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Structural changes in socio-affective networks: Multi-modal MRI findings in long-term meditation practitioners

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ABSTRACT

Our goal was to assess the effects of long-term mental training in socio-affective skills on structural brain networks. We studied a group of long-term meditation practitioners (LTMs) who have focused on cultivating socio-affective skills using loving-kindness and compassion meditation for an average of 40k h, comparing these to meditation-naïve controls. To maximize homogeneity of prior practice, LTMs were included only if they had undergone extensive full-time meditation retreats in the same center. MRI-based cortical thickness analysis revealed increased thickness in the LTM cohort relative to meditation-native controls in fronto-insular cortices. To identify functional networks relevant for the generation of socio-affective states, structural imaging analysis were complemented by fMRI analysis in LTMs, showing amplitude increases during a loving-kindness meditation session relative to non-meditative rest in multiple prefrontal and insular regions bilaterally. Importantly, functional findings partially overlapped with regions of cortical thickness increases in the left ventrolateral prefrontal cortex and anterior insula, suggesting that these regions may play a central role in the generation of emotional states relevant for the meditative practice. Our multi-modal MRI approach revealed structural changes in LTMs who have cultivated loving-kindness and compassion for a significant period of their life in functional networks activated by these practices. These preliminary cross-sectional findings motivate future longitudinal work studying brain plasticity following the regular practice of skills aiming at enhancing human altruism and prosocial motivation.

1. Introduction

There has been a long-standing interest in the neurosciences to study brain plasticity (Huttenlocher, 2002; Kandel, 2012). Plasticity can occur across multiple time scales from short-term to long-term within functional networks, and at the level of structural networks. In humans, most previous studies addressing structural plasticity have focused on training and/or expertise effects on motor, sensory, or memory functions (Draganski et al., 2004; Taubert et al., 2011), but also skills like musicality (Bermudez et al., 2009). Conversely, except for work showing limbic network reorganization in the context of negative affect and fear conditioning (LeDoux, 2000; Phelps and LeDoux, 2005), only a few studies have addressed plasticity in networks mediating socio-affective skills, especially those that require the generation of positive affect, loving-kindness, and compassion.

Behavioral and autonomic research has suggested that cultivating qualities such as loving kindness and compassion are associated with increases in prosocial behavior (Leiberg et al., 2011), and vagal tone as a proxy of physical health (Kok et al., 2013). Furthermore, some studies have supported the application of these strategies in clinical populations, for example as a means to reduce stress, pain, and mood problems (Hofmann et al., 2011). Training of loving-kindness meditation involves the active generation of feelings of warmth, love, kindness, and prosocial motivation for others; confronted with the suffering of others, loving-kindness may furthermore become compassion, associated with a wish to alleviate the suffering (Salzberg, 1995; Singer and Klimecki, 2014). Using short-term longitudinal functional MRI studies, our group recently demonstrated short-term functional plasticity after the training

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of loving-kindness and compassion in meditation-naïve participants (Klimecki et al., 2013a, 2013b). Changes were observed in subcortical networks and fronto-limbic cortices, centred on prefrontal-cingulate as well as insula regions. On a behavioral level, training effects manifested in increased reports of positive affect, even when participants were exposed to distressing videos, and increased helping behavior (Leiberg et al., 2011). While providing initial evidence of subjectively and behaviorally relevant functional changes following the training of compassion and loving-kindness in a network typically associated with positive affect, affiliation, and reward (Grabenhorst and Rolls, 2011; Kringelbach and Berridge, 2009), the short duration of the training protocol and retest-interval did not allow us to robustly study the effects of compassion-related mental practices on brain structure.

In the absence of long-term and sufficiently powered longitudinal mental training studies that involve costly and state-of-the art neuroimaging (Valk et al., in press), cross-sectional assessments of mental training experts, specifically long-term meditation practitioners (LTMs), provides a useful framework to infer brain plasticity following frequent mental training (Ricard et al., 2014). A series of functional MRI studies have shown alterations in the activation of limbic networks, particularly insular regions, in experts relative to controls (Lutz et al., 2008, 2009). Additional analyses of brain structure provide and relatively state-invariant measure of training effects that may not be affected by specific demand characteristics of a given task. Structural changes in LTMs have been reported previously, but generally in cohorts practicing attention and mindfulness (Fox et al., 2014); conversely, practitioners who have explicitly cultivated socio-affective capacities, such as compassion and loving-kindness, are less well studied and we thus do not know about the effects specifically associated to their long-term training expertise.

To study expertise effects related to long-term mental training in socio-affective skills on brain structure, we recruited LTMs from a school of Tibetan Buddhism that focuses on cultivating loving-kindness, altruism, and compassion. Included LTMs have formally practiced meditation more 40k h on average before our study. Moreover, and to furthermore increase the homogeneity in the form of meditative practice, LTMs were included only if they had participated in at least one extensive full-time meditation retreat in the same center by the same group of teachers. In addition to undergoing high-resolution structural MRI acquisitions that were analyzed with surface-based techniques assess between-cohort differences in cortical thickness, our LTMs underwent two counterbalanced functional MRI sessions that were each seven-minutes long. In one session, they were asked to perform a loving-kindness meditation; a counterbalanced non-meditative resting-state session served as control condition. Functional amplitude increases during loving-kindness relative to non-meditative rest were mapped onto cortical surfaces, to assess overlaps with regions of cortical thickness changes. We expected that our LTMs would show alterations in brain structure compared to age-matched controls in regions relevant to the generation of loving-kindness and socio-affective processing, which we hypothesized to likely be situated in prefrontal and insular cortical networks.

2. Material and methods

2.1. Subjects

Participants were tested between November 2011 and March 2013. We studied a cohort of 17 long-term meditators (LTM; 5 women; 45–62 years, mean ± SD age = 56 ± 5 years). All meditators were adepts of the Nyingma tradition of Tibetan Buddhism (one of the schools focussing on the cultivation of loving-kindness, altruism, and compassion). Participants were included only if they had previously participated in multiple intensive full-time meditation retreats (3 months to 3 years of retreat), which all carried out at the Songsen Chanteloube Retreat Center in Dordogne, France by the same group of teachers. The aim of the retreats is to create a protective environment which allows students to concentrate on essential aspects of Buddhist practice, namely, study, reflection, and meditation, expounded according to the teachings of different traditions, but focusing on the Nyingma tradition. The LTMs have emphasized loving-kindness and compassion throughout their training since the so-called *great vehicle* of Buddhism (or *Mahayana*) considers that love and compassion are, together with wisdom, the main driving forces to reach enlightenment. Focused attention, mindfulness and analytical meditation are also used, but mainly as tools to develop wisdom and compassion.

We estimated practice hours from a semi-structured interview with the participants, where they provided information on the year they began meditating, the hours of practice per day, and the consistency of their practice. LTMs reported having practiced meditation for an estimated total of 40k ± 12k h (range = 9–62k h) and practiced for 30 ± 7 years on average (range-20–41 years) before our study, and to practice loving-kindness and compassion meditation daily.

LTMs were compared to 15 healthy controls without prior meditation experience, recruited through advertisement. Controls were matched for age and sex (5 women; 46–63 years, mean ± SD age = 54 ± 6 years), as well as education level (LTM: mean ± SD years of education = 14 ± 3 years; Controls: 14 ± 3 years). We assessed the IQ of all participants using Raven's Progressive Matrices (Raven, 2000). LTMs and controls did not differ with respect to IQ (103.6 ± 20.0 *vs* 102.4 ± 28.5). Furthermore, LTMs and controls scored similar on the Beck's Depression Inventory [BDI-2; (Beck et al., 1996); mean ± SD in LTM: 5.9 ± 3.9; controls: 6.6 ± 7.0; $p > 0.7$] and the Spielberger Trait Anxiety Scale [STAI-T; (Spielberger et al., 1983); mean ± SD in LTM: 37.3 ± 9.9; controls: 37.0 ± 9.1; $p > 0.7$]. One LTM was Asian, while sixteen LTMs and all controls were Caucasian. All were EU citizens (all controls German; LTMs: 1 German, 2 British, 14 French). The study was approved by the Ethics Committee of the University of Leipzig and was carried out in compliance with the Declaration of Helsinki. All participants gave written informed consent, were paid for their participation, and were debriefed after the study.

2.2. Structural MRI

2.2.1. Acquisition

Structural MRI data were acquired on a Siemens 3T Verio scanner (Siemens Medical Systems, Erlangen, Germany) using a 32-channel head coil. Images were acquired using a T1-weighted 3D-MPRAGE sequence (repetition time [TR] = 2300 ms, echo time [TE] = 2.98 ms, flip angle = 7°, 176 sagittal slices, field of view [FOV] = 240 × 256 mm^2, matrix = 240 × 256, 1 × 1 × 1 mm^3 voxels).

2.2.2. Cortical thickness measurements

FreeSurfer (5.0.0; http://surfer.nmr.mgh.harvard.edu) was used to generate cortical surface models and to measure cortical thickness on T1-weighted images. Previous work has validated FreeSurfer by comparing it with histological analysis (Rosas et al., 2002) and manual measurements (Kuperberg et al., 2003). Processing steps have been described in detail elsewhere (Dale et al., 1999; Fischl et al., 1999a, 1999b). Following surface extraction, sulcal and gyral features across individuals were aligned to an average spherical representation that allows for accurate matching of cortical thickness measurement locations among participants. The entire cortex in each participant was visually inspected and a single rater manually corrected segmentation inaccuracies (LS). For whole-brain analysis, thickness data were smoothed using a 15-mm full-width-at-half-maximum (FWHM) surface-based Gaussian kernel. Surface-based smoothing reduces measurement noise while preserving the capacity for anatomical localization, as it respects cortical topology (Lerch, 2005; Lerch and Evans, 2005).

2.3. Functional MRI

2.3.1. Paradigms

We analyzed two fMRI sessions that were each seven-minutes long. Participants were instructed to lie still in the scanner with open eyes, and to fixate a white cross in the center of a black screen. *Session A* was a meditation-free *resting-state* run, where participants instructed to not think about anything in particular, and specifically not to meditate. *Session B* was a loving-kindness meditation session, similar to previous work (Klimecki et al., 2013a, 2013b; Lutz et al., 2008). The specific instructions for the loving kindness meditation session were developed in cooperation with a Buddhist monk (Dr M. Ricard), and aimed at reflecting the practice of the LTMs:

> *"In this condition, we want you to cultivate loving-kindness, 'Metta' in Pali. Altruistic love, loving-kindness or, more simply, warm-heartedness, is the wish that others be happy and well. How to generate and cultivate that state of mind? The easiest way is to begin by bringing to the mind someone who is very dear to us, someone for whom this feeling of affection arises effortlessly and spontaneously. Imagine that a young child approaches you and gives you a look that is joyous, confident, and full of innocence. You stroke his head, look at him with tenderness, and take him in your arms. You feel a sense of unconditional benevolence and love. Let yourself be entirely pervaded by this love that wishes only for his wellbeing. Then cultivate, sustain, and nourish this feeling of loving-kindness. When it declines, revive it. Think repeatedly, 'May that person be safe, may that person be healthy, may that person find happiness and flourish in life'. You could also choose someone else toward whom you feel great tenderness and deep gratitude. Wish with all your heart that this person will find happiness and be well. "*

It should be noted that this instruction specifically requested participants to focus on individuals close to them. This differs somewhat from standard practice, where participants might also focus on other, unknown individuals, or even engage in non-referential forms of meditation. However, as these are arguably, more difficult states to attain and sustain, we opted for this more constrained instruction in the current experiment, to ensure comparability of implementation across participants. Participants also performed an open-presence meditation *Session C*; this session was, however, not the focus of the current work and will be reported elsewhere. *Sessions A*, *B*, and *C* were presented in a counterbalanced order across subjects, and participants could rest between them for 1–2 min and to prepare themselves for the next session.

Post-scan debriefing (on a scale from 1 to 10) was carried out in all individuals and indeed confirmed no difficulties in the LTMs to maintain the state (mean ± SD ease: 6.5 ± 1.9; difficulty: 3.2 ± 1.8). They found themselves in the state most of the time (mean ± SD: 6.8 ± 1.7) and did not report high mind-wandering (mean ± SD: 2.6 ± 1.18). Thus, participants reported both subjective success in achieving a state of loving-kindness in the scanner, and no difficulties with refraining from meditation in the resting state scan.

2.3.2. Acquisition

Functional MRI data were acquired on the same scanner as the functional signals using a 12-channel head-coil. We recorded fMRI time-series using T2*-weighted gradient EPI sequence (TR = 2000 ms, TE = 27 ms, flip angle = 90°, 37 slices tilted at approximately 30° from an axial orientation, FOV = 210 × 210 mm^2, matrix = 70 × 70, 3 × 3 × 3 mm^3 voxels, 1 mm gap; 210 volumes per session). Data were available for all participants except one LTM.

2.3.3. Preprocessing

Processing was based on DPARSF/REST for Matlab [http://www.restfmri.net (Song et al., 2011)]. We discarded the first 5 volumes to ensure steady-state magnetization, performed slice-time correction, motion correction and realignment, and co-registered functional time series to the corresponding T1-weighted MRI. Images underwent DARTEL-based segmentation and registration, followed by nuisance covariate regression to remove effects of average WM and CSF signal, as well as 6 motion parameters (3 translations and 3 rotations). To increase the robustness against motion confounds, we included a *scrubbing* (Power et al., 2012). In brief, we modeled time-points with a frame-wise displacement > 0.5 mm, together with one time-point before and one after each such time-point, as separate regressors during covariate correction. Time-series were band-pass filtered (0.01–0.08 Hz). We computed the amplitude of low frequency functional fluctuations in this frequency band, a marker of overall local functional activation. The amplitude of low frequency functional fluctuations has been suggested to be a measure of changes of activation of brain regions in task-free paradigms (Zuo et al., 2010). Additionally, it has been shown to relate to the contents of thoughts in the resting state (Gorgolewski et al., 2014), suggesting that it provides a measure of the activation patterns supporting different brain states. Amplitude measures were z-scored within the brain mask of each participant prior to analysis.

2.3.4. Surface mapping

Using a boundary-based registration, we mapped functional time-series to cortical surface models (Greve and Fischl, 2009). Like the cortical thickness data, functional data were registered to a surface-based template based on cortical folding patterns and smoothed on tessellated surfaces.

2.4. Statistical analysis

As in previous work (Bernhardt et al., 2015, 2014a, 2014b; Valk et al., 2017), analysis was performed using SurfStat for Matlab (Worsley et al., 2009). All structural and functional MRI analyses were corrected for age and sex.

2.4.1. Cortical thickness comparisons

We carried out a surface-based comparison of thickness between LTMs and controls using Student's *t*-tests at each cortical point. In regions of findings, statistical group comparisons were complemented by individual analysis that provides prevalence estimates in LTMs for markedly different thickness values relative to controls.

2.4.2. Functional analysis

We applied linear mixed effects models to compare differences in amplitude between loving-kindness and resting-state conditions within LTMs and to study the interaction between this contrast and our study groups. The model included *subject* as a random effect and *age*, *sex*, *group*, *state*, as well as a *group* × *state* interaction as fixed effects.

2.4.3. Correction for multiple comparisons

Surface-based structural and functional findings results were controlled at $p_{FWE} < 0.05$, using random-field theory for non-isotropic images (Worsley et al., 1999) using cluster-defining thresholds of p = 0.025.

2.4.4. Structure-function overlap analysis

Following correction for multiple comparisons (see Section 2.4.3), we geometrically intersected significant structural with functional findings (Friston et al., 2005). This allowed us to identify regions that showed significant differences in cortical thickness between LTMs and controls, as well as significant increases in functional amplitude in loving-kindness meditation compared to rest. Thus, these regions are both functionally involved in the generation of the meditative state and structurally affected by extended training in generating these states, suggesting they play a key role in loving-kindness generation.

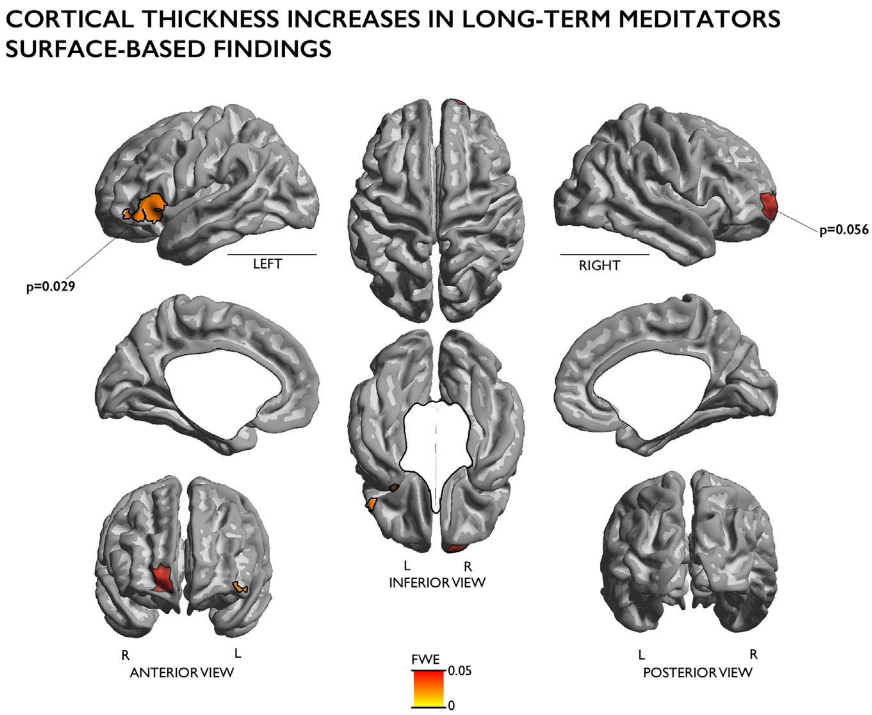

Fig. 1. Cortical thickness increases in long-term meditators (LTMs) relative to controls: surface-based findings. Significant increases after multiple comparisons correction at a cluster-level of $p_{FWE} = 0.05$ are surrounded by bold black outlines.

3. Results

3.1. Cortical thickness increases in LTMs: surface-based findings

Surface-based analysis revealed increased cortical thickness in LTMs compared to controls in a right prefrontal cluster encompassing fronto-polar cortex and left ventrolateral (cluster-wise $p_{FWE} < 0.029$) as well as a trend in right fronto-insular cortices (cluster-wise $p_{FWE} = 0.069$, Fig. 1, Supplementary Table 1). There were no significant cortical thickness decreases in LTMs. Inter-hemispheric asymmetry analysis (following the mapping of the thickness data to a hemisphere-symmetric surface template) did not yield significant differences in asymmetry between LTMs and controls, both at the level of mean thickness ($t < 0.5$, $p > 0.2$) nor at the level of individual surface points following the correction for multiple comparisons ($P_{FWE} > 0.7$).

3.2. Loving-kindness related amplitude increases in LTMs

Comparing state-related differences between LTMs and controls, we observed greater within-subject increases in LTMs than in controls when comparing loving-kindness meditation to non-meditative rest in medial and anterior prefrontal regions (cluster-wise $p_{FWE} < 0.001$), while amplitude in posterior regions decreased (Fig. 2, Supplementary Table 2). When analyzing state-differences in LTMs only, we observed virtually identical findings (Supplementary Fig. 1, Supplementary Table 3).

3.3. Overlap of functional and structural findings

Intersecting FWE-corrected structural and functional findings (Fig. 3), we observed overlaps in ventrolateral and insular regions (Supplementary Table 4).

3.4. Relation to hours and years of practice

Following correction for multiple comparisons, we did not observe any significant associations between cortical thickness or functional amplitude on the one hand, and hours or years of practice on the other hand.

4. Discussion

The focus of the current work was to investigate structural brain changes in a unique cohort of long term meditators (LTMs), with extensive and homogenous experience in compassion and loving kindness meditation – a family of meditation techniques that has only received relatively little attention in the literature. Using structural MRI, the LTMs were compared with an age- and sex-matched meditation-naïve control group, allowing us to identify changes in gray matter thickness in the cortical mantle associated with meditation practice. To differentiate general effects of meditation as reported previously (*e.g.,* Fox et al., 2014) from those specifically associated with loving-kindness meditation, we additionally investigated the amplitude of low frequency functional fluctuations (Zang et al., 2007, Zhang et al., 2010) during a loving-kindness meditation session relative to non-meditative rest. This allowed us to investigate both functional and structural signatures of loving-kindness meditation, thereby differentiating general expertise effects from those specific to the meditative state.

Using an unconstrained whole-cortex approach, we observed thickness increases in LTMs relative to novices in a left-lateralized cluster ranging from ventrolateral prefrontal regions into anterior insular cortex, together with a trend for changes in a right-lateralized fronto-polar cluster. Notably, the location of morphological changes was partially overlapping with regions highlighted by a recent meta-analysis on structural imaging studies of meditation effects on brain morphology (Fox et al., 2014), which however pooled across many different forms of meditation and mainly included attention-oriented practices. Alterations in insular morphology were observed in earlier work in participants with experience in insight meditation (Lazar et al., 2005) and by a more recent study showing increased cortical folding complexity in Samata and Vipassana practitioners (Luders et al., 2012). In addition to its well-established role in socio-affective processing (Bernhardt and Singer, 2012; Krach et al., 2015; Lamm and Singer, 2010; Singer et al., 2009), anterior insula is heavily implicated in interoception, general awareness, and attentional processing (Craig, 2009), possibly suggesting the increases we observed here might reflect

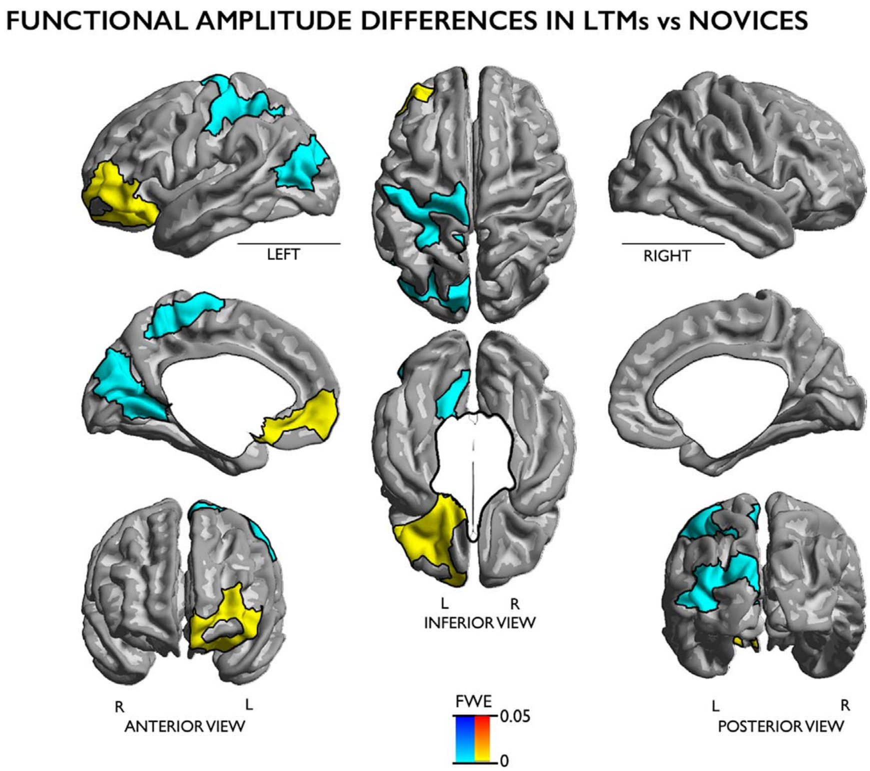

Fig. 2. Between-group interaction of functional amplitude differences during a 7 min-long loving-kindness meditation session relative to non-meditative resting-state session in LTMs relative to controls. *Increases/decreases* after multiple comparisons correction at a cluster-level of $p_{FWE} < 0.05$ are shown in *red/blue*.

more general effects of meditation practice on attentional capacities. Similarly, frontopolar cortical changes may relate to more general effects of mediation training on higher-order cognition, as this region is generally thought to support integrative cognition related to metacognitive awareness (Baird et al., 2013; Fleming et al., 2012; Valk et al., 2016), a skill heavily emphasized in all meditation practices (Dahl et al., 2015).

The functional signature of loving-kindness meditation included amplitude increases during in portions of anterior and medial ventrolateral prefrontal cortex, medial and lateral orbitofrontal regions, together with anterior cingulate, perigenual cingulate, and insular subregions, together with deactivations in posterior parietal, occipital and somatosensory regions. Importantly, we observed an overlap between our structural and functional findings in the left ventrolateral prefrontal cortex. Although the exact biological mechanisms of this structure-function relation remain to be established, frequent activation

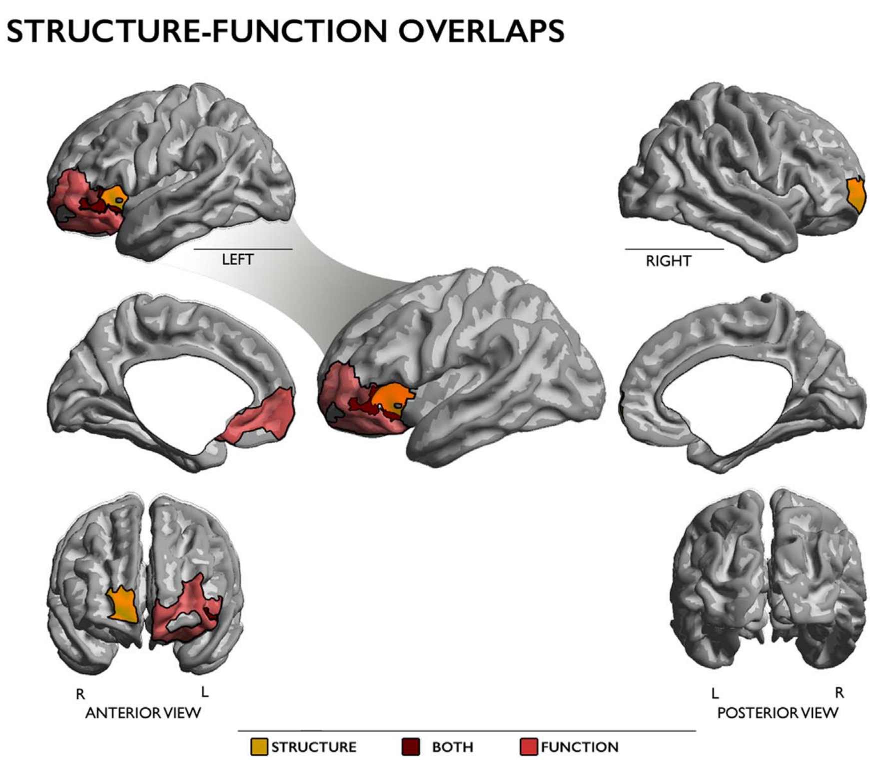

Fig. 3. Overlap analysis between structural and functional findings.

of a brain region as a result of practice and learning may potentially influence synaptic morphology and dendritic arborization, which may ultimately relate to brain structure (Zatorre et al., 2012). If this is the case, our findings may suggest a role of this region in both acute and long-term implementations of loving-kindness. While its precise function is still a subject of debate, this region has been implicated in processes requiring the internal generation of information and has been proposed to support controlled retrieval processes (Badre and Wagner, 2007). This is in line with its frequent involvement in emotion regulation, particularly forms requiring the active generation of alternate meanings of emotional stimuli, such as cognitive reappraisal (Buhle et al., 2014; Wager et al., 2008). Interestingly, reappraisal appears to be somewhat unique among emotion regulation strategies in recruiting left lateralized ventrolateral prefrontal cortex (Dörfel et al., 2014), particularly the inferior frontal gyrus (IFG). We have previously found that a similar region was one of few areas showing activation during regulation of negative emotion using positive reappraisal and compassion mediation (Engen and Singer, 2015). Similarly, in a recent study (Engen et al., 2017), we could show that a region of the left IFG closely overlapping with the region implicated in the present study was central to the generation of emotional states based on endogenous sources of emotion. The left ventrolateral regions observed in the current study might thus be playing a key role in orchestrating generation of positive emotional states associated with loving-kindness meditation. The capacity to generate such states of positive emotion in a volitional manner help foster resilience, potentially offering a "buffer" against negative stressors. Future research could test this hypothesis in at least three ways: First, if our interpretation is correct, one would expect that individual differences in the capacity to endogenously generate emotions should be associated with IFG characteristics. In Engen et al. (2017), we found some support for this, showing that trial-level success at generating emotional states (irrespective of valence) based on endogenous sources of emotion was associated with IFG activation. Second, one would expect that interventions aimed at training loving-kindness or training to enhance the ability to self-generate emotions in general would be associated with morphological and structural changes to this region. Finally, using virtual lesioning techniques like transcranial magnetic stimulation, one would expect that disrupting left IFG activity should interfere with emotion generation abilities more than, for instance, equivalent interference with the right IFG.

Another possibility is that the left ventrolateral activations involve the role of language production, as loving-kindness mediation centrally involves the recitation of a mantra using inner speech (Garrison et al., 2015). It should be noted that these are not necessarily mutually exclusive accounts: The ventral portion of the ventrolateral prefrontal cortex we show to be associated with loving-kindness meditation is known to be part of the "ventral language pathway" (Friderici and Gierhan, 2013), which is thought to be involved in semantic processing, and in the process by which language provides context for emotional perception (Barret et al., 2007). Thus, ventral prefrontal engagement could reflect the generation of a specific emotional context using a linguistic processing modality. It should be noted that a previous functional MRI study did not find evidence for such modality-specificity of these areas (Engen et al., 2017), suggesting it might rather serve domain general processes, for example controlled retrieval of information from memory (Badre and Wagner, 2007). We also observed overlap of functional and structural findings in the ventral anterior insular cortex. Recent work suggests that these regions partake in dissociable large-scale intrinsic connectivity networks serving interoceptive awareness and regulation (Touroutoglou et al., 2012). Thus, our data could suggest that ventrolateral prefrontal and ventral anterior insular regions cooperate in the volitional generation of affective states and, potentially, meditation practices requiring the construction of affective states in general.

It should be noted that we observed decreases in posterior midline regions, a finding supportive of earlier studies suggesting default mode network deactivation that could relate to a reduction of self-related processing during meditation (Garrison et al., 2014, 2015). Thus, loving-kindness meditation may involve the generation of positive affective states combined with a decrease in self-related processing, consistent with the altruistic, other-related motivation.

A related, albeit more speculative, interpretation of the current findings comes from the broader literature on social cognition. This generally converges on at least two core routes underlying our ability to understand others: a socio-cognitive route that enables understanding others' believes and intentions [also referred to as "Theory of Mind" or "mentalizing" (Frith and Frith, 2003; Premack and Woodruff, 1978)] and a socio-affective route encompassing social emotions, such as empathy [the sharing of affect with others (de Vignemont and Singer, 2006)], but also motivational states such as compassion (Singer and Klimecki, 2014). Previous functional imaging studies have identified that Theory of Mind engages a network including medial prefrontal, temporo-parietal, superior temporal, and midline parietal regions (Bzdok et al., 2012; Frith and Frith, 2003). Conversely, socio-affective competences such as the ones targeted in the current assessment generally involve more limbic/paralimbic cortical areas, such as AI, ACC (Lamm et al., 2011), together with orbital frontal cortex - regions also highlighted in the current work based on the analysis of brain structure and intrinsic functional networks. Interestingly, these regions are particularly rich in opioid receptors, and play a crucial role in pain analgesia. Notably, recent studies combining functional MRI and neuropharmacological interventions suggested a direct role of opioid release in empathy for pain (Rutgen et al., 2015a, 2015b). This opens for the intriguing possibility that loving-kindness meditation might enable an individual to volitionally engage this affective route of social processing, and by extension effect the release of endogenous opioids that might have analgesic as well as hedonic effects.

Given the rather stringent inclusion criteria, study cohort size was rather small and findings thus await replication in larger samples. Notably, as we present findings at rather liberal cluster-defining thresholds and FWE-levels of 0.05, our results and especially the overlap findings (which may be influenced by overall sensitivity/specificity of the statistical analysis procedures) need to be interpreted relative to these considerations. Given the rather homogeneous practice of the LTMs, generalizability to populations with none or other forms of meditative experience will also need to be assessed. In this context, it may be of interest to compare LTMs with expertise in compassion to controls from "caring" professions, including doctors and nurses who may have dedicated their lives to helping others and may thus also have altruistic dedication yet a completely different training. Such studies demand further control of potential influences of life-style factors (diet, sleeping, stress, life purpose) on markers of brain structure and function. Our findings may, nevertheless, have implications for the understanding of the brain basis of both constructive meditation practices and meditation in general, suggesting that structural consequences of meditation training may include practice-specific as well as meditation-general processes that need to be dissociated for a more comprehensive understanding of how meditation training relates to long-term changes in brain structure. Additionally, our findings suggest that care must be taken to dissociate functional and structural markers of plasticity, as demonstrated by the relatively circumscribed overlap between structural changes we identified and the extended functional network we found to support the implementation of the loving-kindness state in our meditators. As such, our findings motivate ongoing and future longitudinal investigations, where training and retest cohorts are enrolled in a randomized and controlled fashion, to directly address the causality between mental training, brain changes, and markers of affect and prosociality.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.neuropsychologia.2017.08.024.

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