tibetan, and two were Caucasian. Two MHEMs were Tibetan, and two were Caucasian. All NMs were Caucasian native English speakers. For INM we made efforts to culture-match participants, but, despite advertisement in Asian newspapers and localities, there was only one Asian (Indian, nonnative English speaker). In addition, there was one African American and eight Caucasians. Experts were contacted by M. Ricard, an interpreter for the Dalai Lama who is a Western Buddhist monk with years of meditative training in Nepal and Western scientific training in France. Experts had previously completed mental training in the similar Tibetan Nyingmapa and Kagyupa traditions for 10,000-50,000 h. Although this estimate includes a variety of meditation practices, the attentional stability cultivated by concentration meditation is considered to be integral to all of their meditation practices. The length of their training was estimated based on their daily practice and the time they spent in meditative retreats. Ten hours of meditation per day of retreat was estimated as an average. The control participants were recruited by means of advertisements in local newspapers. The advertisement specifically recruited participants who had an interest in meditation but who had no prior meditative training. Written informed consent was obtained before scanning, in accordance with procedures and protocols approved by the University of Wisconsin, Madison, Institutional Review Board. A proficient Tibetan-speaking translator gave detailed procedural instructions and read the consent form to non-English-speaking participants.

Imaging and Task Parameters. MR images were collected with a GE Signa 3.0 Tesla scanner equipped with a high-speed, whole-body gradient and a whole-head transmit-receive quadrature birdcage headcoil. Whole-brain anatomical images were acquired at the end of each session by using an axial 3D T1-weighted inversion-recovery fast gradient echo (or IR-prepped fast gradient echo) sequence. The field of view (FOV) was

240 × 240 mm with a 256 × 256 matrix. The slice thickness was 1-1.2 mm, with 0.9 by 0.9 mm in-plane dimensions. Functional data were collected by using whole-brain EPI (TR = 2000 ms, TE = 30 ms). For functional images, sagittal acquisition was used to obtain 30 interleaved 4-mm slices with a gap of 1 mm between slices. The resulting voxel size was 3.75 by 3.75 by 5 mm (FOV = 240 mm, matrix = 64 × 64).

To ensure a high signal-to-noise ratio in areas prone to susceptibility artifacts, the field inhomogeneities were lessened during data collection using high-order shim coils that applied small correction gradients. In addition, acquisition of a 3D field map of the magnetic field provided a complementary strategy to further reduce distortion (these data were not acquired for the first three EMs). Based on these field maps, echo planar imaging (EPI) data were warp-corrected so that accurate alignment to anatomical images could be made (2, 3).

During the fMRI session, head movement was restricted by using a vacuum pillow (Vac Fix System; S&S Par Scientific). A Silent Vision system (Avotec, Jensen Beach, FL) displayed the fixation point. Eye movements, fixations, and pupil diameter were recorded during the fMRI scan by using an iView system with a remote eye-tracking device (SensoMotoric Instruments, Needham, MA).

During the MRI scanning session, participants entered three meditative states: one, concentrative meditation on a visual object, and two other meditations (compassion and open presence). The INMs, however, participated in only concentration and compassion meditation. After each scan run, participants reported verbally on the quality of the meditation. Two scans were run on two separate days (1 day apart for EMs, about 1 week apart for NMs) because of the length of the scan run. INMs were run on only 1 day but had two concentration meditation scan runs on that day.

Before the MRI scanning session, participants underwent a simulation session (1 day before for EMs, 1 week before for NMs) during which they viewed an abbreviated version of the experimental paradigm while lying in a mock MRI scanner (including headcoil and digitized scanner sounds). This simulation session served to acclimate all participants to the fMRI environment. There was an average per scan run of 643 seconds of meditation and 550 seconds of neutral state (264 seconds and 190 seconds, respectively, for EM participant 2). Block length was varied to better distinguish neural activity from physiological activity in analyses. Because entering the state of concentration was unlikely to be instantaneous, a longer meditation block was chosen despite a possible reduction in signal to noise.

Because of excessive head motion, three EMs were omitted from the meditation block data (whole brain motion correlating with block presentation), one EM was omitted from the distracting sound data (head motion during sound presentations, also one EM did not have sounds presented), three NM were omitted from the meditation block data, two NMs from the distracting sound data, and one INM from both the block and sound data. Only the best day (based solely on amount of head motion) was used in the final analysis for the meditation block data. If possible, both days were used in the distracting sound data analysis. The number of time points removed because of head motion was not significantly different between EMs and the other two groups, although INMs had significantly fewer time points removed than NMs at $P < 0.042$. In a voxel-wise analysis of the motion parameters, participant groups showed no significant differences in the brain, at $P < 0.05$ corrected.

Analysis. Analysis techniques were similar to those described previously in our laboratory (4). Briefly, data reconstruction was implemented via Analysis of Functional Neural Images (AFNI), version 2.31 software (5). Data processing steps included offline image reconstruction in conjunction with smoothing in Fourier space via a Fermi window (full width at half maximum = 1 voxel), correction for differences in slice-timing, and six-parameter rigid-body motion correction. The motion estimates over the course of the scan for translation (inferior-superior, right-left, and anterior-posterior) and rotation (yaw, pitch, roll) parameter estimates were charted. Data sets were analyzed for head motion, and time points with >0.5 mm of motion, as well as time points in which head motion correlated with the presentation of the block (which could be mistaken for brain activation) were removed from the analysis.

The time series of meditative blocks and resting blocks was analyzed with a least-squares general linear model (GLM) fit that modeled the mediation block data and the event-related, distracting sounds data (both with gamma variant models of the hemodynamic response), and the head motion parameters. The resultant beta-weights were converted to percentage signal change by using the mean overall baseline and spatially smoothed by using a 4-mm Gaussian filter. These percentage signal change maps were transformed into the standardized Talairach space via identification of anatomical landmarks on the high-resolution anatomical image.

For meditation block data, a voxel-by-voxel parametric two-tailed t test was used on the percentage signal change maps within each population (percent signal change vs. zero baseline). P value correction for multiple comparisons was based on a combination of threshold cutoff and cluster extent using 3dmerge (AFNI). A similar analysis was used in comparing groups for the meditation block data (EMs vs. NMs). Minimal cluster size was calculated by using Monte Carlo simulation program AlphaSim (AFNI). For a masked Afni image, AlphaSim ran 1,000 iterations, with a radius connectivity of 5.1 (because slice thickness was 5 mm) and image defined Gaussian filters with FWHM determined with 3dFWHM. The minimal cluster size to avoid false cluster detection was for $P < 0.05$, 1,409 voxels, for $P < 0.02$, 680 voxels, for $P < 0.01$, 475 voxels, and $P < 0.001$, 215 voxels. Some clusters reported in tables were smaller than allowed for in multiple comparison correction and were marked with the symbol † in tables and a white ‡ in the figures. For the event-related analysis, similar t tests were performed, in addition to a mixed effects state-by-group ANOVA between sounds vs. silence in Med. vs. Rest (state) and EMs vs. NMs (group). (Because Rest was used as the baseline contrast, a state-by-group ANOVA could not be run for the Med. block data). In ANOVAs including the IM group, three participants each from the EM and NM group had to be removed in order to obtain a balanced ANOVA (inclusion was determined by head motion parameters).

Although ROI volumes and coordinates were determined by the balanced ANOVAs, the t-value statistics in the tables included all 13 EMs and 13 NMs.

Two types of ROIs were used: independent of our study, “attention-shifting” ROIs obtained from a metaanalysis of 31 studies (6), and data-based ROIs based on significant activation from analyses in our study. One subject had a left frontal lobe anatomical abnormality (SFG), and data from this region were not included in ROI analyses. The average time course of the MR BOLD response in the resting and meditation blocks was generated by using the AFNI 3dDeconvolve program. The average time course for each meditation and resting block was averaged within each ROI and normalized across data sets. Differences in the shape of response profile were examined in the four subjects with the most hours (MHEMs) vs. four subjects with the least hours (LHEMs) and all NMs.

Correlations and partial correlations with hours of practice and with age were calculated for all ROIs for EM participants (Statistica; StatSoft, Tulsa, OK). One participant for the distracting sound data was an outlier (>2 SD from mean) for multiple ROIs and was not used in the correlation analyses. In addition, voxel-wise regression analysis for hours of practice and age was performed by using AFNI’s 3dRegana. Regions that showed correlation (all positive) with age of EM participants include bilateral cerebellum, left SMA/MeFG, left basal ganglia, left insula, right IFG, right precuneus, and right posterior STG (data not shown). Steiger’s z, on correlation r values of ranked data sets, was calculated to determine whether two r values were significantly different (http://www-class.unl.edu/psycrs/statpage/comp.html).

Horizontal pupil diameter sampled continuously at 60 Hz was processed by algorithms implemented in Matlab (MathWorks, Natick, MA) by Siegle, Granholm, and Steinhauer (2002, unpublished Matlab code) and modified by Greischar (2003, unpublished Matlab code). Blinks and other artifacts identified by steep local regression slopes and extreme values were replaced by linear interpolation of neighboring data. Data were then smoothed twice by using a five-point moving average. To avoid erroneous interpolation, interpolation continuing for >4 seconds or manually judged as deviating from raw data were excluded from further analysis. Median pupil diameter was extracted for each second, and each participant's data were autonormalized to obtain a common scale. An impulse response for each condition was formed by pooling across trials within blocks and pooling again across blocks and sound types to give unweighted means. For each impulse response the value in the second before sound onset was subtracted from all values, and peak pupil diameter was defined as the maximum value within the first six seconds of sound onset. Statistical analysis took the form of a two-factor, mixed-design ANOVA, with a within-participant factor of state (meditation, rest) and a between-participant factor of group (NM, INM, EM), with nine participants in each group.

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