

Investigation of mindfulness meditation practitioners with voxel-based morphometry

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Mindfulness meditators practice the non-judgmental observation of the ongoing stream of internal experiences as they arise. Using voxel-based morphometry, this study investigated MRI brain images of 20 mindfulness (Vipassana) meditators (mean practice 8.6 years; 2 h daily) and compared the regional gray matter concentration to that of non-meditators matched for sex, age, education and handedness. Meditators were predicted to show greater gray matter concentration in regions that are typically activated during meditation. Results confirmed greater gray matter concentration for meditators in the right anterior insula, which is involved in interoceptive awareness. This group difference presumably reflects the training of bodily awareness during mindfulness meditation. Furthermore, meditators had greater gray matter concentration in the left inferior temporal gyrus and right hippocampus. Both regions have previously been found to be involved in meditation. The mean value of gray matter concentration in the left inferior temporal gyrus was predictable by the amount of meditation training, corroborating the assumption of a causal impact of meditation training on gray matter concentration in this region. Results suggest that meditation practice is associated with structural differences in regions that are typically activated during meditation and in regions that are relevant for the task of meditation.

Keywords: meditation; mindfulness; voxel-based morphometry; gray matter concentration

INTRODUCTION

In recent years, the clinical application of mindfulness and its role in understanding human consciousness have come into the focus of psychological research (Brown *et al.*, 2007, Psychological Inquiry). The practice of mindfulness requires attentiveness to the internal experiences that arise at each moment, adopting an attitude of non-judgmental acceptance (Hart, 1987; Kabat-Zinn, 2003). The detached observation of bodily sensations, emotions and thoughts is assumed to interrupt automatic responding and to increase behavioral flexibility (Bishop *et al.*, 2004). Repeated training should facilitate self-regulation of attention and improve emotion regulation (Hart, 1987). In fact, empirical behavioral studies support the idea that mindfulness training modifies subsystems of attention (Jha *et al.*, 2007), and enhances well-being (Brown and Ryan, 2003). Clinical interventions that incorporate mindfulness practices have proven effective in the treatment of a broad spectrum of disorders (Baer, 2003; Grossman *et al.*, 2004).

Recent studies showed that altered cognitive functions in mindfulness meditators are associated with a difference in the corresponding brain activations (Brefczynski-Lewis *et al.*,

2007; Hölzel *et al.*, 2007; Slagter *et al.*, 2007). While several studies have investigated the effects of meditation training on brain function, little is known about the modification of the brain structure that accompanies meditative practice.

In several domains, intensive training has been found to increase gray matter in regions relevant for the respective task, detectable in anatomical MRI scans. For instance, brains of volunteers who have learned to juggle showed an expansion in gray matter in the mid-temporal area and in the left posterior intraparietal sulcus (Draganski *et al.*, 2004). Learning a second language increases the density of gray matter in the left inferior parietal cortex (Mechelli *et al.*, 2004).

These recent studies challenged the traditionally held view that learning changes only the way the brain functions, and instead showed that structural changes at the macroscopic level are possible. Presumably, the repeated activation of certain regions leads to structural changes. A study by May *et al.* (2007) shows that even repetitive transcranial magnetic stimulation can cause cortical changes in gray matter within 5 days of intervention.

The only study published to date investigating the effects of meditation training on brain structure (Lazar *et al.*, 2005) compared cortical thickness between mindfulness meditators and non-meditators. Meditators had a significantly thicker cortex in the right anterior insula, the left superior temporal gyrus and the right middle and superior frontal sulci. The right anterior insula is commonly activated during tasks

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of interoceptive awareness and its local gray matter volume correlates with interoceptive accuracy and visceral awareness (Critchley *et al.*, 2004). The observation of bodily sensations, constantly performed during mindfulness meditation, might thus be reflected in increased insular thickness in meditators. According to Lazar *et al.* (2005), regular meditation is thought to promote structural changes in a subset of cortical regions in areas of interoceptive and somatosensory processing and attention regulation. The method of cortical thickness analysis employed by Lazar *et al.* (2005) bears the disadvantage that changes in subcortical structures cannot be detected. The purpose of the present study was to extend the findings of Lazar *et al.* (2005) by comparing the regional gray matter concentration of mindfulness meditators in the whole brain to that of people without meditation experience. We used voxel-based morphometry (VBM; Ashburner and Friston, 2000; Mechelli *et al.*, 2005), which permits an automated voxel-wise whole-brain statistical comparison of MRI scans. The VBM procedure involves normalizing structural images to a standard template, segmenting those into gray and white matter and smoothing them (Ashburner and Friston, 2000).

We expected that meditators would show greater gray matter concentration in regions that are activated during meditation. Although only relatively few studies have investigated the brain activation during meditation to date (cf. Cahn and Polich, 2006; Lutz *et al.*, 2007), some relevant regions have been identified across different studies. Typical patterns of activation during self-directed meditation involve the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC; Newberg and Iversen, 2003; Cahn and Polich, 2006). This activation is thought to reflect the active regulation of attention. Further regions found to be involved in meditation include the hippocampal/parahippocampal formation (Lou *et al.*, 1999; Lazar *et al.*, 2000; Hölzel *et al.*, 2007). The hippocampus is thought to be important for meditation because of its involvement in the modulation of cortical arousal and responsiveness (Newberg and Iversen, 2003). Furthermore, activation in the left inferior temporal gyrus and the left postcentral gyrus was found during mindfulness exercises (Hölzel *et al.*, 2007).

In addition to regions that are typically activated during meditation, we tested the right anterior insula, an area previously shown to differ between experienced meditators and people without meditation experience (Lazar *et al.*, 2005). Group differences were tested in regions of interest (ROI) analyses. We hypothesized that the DLPFC, ACC, hippocampus, left inferior temporal gyrus, left postcentral gyrus and right anterior insula would show greater gray matter concentration in meditators than in controls. Moreover, to test for changes in gray matter dependent on the amount of meditation training, the accumulated number of hours of meditation practice was used as a predictor in a regression analysis.

METHODS

Study participants

Forty healthy, right-handed participants took part in the study: 20 meditators (mean age: 34.1 years; s.d.: 4.7 years) and 20 non-meditators (mean age: 34.0 years; s.d.: 5.1 years), matched for sex, age, education and handedness. Each group consisted of 16 male and four female participants. Meditators were recruited from a Vipassana Center in Germany, where meditation is taught in the tradition of S.N. Goenka (Hart, 1987). Participants practiced meditation 2 h daily and their duration of meditation practice ranged between 2.1 years and 16.2 years with a mean of 8.6 years (s.d.: 5.0 years). On the basis of the daily practice and the time spent in meditation retreats, the total amount of hours of meditation training was estimated. Overall, participants' meditation training ranged between 1120 and 17 700 h with a mean of 6254 h (s.d.: 4529 h). Controls had no experience with meditation. Written informed consent was obtained from all participants.

Imaging data

High-resolution MRI data were acquired with a Siemens Symphony 1.5 T scanner with standard head coil. Three-dimensional data sets of the whole brain were collected using a T1 weighted, magnetization prepared rapid acquisition gradient echo (MP-RAGE) sequence, consisting of 160 sagittal partitions (slice thickness 1 mm, TI/TE/TR (inversion/echo/repetition time) 1100/4.18/1990 ms, flip angle 15°, matrix 256 × 256, field of view 250 mm).

Image analysis was performed using the voxel-based morphometry toolbox by Gaser (2007) for SPM2 software (Wellcome Department of Cognitive Neurology, London) in MATLAB 6.5, release 13 (Mathworks Inc., Natick, MA, USA). The toolbox by Gaser is an extension to the segmentation algorithm of SPM 2 and is based on the so-called optimized VBM protocol first proposed by Good *et al.* (2001; see also Mechelli *et al.*, 2005).

All images were spatially normalized (12 parameter affine registration followed by a non-linear registration) to the same stereotaxic space using a customized template and customized priors, which were created based on the 40 images of the whole sample. Images were then segmented into gray matter, white matter and cerebrospinal fluid. For our study, unmodulated images (Good *et al.*, 2001) were analyzed, as they do not take into account alterations in volume due to normalization effects. Unmodulated images contain the probability within each voxel for being gray matter, i.e. the proportion of gray matter to other tissue types within a region (Good *et al.*, 2001). A 12 mm full width at half maximum (FWHM) Isotropic Gaussian Kernel was used to smooth images. The final probability maps were tested voxel-wise using a two sample *t*-test for independent samples in SPM2 to measure group differences in local gray matter concentration. For definition of the ROIs, the anatomical parcellation of the normalized brain

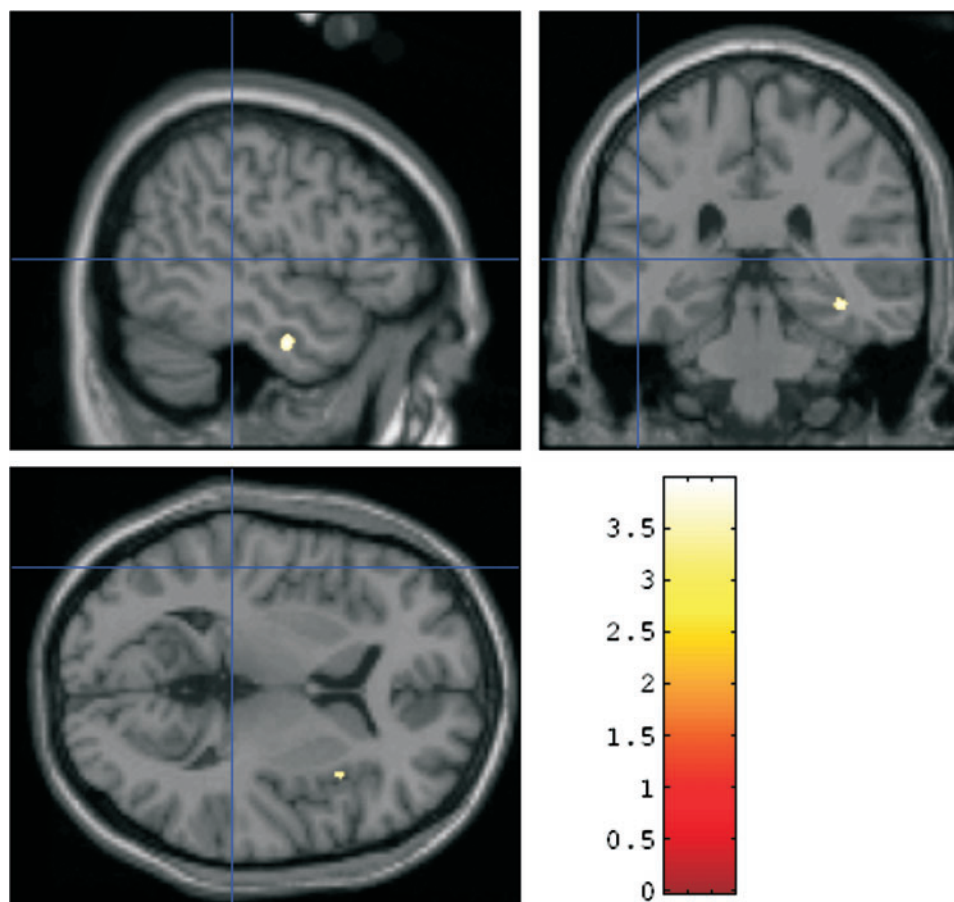


Fig. 1 Greater gray matter concentration for meditators compared to non-meditators ($P < .001$; uncorrected; > 20 voxels) in the left inferior temporal gyrus (upper left panel; cluster size: 157 voxels), right anterior insula (lower left panel; cluster size: 22 voxels) and right hippocampus (upper right panel; cluster size: 106 voxels). MNI coordinates of shown slices: $x, y, z = -49, -32, 6$; color bar indicates t -values.

(single-subject high-resolution T1 volume of the Montreal Neurological Institute) was used (Tzourio-Mazoyer *et al.*, 2002). Masks were created using MARINA software (Walter *et al.*, 2003). ROI analyses (family wise error corrected) were calculated for the bilateral DLPFC, ACC, hippocampus, left inferior temporal gyrus and left postcentral gyrus. In order to obtain a mask for the right anterior insula, the standard mask for the right insula was restricted to those voxels anterior to MNI coordinate $y = 0$.

For regions that yielded a group difference in local gray matter concentration, we tested whether the amount of meditation training could predict the gray matter value for meditators. A positive correlation would corroborate the causal role of meditation practice on the increase of gray matter concentration. Within the identified regions, those voxels were selected that showed t -values greater than $t = 3.32$ (corresponding to $P = 0.001$, uncorrected for multiple comparisons) in the group comparison. Mean values of gray matter probabilities in those areas for each of the meditators were then used as the dependent variable and the total number of hours of meditation practice served as the predictor. Regression analyses were computed in

SPSS for Windows (Statistical Package for Social Sciences, Release 12.0.2, 2004. Chicago: SPSS Inc.).

Finally, an exploratory whole-brain regression analysis in SPM (threshold: $P = 0.001$, uncorrected; cluster size > 20 voxels) was performed for the meditators, using the accumulated hours of meditation practice to predict the gray matter concentration in every voxel.

RESULTS

Comparison of meditators and non-meditators

In ROI analyses, gray matter concentration in the right hippocampus (peak $x, y, z = 38, -32, -12$; $t = 3.77$; $P = 0.027$; Figure 1) and right anterior insula (peak $x, y, z = 36, 12, 6$; $t = 3.51$; $P = 0.022$) was significantly greater in meditators. The cluster at the left inferior temporal gyrus (peak $x, y, z = -49, -9, -28$; $t = 3.88$; $P = 0.058$) showed a trend towards significance.

We then created a mask for the left inferior temporal gyrus based on the activation we found during mindfulness of breathing meditation reported in Hölzel *et al.* (2007). To this end, the ROI was restricted to those voxels with

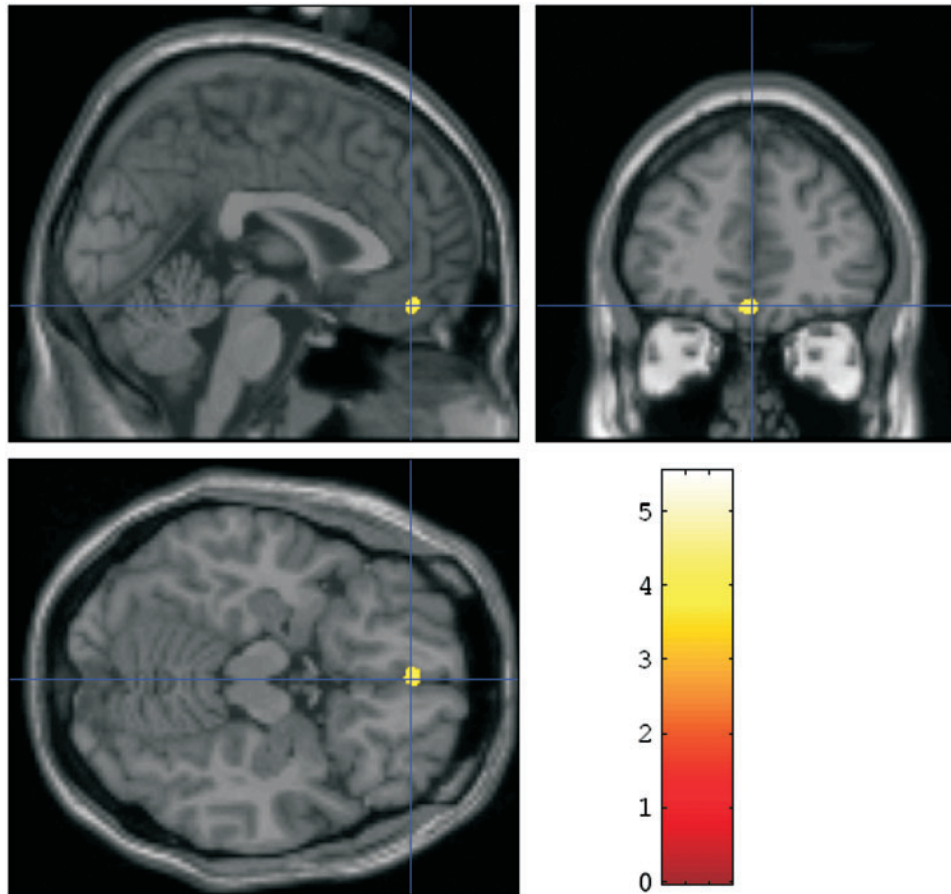


Fig. 2 Gray matter concentration at the medial orbitofrontal cortex correlates with total hours of meditation training. MNI coordinates of shown slices: x, y, z : 1, 45, -16; cluster size: 185 voxels; color bar indicates t -values.

$t > 4.25$ in the functional data (corresponding to the significance level reported in Table 3 in Hölzel *et al.*, 2007). When investigating this search region, the greater gray matter concentration for meditators was clearly significant (peak x, y, z : -49, -8, -30; $t = 3.58$; $P = 0.023$).

The other structures tested in ROI analyses, namely the DLPFC, ACC and left postcentral gyrus were not significant. Controls did not show any cluster of greater gray matter concentration compared to meditators.

An additional exploratory analysis of the group difference in gray matter concentration at an α level of 0.05 corrected for multiple comparisons for the whole brain yielded no significant results.

Amount of practice

Masks derived from group comparison. We first tested the effect of the amount of meditation training on gray matter concentration in those areas found to differ between the groups. In the identified regions within MARINA masks of the left inferior temporal gyrus, right hippocampus and the right anterior insula, mean values of gray matter probabilities were used as dependent variables and were predicted by the total number of hours of meditation practice. A positive

and significant regression weight was obtained for the left inferior temporal gyrus ($r = 0.40$; $P = 0.04$; one-tailed). The correlation of mean values at the right anterior insula and the hours of meditation training showed a trend toward significance ($r = 0.36$; $P = 0.06$; one-tailed). Mean values in the right hippocampus did not correlate with the hours of meditation training.

Whole-brain. In order to detect further regions that correlated with the amount of practice, voxel values within the whole brain were correlated with the hours of meditation training on an exploratory basis. A cluster of gray matter concentration that was found to correlate with the amount of meditation training was located in the bilateral gyrus rectus and medial orbitofrontal cortex (OFC) (peak x, y, z : 1, 45, -16; $t = 4.28$; cluster size: 185 voxels; Figure 2). It has to be emphasized that this region was not significant when correcting for the whole brain.

DISCUSSION

Meditation techniques are applied to train specific cognitive functions (Hart, 1987). Task demands of different techniques are reflected in characteristic brain activation patterns (Cahn and Polich, 2006; Lutz *et al.*, 2007). The present study

revealed that meditators show increased gray matter concentration in regions that are relevant for meditation. Gray matter concentration was significantly greater for meditators in the right hippocampus and the right anterior insula and showed a trend towards significance in the left inferior temporal gyrus. When isolating the area within left inferior temporal gyrus previously shown to be active during mindfulness meditation (Hölzel *et al.*, 2007), meditators showed significantly greater gray matter concentration in this region. Although the clusters that showed a group difference were relatively small (Figure 1) compared to other VBM studies (Draganski *et al.*, 2004; Mechelli *et al.*, 2004), results are clearly significant. Meditators mean gray matter concentration within the relevant regions in the left inferior temporal gyrus was predictable by the amount of meditation training, corroborating the assumption of a causal impact of meditation training on gray matter concentration. The correlation of gray matter concentration at the right insula and the amount of meditation practice showed a trend towards significance. In an exploratory whole brain regression analysis, the cumulated hours of meditation training predicted gray matter concentration in the medial OFC. The expected differences at the DLPFC, ACC and left postcentral gyrus were not confirmed.

The right insula, which showed greater gray matter concentration in meditators in our study and greater thickness in the study by Lazar *et al.* (2005), is involved in interoception and visceral awareness (Critchley *et al.*, 2004). This difference between meditators and non-meditators presumably reflects the specific training during Vipassana meditation, namely the awareness of bodily sensations (Hart, 1987). In a recent study, Farb *et al.* (2007) found that participants trained in mindfulness showed increased activation of viscerosomatic areas, including the right insula, during a momentary experiential focus. This finding further supports the crucial role of the insula for the experience of a mindful state.

Further, our data confirmed the hypothesis that the hippocampus exhibits greater gray matter concentration in meditators. An investigation of subcortical structures was not possible with the methods employed by Lazar *et al.* (2007). The usage of VBM in the present study enabled us to go beyond these limitations and made it possible to confirm the group difference in the hippocampus. In their neuropsychological model of meditation, Newberg and Iversen (2003) ascribe an important role for meditative experiences to the hippocampus, due to its involvement in modulating cortical arousal and responsiveness. The hippocampus also modulates amygdalar activity and its involvement in attentional and emotional processes. The region identified in the group comparison extends into the parahippocampal region, which participates in emotional memory and sensory functions (Suzuki, 1996) and is the essential link between the hippocampus and neocortex (de Curtis and Paré, 2004). As activation of the

hippocampal/parahippocampal region has been found in several meditation studies, including ours, we assume that the repeated activation during meditation practice leads to the altered gray matter structure in meditators.

Gray matter concentration in the left inferior temporal gyrus not only differed between groups, but also significantly correlated with the hours of meditation training, supporting the presumption that meditation training favors an increase in gray matter in the left inferior temporal gyrus resulting from repeated activation. Temporal lobe activation during meditation has been found in several studies (Lou *et al.*, 1999; Lazar *et al.*, 2000; Hölzel *et al.*, 2007), suggesting it plays an important role in meditation (Previc, 2006). The temporal lobe has been implicated in religious activity and mystical experiences (Saver and Rabin, 1997), which are characterized by the feeling of deep pleasure and the experience of insight into the unity of all reality. While we do not assume that the participants of the present study experience mystical states, similar experiences are reported by meditators during deep stages of meditation (Piron, 2001) and might be related to the structural differences. However, this explanation remains tentative and has to be tested in future investigations.

Data did not confirm the expected differences at the DLPFC, ACC and left postcentral gyrus. Also, no effect of meditation training has been found on the cortical thickness in these regions (Lazar *et al.*, 2005). Possibly, the activation of these regions during meditation training does not lead to alterations in cortical structure. It is conceivable that some brain regions are more amenable to structural modifications than others. It will be the task of future studies to investigate this issue. It also has to be kept in mind that the evidence on the functional neural correlates of mindfulness practice is still limited. Different types of meditation may recruit different neural networks. Only few studies have investigated brain activation during mindfulness practice so far and more research is strongly needed to identify relevant regions.

The exploratory whole-brain regression analysis revealed that gray matter concentration in the medial OFC was positively correlated with the cumulated hours of meditation training. The OFC plays a crucial role in emotion regulation (Quirk and Beer, 2006), during which it is thought to down-regulate activity of the amygdala. Thickness of the medial OFC is directly correlated with extinction retention after fear conditioning, suggesting that its size might explain individual differences in the ability to modulate fear (Milad *et al.*, 2005). It is thus critical for the modification of learned emotional responses. Greater gray matter concentration in the medial OFC dependent on meditation training might reflect the improved ability to modify emotional responses.

In an fMRI study, dispositional mindfulness was found to predict activation of multiple PFC sites, including the medial OFC, during an affect labeling task of facial

expressions (Creswell *et al.*, 2007). These data suggest a crucial role of the medial OFC for emotion regulation capacities associated with dispositional mindfulness. Mindfulness meditation training, which leads to greater dispositional mindfulness (Brown and Ryan, 2003), might strengthen those structures.

It has to be kept in mind that gray matter in the OFC was not found to be greater in meditators than in non-meditators in the present study. The absence of a group difference in gray matter concentration in the OFC might be a sample artifact. It is, however, also possible that meditators compensate for an initially lower gray matter concentration in the OFC with meditation practice. Future studies should clarify this question. Finally, it has to be emphasized that this effect is very small and a replication on a larger sample is needed.

We assume the difference in gray matter concentration between the groups to be an effect of the extensive meditation training of meditators. However, the causal direction of influence cannot be determined by this study. Alternatively, people with greater gray matter in the relevant regions might be prone to take up meditation practice. Though correlational data provide support for the causal role of meditation practice, it is possible that meditators with greater gray matter concentration in the relevant regions maintain practice for a longer period of time. Randomized longitudinal studies could adequately address this question. While groups were matched on gender, age, education and handedness, uncontrolled systematic differences between the two groups could have accounted for the structural differences. Possible confounding variables, such as personality profiles, drug and alcohol consumption were not considered here, but should be controlled in future research.

Both, the present study and the study by Lazar *et al.* (2005) investigated participants who practice mindfulness meditation. Samples had similar durations of meditation practice. Yet, it has to be emphasized that our results are not directly comparable to those of Lazar and colleagues because different variables were measured: Lazar investigated cortical thickness while our study examined gray matter concentration. Only very few studies have compared cortical thickness and gray matter concentration analyses so far (Narr *et al.*, 2005; Chung *et al.*, 2007), and more investigation is required on the concordance of both measures. To date, the physiological underpinnings of alterations in gray matter structure on a histological or cellular level are not yet sufficiently understood (May and Gaser, 2006) and further research is strongly needed to investigate whether modifications of cortical thickness and gray matter concentration are related to changes in neuropil, neuronal size, dendritic or axonal arborization. Direct comparison of anatomical MRI data and histological data could advance the understanding of the mechanisms of structural reorganization.

CONCLUSION

The present study showed a distinct pattern of gray matter concentration in meditators, who spent a significant part of their lifetime training non-judgmental acceptance towards internal experiences that arise at each moment. Regular meditation practice is associated with structural differences in regions that are typically activated during meditation, such as the inferior temporal gyrus and hippocampus as well as in regions that are relevant for the task of meditation, such as the insula and OFC. Using the whole-brain unbiased objective technique of VBM (Mechelli *et al.*, 2005), the present study extends the findings by Lazar *et al.* (2005) and suggests a wider network of brain regions involved in meditation show long-term structural differences. Given the rising interest in mindfulness-based programs for the treatment of psychological disorders (Grossman *et al.*, 2004), the identification of structural correlates of intensive mindfulness training offers a promising approach for research on the neurophysiological mechanisms underlying such programs. Future studies should combine neuroscientific and clinical streams of research for a better understanding of the effects of such treatments.

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