**Supplementary Materials**

For “Mindfulness-based therapy regulates brain connectivity in major depression”.

Participants

Participants were adult patients with chronic or recurrent MDD recruited from a larger clinical trial examining the impact of mindfulness training (ClinicalTrials.gov NCT02801513) [1]. Thirty-seven patients who had fully adhered to the treatments participated in the resting-state fMRI assessments at pre- and post-treatment, 17 of whom had been randomized to the mindfulness therapy condition and 20 to the relaxation control condition. Six of these participants were excluded due to excessive movement during the fMRI assessments (three in the mindfulness intervention, three in the control intervention), leaving a final sample of *n* = 14 in the mindfulness group and *n* = 17 in the control group.

Inclusion criteria at initial assessment for the trial were (a) a current diagnosis of MDD as assessed by Structured Clinical Interview for DSM-IV-TR (SCID) [2] (b) a lifetime history of depression with onset before age 19 and either chronic persistence of symptoms or a history of at least three previous episodes of depression, two of which needed to have occurred during the last two years, (c) self-reported severity of current symptoms on a clinical level as indicated by BDI-II scores above 19, (d) age 25 to 65, (e) right handedness (adopted in order to control for laterality effects), and (f) fluency in spoken and written German. Exclusion criteria were (a) history of psychosis or mania, current eating disorder, obsessive compulsive disorder, current self-harm, current substance abuse or dependence, (b) history of traumatic brain injury, and (c) current treatment with cognitive behavioral therapy. Patients who were currently taking antidepressants were allowed into the study provided that the medication had not been changed during the four weeks before entry into the study. Interviews using the Research Version of the Structured Clinical Interview for DSM-IV-TR Axis I Disorders, a well-validated semi-structured interview to determine current and past DSM-IV-TR axis-I diagnoses, were conducted by one of two trained clinical psychologists. The SCID was used to assess current and past diagnostic status at pre-treatment and current diagnostic status at post-treatment. To facilitate assessment of past episodes of depression, interviewers guided patients to construct visual timelines of depression lifetime history in order to identify episodes before assessment of criteria. Note that the time frame for the post-assessment, two weeks, partly overlapped with the intervention period.

Five of the 14 participants in the final mindfulness group reported taking antidepressants at entry into the study (35%; 2 tricyclics, 3 selective serotonin reuptake inhibitors). In the control group, 5 of 17 participants were on antidepressants (29%; 4 selective serotonin reuptake inhibitors, 1 selective serotonin norepinephrine reuptake inhibitors), χ2(1) = .14, *p* = .70. Mean age of onset was 17.6 (*SD* = 8.3) years in the mindfulness group and 15.0 (6.6) years in the control group, *t*(29) = .97, *p* = .34. Median number of previous episodes was 7.5 (range: 4, 14) in the mindfulness group and 7 (range: 3, 35) in the control group, Independent Samples Median Test *p* = .89. Nine of the participants in the mindfulness group (64%) and 8 of the participants in the control group (47%) suffered from comorbid anxiety disorders, χ2(1) = .92, *p* = .33.

The two groups did not differ in terms of age, gender distribution, education (*p*s > .05, see Supplementary Table 1). In addition, we found no significant differences in fMRI head motion (maximum frame displacement) between the pre- and post-intervention scans in either group, or when comparing between groups at either pre- or post-intervention time points (*p*s > .05, see Supplementary Table 2).

Interventions

The interventions lasted two weeks, including three 1.5-hour individual sessions with trained clinical psychologists (MF and EW) as well as intensive daily home practice. The three sessions followed a set and manualized structure. During the first session, the therapist introduced the rationale of the treatment and familiarized the participant with the main practices for the coming week. The second session started with a review of experiences from the first week. The therapist addressed any questions and difficulties with the practices that had arisen during the previous week, and then introduced the main practices for the second week and their rationale. The third session served to debrief participants and to help them establish ways of continuing the practices on their own following the end of the study should they wish to do so. In addition to the individual face-to-face sessions, participants received a booklet that described in detail the practices for each day along with their rationale and related psycho-educational material.

*Mindfulness training:*

Participants in the mindfulness therapy group were asked to engage in formal meditation practice for about 25 minutes twice per day on all seven days of each week (14 days total) using recorded guided meditations. Practices were drawn from standard Mindfulness-Based Cognitive Therapy (MBCT) [3], although they were shorter than usual in order to facilitate practice in light of the fact that patients currently suffered from depression. Nonetheless, practices followed the standard MBCT sequence leading from body scan meditation and mindful movement to sitting meditations focusing on the breath, body sensations, sounds, thoughts, and open awareness, to practices that were more specifically focused on relating to difficult experiences with acceptance and compassion. In addition to formal meditation, participants were asked to engage in shorter informal practices, such as breathing spaces, that served to generalize a mindful stance to activities in daily life.

*Relaxation control:*

Participants in the active control condition were asked to schedule regular rest periods as a means of deliberately retreating from the activities of the day. Participants received audio files with ambient music that they were free to listen to should they feel that the music might facilitate relaxation. Length and frequency of the rest periods mirrored the time demands of the meditation training. Participants received a plausible rationale for the control training that linked acute depression to stress and suggested rest, relaxation, and disengagement from negative thinking as an initial step towards recovery.

Clinical outcome measure

Severity of depressive symptoms was assessed with the widely used self-report Beck Depression Inventory-II (BDI-II) [4]. This measure consists of 21 groups of statements referring to the presence of symptoms of depression over the past two weeks.

Procedure

The imaging study was embedded in a larger trial testing the effects of brief mindfulness training in chronically depressed patients (ClinicalTrials.gov NCT02801513). Potential trial participants were screened over the phone by the recruitment team for the main inclusion and exclusion criteria and those likely to meet eligibility were invited to an initial assessment session during which the Structured Clinical Interview for DSM-IV was conducted. Participants who met inclusion criteria continued this session to fill in self-report questionnaires and to then partake in EEG assessments, the results of which have been reported elsewhere [1, 5]. Self-reported severity of depressive symptoms was assessed using the BDI-II. MRI assessments were conducted in a separate session within one week after the initial assessments. Participation in the MRI assessments was offered as a voluntary extra to patients who took part in the larger trial. After the pre-treatment assessment sessions, depressed participants were randomly allocated to receive either mindfulness-based therapy or a relaxation control intervention. After the end of the intervention, participants took part in the post-treatment assessment sessions, which followed the same sequence as the pre-treatment sessions. Individuals who had been randomized into the relaxation control were offered to take part in the mindfulness-based therapy after their last assessment for the study. Randomization for the larger trial was conducted following a simple randomization protocol using a computer-generated randomization sequence (permuted blocked randomization with blocks of size 4) and sealed envelopes that remained concealed until assignment to the groups.

Participants recorded adherence to the daily practice on protocol sheets. Given the brief duration of the interventions, we defined the adequate minimum dose for the mindfulness training as having completed at least 75% of formal meditation practices. The mean rate of compliance with formal home practice was 93.8 (*SD* = 10) in the mindfulness group and 92.2 (*SD* = 7.1) in the control group.

Resting-state fMRI data acquisition and preprocessing

Following a structural scan, participants underwent an 8 min resting-state fMRI assessment, during which they were asked to rest silently while watching a white fixation cross displayed against a black background, and to remain “relaxed and awake”.

Structural and functional MRI data were acquired on a Siemens Trio 3T scanner using a 12-channel radio-frequency (RF) head coil. T1-weighted structural images were acquired with the following parameters: 176 sagittal slices covering the whole brain, repetition time (TR) = 1900 ms, echo time (TE) = 2.52 ms, flip angle = 9°, 256 x 256 matrix, voxel size 1 x 1 x 1 mm3. For each resting-state measure, 257 volumes of T2\*-weighted echo-planar images (EPIs) were acquired with the following parameters: 37 axial slices covering the whole brain, TR = 2300 ms, TE = 30 ms, flip angle = 70°, 64 x 64 matrix, field of view = 192 x 192 mm2, 37 slices, slice-timing: interleaved ascending, voxel size = 3 x 3 x 3 mm.

Functional images were preprocessed using MATLAB 2012 (The Mathworks Inc., Natick, MA, USA), SPM12 (Statistical parametric mapping software, SPM; Wellcome Department of Imaging Neuroscience, London, UK; <http://www.fil.ion.ucl.ac.uk>), DPABI v2.1 (toolbox for Data Processing & Analysis for Brain Imaging; <http://rfmri.org/dpabi>) [6] and Analysis of Functional Neuroimages (AFNI; National Institutes of Health, Bethesda, MD, USA; <https://afni.nimh.nih.gov/>) [7]. We reoriented functional and T1 anatomical images to oblique space, then removed the first 5 functional volumes, slice time corrected and realigned the functional scans, coregistered the T1 to functional data, segmented the T1 using DARTEL, normalised using DARTEL, performed nuisance covariate regression using the six rigid body head movement parameters and the first five principal components from white matter and cerebrospinal fluid signal according to the CompCor algorithm (component based noise correction method) [8]. The AFNI program 3dBlurInMask with the automask option was then used to smooth the data to 4 mm FWHM.

During realignment, we flagged bad time points as frames with displacement exceeding 0.5 mm [9]. We excluded participants if their bad time point rate exceeded 15% or if any of the six rigid body head movement parameters exceeded 3 mm or degrees. These criteria resulted in four participant exclusions (see Supplementary Table 2).

Resting-state functional connectivity analysis

Functional connectivity analyses were conducted using AFNI. Brain systems of interest were identified *a priori* based on the depression and meditation neuroimaging literatures, and included the frontoparietal, salience, and default systems. We investigated these systems using a standard seed-based connectivity approach. Seed coordinates were selected for the placement of 10mm spheres placed as followed: bilateral dorsolateral prefrontal cortex (DLPFC; MNI coordinates: -6, -50, 18), bilateral anterior insula (aINS; MNI coordinates: left -34, 22, 0; right 40, 18, 2), and bilateral posterior cingulate cortex (PCC; MNI cooridnates: left 40, 40, 36; right -46, 38, 30) for the frontoparietal, salience, and default networks, respectively. Coordinates were identified as the peaks of the Neurosynth reverse inference maps for “DLPFC”, “anterior insula”, and “posterior cingulate”. Timecourses for each network were extracted as averages from network-associated seeds and correlated against every voxel in the brain, and subsequently converted to *z*-scores. We implemented a spreading interaction approach [for example, as in 10] to specifically statistically test for voxels in which the meditation group exhibited statistically significant change from pre- to post-treatment while the control group did not. Explicitly, the spreading interaction was modeled as [−1 (pre-treatment, meditation), −1 (pre, control), 3 (post, meditation), −1 (post, control)].

We tested three *a priori* hypotheses, namely, that connectivity of the (1) DLPFC, (2) aINS, and (3) PCC would change as a result of meditation training. Significance of the spreading interaction maps were assessed using a cluster-simulation method. Taking into account recent concerns regarding null-hypothesis modeling [11] we used a spatial auto-correlation function for generating simulated noise volumes. Noise volumes were simulated with smoothness values estimated from the smoothed data for all participants. 5000 simulated datasets with bi-sided *NN*=3 thresholding indicated that *k*=67, that is, that 67 or more clustered voxels with a voxel-wise *p*-value of less than 0.01 (*t*-stat > 2.757) are required to reach a *p*-value of less than 0.05 corrected at the whole-brain level. *Z*-scores were subsequently extracted for each participant from identified statistically significant clusters. These values were then plotted to interpret the spreading interaction (see Figure 1B in the main manuscript). To determine brain-network membership when interpreting the resulting clusters, we situated identified clusters against a common 7-network functional connectivity parcellation atlas that was previously developed based on fMRI data from 1,000 individuals [12].

Correlating neural and symptom measures

Subsequent to our primary functional connectivity analysis, we additionally examined whether reductions in depressive symptoms were correlated with the observed mindfulness-related changes in functional connectivity. We conducted three Pearson’s correlations in the mindfulness group to examine the relationship between reductions in depressive symptoms (change in BDI-II score from pre- to post-intervention) and reductions in functional connectivity (change in Z-score from pre- to post-intervention) for each of the three significant clusters identified in the primary analysis (i.e., right fusiform gyrus, left fusiform gyrus, and angular gyrus). Counterintuitively, these analyses showed that, in the mindfulness group, decreases in connectivity were inversely correlated with decreases in BDI-II scores (angular gyrus: r = -.505, p = .065; right fusiform: r = -.675, p = .008; left fusiform: r = -.543, p < .045). That is, while reductions in connectivity with the frontoparietal control network emerged as a signature of the early effects of mindfulness meditation, such effects were more pronounced in those who had shown relatively smaller reductions in symptoms. It is possible that the observed signatures might reflect the initial effort patients use in responding mindfully to existing symptoms and that signatures might change once patients have reached a more stable state of remission. Nonetheless, it is important to note that all but the strongest of correlations are unstable at a sample size of 14 [13]. Future studies will be necessary to confirm and potentially qualify the stability and meaning of these results.

**Supplementary Materials References**

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**Supplementary Table 1.** Sociodemographic characteristics of depressed patients in the mindfulness-based therapy (*n* = 14) and the relaxation control group (*n* = 17).

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|  | **Mindfulness Therapy** | **Relaxation Control** | **Test** |
| **Age** | 43.4 [11.3] | 37.3 [12.0] | *t*(29) = 1.48, *p* = .15 (two-tailed) |
| **Gender, n female (% female)** | 8 (57) | 11 (64) | *χ*2(1) = .18, *p* = .66 |
| **Education, n higher education**  **(% higher education)** | 10 (71) | 10 (58) | *χ*2(1) = .53, *p* = .46 |

Square brackets show standard deviation.

**Supplementary Table 2.** Maximum frame displacement (movement metric) for resting-state fMRI scans at each timepoint in the mindfulness-based therapy (*n* = 14) and the relaxation control group (*n* = 17).

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|  | **Mindfulness-Based Therapy** | **Relaxation Control** | **Test**1 |
| **Pre-treatment** | 1.07 [.42] | 1.00 [.52] | *t*(29) = 0.41, *p* = .69 |
| **Post-treatment** | 1.05 [.41] | .88 [.44] | *t*(29) = 1.1036, *p* = .28 |
| **Test**1 | *t*(13) = 0.2, p = .84 | *t*(16) = 0.97, p = .35 |  |

Square brackets show standard deviation.

1T-tests were two-tailed.