Gray Matter and Functional Connectivity in Anterior Cingulate Cortex are Associated with the State of Mental Silence During Sahaja Yoga Meditation

Sergio Elías Hernández, Alfonso Barros-Loscertales, Yaqiong Xiao, José Luis González-Mora and Katya Rubia

Abstract—Some meditation techniques teach the practitioner to achieve the state of mental silence. The aim of this study was to investigate brain regions that are associated with their volume and functional connectivity (FC) with the depth of mental silence in long-term practitioners of Sahaja Yoga Meditation. Twenty-three long-term practitioners of this meditation were scanned using Magnetic Resonance Imaging. In order to identify the neural correlates of the depth of mental silence, we tested which gray matter volumes (GMV) were correlated with the depth of mental silence and which regions these areas were functionally connected to under a meditation condition. GMV in medial prefrontal cortex including rostral anterior cingulate cortex were positively correlated with the subjective perception of the depth of mental silence inside the scanner. Furthermore, there was significantly increased FC between this area and bilateral anterior insula/putamen during a meditation-state specifically, while decreased connectivity with the right thalamus/parahippocampal gyrus was present during the meditation-state and the resting-state. The capacity of long-term meditators to establish a durable state of mental silence inside an MRI scanner was associated with larger gray matter volume in a medial frontal region that is crucial for top-down cognitive, emotion and attention control. This is furthermore corroborated by increased FC of this region during the meditation-state with bilateral anterior insula/putamen, which are important for interoception, emotion, and attention regulation. The findings hence suggest that the depth of mental silence is associated with medial fronto-insular-striatal networks that are crucial for top-down attention and emotional control.

INTRODUCTION

Meditation is essentially a physiological state of demonstrated reduced metabolic activity – different from sleep – that elicits physical and mental relaxation and is reported to enhance psychological balance and emotional stability (Young and Taylor, 1998; Rubia, 2009; Jevning et al., 1992). In western psychology, three states of consciousness are described: sleep, dream, and wakefulness. In eastern philosophy and in several western religious and mystical traditions, an additional and supposedly “higher” state of consciousness has been described, the so-called “fourth state of consciousness”, the state of “mental silence” or “thoughtless awareness” (Ramamurthi, 1995). This state can be achieved by the practice of meditation. According to the Yoga Sutras of Patanjali, one of the oldest recorded scriptures on meditation, “Yoga is the suppression of the modifications of the mind” (Rubia, 2009; Kokodoko, 2014).

Meditation has been proposed as a therapy for stress, anxiety, depression and other mental disorders which are typically characterized by problems with affective and attention systems (Rubia, 2009; Manocha et al., 2011; Platt et al., 2016). Meditation has important advantages compared with other therapies including lack of side effects compared to pharmacological treatments, cost-effectiveness in sanitary programs, ease of implementation, and no need for complex instrumentation, technology or infrastructures.
It has been shown that most psychiatric disorders suffer from increased activity of the default mode network, which has been associated with mind wandering and mental clutter (Shim et al., 2010; Whitfield-Gabrieli and Ford, 2012). A reduction in mind wandering may hence be therapeutic for psychiatric disorders (Rubia, 2009). In fact, mindfulness meditation and other meditation techniques that reduce mind wandering or random thinking processes have been shown to decrease the default mode network and to improve clinical symptoms in a variety of disorders (Aftanas and Goloecheikine, 2001; Brewer et al., 2011; Sood and Jones, 2013).

The goal of Sahaja Yoga Meditation (SYM) is the achievement and establishment of mental silence or thoughtless awareness. In this state, the mind is calm and has none or very few thoughts interfering with the state of pure consciousness. The predominant feelings during this state are those of peace and inner joy and the conscious mind is crystal clear, fully aware of each present moment, living in the here and now, in a moment-by-moment basis (Manocha, 2011). It has been shown that the frequency of the perception of mental silence is associated with the improvements of mental and physical health in long-term meditators of SYM (Manocha et al., 2012).

It has also been shown that SYM, with its inherent state of mental silence, has health benefits in mental disorders that are often associated with mind wandering and rumination, i.e., recurrent or repetitive negative thoughts, such as depression, stress, anxiety, and attention-deficit/hyperactivity disorder (Morgan, 2001; Chung et al., 2012; Harrison et al., 2004; Manocha et al., 2011; Rubia et al., 2007). Further, research also found that SYM has beneficial effects in treating physiological and neurological diseases such as asthma (Manocha et al., 2002), high blood pressure (Chung et al., 2012), menopause (Manocha et al., 2007) and epilepsy (Panjwani et al., 1995, 2000, 1996).

Our group has previously conducted two studies to investigate the effects of SYM on brain structure and function. In 19 long-term meditators, using functional Magnetic Resonance Imaging (fMRI), brain activations were compared during three short consecutive meditation conditions with that of a control condition where meditators focused their attention on their breathing (Hernandez et al., 2015). The meditators' effortful process to silence their mind throughout the three meditation states was characterized by neuronal activity in bilateral inferior frontal and temporal regions which became progressively more reduced with the depth of the state of mental silence. In the last and deepest state of meditation, relatively weaker activation remained in right inferior frontal gyrus extending to right anterior insula (AI) and right middle-superior temporal lobe, with the right inferior frontal gyrus activation being specifically correlated with the depth of mental silence. In the structural MRI study, gray matter volumes (GMV) of 23 long-term meditators of SYM were compared to those of a group of non-meditators matched on age, gender, and education level (Hernandez et al., 2016). An ANCOVA with total intracranial volumes, gender, and age as nuisance covariates showed that overall whole brain GMV was significantly different between groups ($F = 10.445, p = 0.002$), due to GMV being on average 7% larger in meditators. Furthermore, GMV difference was larger in several regions, including bilateral insula, left ventrolateral prefrontal cortex, right inferior temporal and parietal cortices. However, none of these brain regions that differed between groups was correlated with measures of meditation such as years of meditation, overall hours dedicated to meditation, or the frequency of the perception of mental silence. This lack of correlations was attributed to the fact that neuronal plasticity could have a saturation effect on GMV expansion after years of practice and potential statistical power limitations, something that needs further research in a longitudinal GMV study.

In this study, we aimed to assess which brain regions were structurally and in their functional connectivity (FC) associated with the state of mental silence in long-term meditators of SYM. For this purpose, we tested the correlations between GMV and the subjective depth of mental silence perceived by meditators during an fMRI meditation-state (MS). At a second stage, we used the resulting region as seed region and tested the FC of these regions with the rest of the brain during a resting-state (RS) and the MS.

### EXPERIMENTAL PROCEDURES

#### Participants

Twenty-three long-term SYM practitioners participated in this study. Although the main analysis of this study is focused on correlations between the depth of mental silence and its association with brain structure and FC during the MS, and hence focused on the meditation group only, we also included 23 healthy volunteers as a reference group to test whether any of the resulting structural brain regions or functionally interconnected regions that were associated with the state of mental silence were different between meditators and non-meditators.

Volunteers of the reference group were matched with meditators on the following parameters: age (years) [Meditators (M) mean (SD) 46.5 (11.4), Controls (C) 46.9 (10.9)], age range (years), [M 20.3–63.1, C 21.3–63.3], gender [both groups 17 females, 6 males], education level (from 0 to 6) [M 3.78 (1.2), C 4.04 (1.36)], height (cm) [M 167.0 (8.8), C 167.2 (7.6)], weight (kg) [M 69.5 (14.6), C 71.7 (14.5)] and body mass index [M 24.9 (4.5), C 25.5 (3.9)]. The p-values representing group differences between meditators and controls, using two-tailed independent samples t-tests, were higher than 0.5 ($p > 0.5$) in all mentioned parameters.

Volunteers, in both groups, had no history of neurological disorders, and no addiction to alcohol, nicotine or drugs. The 23 meditators and 23 non-meditators who participated in this study are identical to those reported in our Voxel-Based Morphometry (VBM) study (Hernandez et al., 2016).
All meditators were experienced practitioners of SYM with 5–26 years of meditation practice (mean (SD) 14.1 (6.1) years). The daily time dedicated to meditation was a mean (SD) of 84.7 (32.2) minutes.

All subjects filled in questionnaires to inform about their demographic data, health status, education, and age. Additionally, meditators informed in another questionnaire about their previous experience of SYM including years of meditation practice, total hours dedicated to SYM in their life, average time per day dedicated to SYM and the frequency of the perception of the state of mental silence. Following the scanning session, meditators reported their perception of the subjective experience of the duration of the depth of mental silence they could reach inside the scanner at the MS.

All participants signed an informed consent to participate freely. This study was approved by the Ethics Committee of the University of La Laguna.

Protocols for structural and functional MRI sessions

All volunteers were asked to follow one of the two following paradigms, according to their group:

Meditators were instructed to follow a paradigm sequenced in three phases. (1) The first 6 min were dedicated to a RS where they were instructed to keep their eyes closed, relax, lie still, not to think of anything in particular, and not to fall asleep. (2) After this, structural morphometry data were collected during 13 min, and meditators were instructed to start meditating during this time using their usual practice. (3) The last phase was dedicated to the MS for 6 min also with eyes closed. We thus used the structural MRI acquisition to allow meditators to pass from the RS to the MS, in order to give meditators enough time to enter into deep meditation.

Controls were instructed to follow a paradigm sequenced in two phases. (1) The first 6 min, identical to the meditators’ paradigm, were dedicated to the RS. (2) They watched an entertainment video during the second phase when structural morphometry data were collected during 13 min using identical acquisition parameters as in the meditator’s group.

Because the registration of structural morphometry data is not altered by the neuronal activity volunteers may have inside the scanner, an entertaining video about surf was shown to the control group, so that they would feel more comfortable in this part of the paradigm. Meditators were asked to start meditating during the structural morphometry scan so that their subsequent MS would be more efficient. The group differences in behavioral activities during the structural scan, however, did not affect the structural data, as these are independent of the behavioral activity during the structural scan.

The GMV comparison between meditators and non-meditators has been published elsewhere (Hernandez et al., 2016). In this study, our focus was on the correlation between the depth of mental silence and the structural imaging data and the FC data during the MS.

MRF acquisition

All acquisitions were obtained by a 3 T Signa HD MRI scanner (GE Healthcare, Waukesha, WI, USA).

Structural MRI: High resolution sagittally oriented anatomical images were collected for VBM analysis and anatomical reference. A 3D fast spoiled-gradient-recalled pulse sequence was obtained (TR = 8.761 ms, TE = 1.736 ms, flip angle = 12°, matrix size = 256 × 2 56 pixels, 0.98 × 0.98 mm in plane resolution, spacing between slices = 1 mm, slice thickness = 1 mm).

FC fMRI: Axially oriented functional images were acquired using an echo-planar-imaging gradient-echo sequence and an eight channel head coil (TR = 2000 ms, TE = 21.6 ms, flip angle = 90°, matrix size = 64 × 6 4 pixels, 37 slices, 4 × 4 mm in plane resolution, spacing between slices = 4 mm, slice thickness = 4 mm, interleaved acquisition). The slices were aligned to the anterior commissure-posterior commissure line and covered the whole brain. Functional MRI scanning was preceded by 18 s of dummy scans to ensure tissue steady-state magnetization. For every participant, 180 volumes were taken during each run of the RS. Meditators did a second run with the same parameters as those used at the RS for their MS.

Subjective depth of mental silence perceived inside the scanner

The subjective depth of mental silence was obtained by means of a questionnaire that meditators filled in just after their scanner session. Meditators rated their state of mental silence based on the following scale from 1 to 5: 1. No mental silence; 2. Less than a minute of mental silence, only seconds of mental silence; 3. Few minutes of mental silence, lasting less than three minutes; 4. Several minutes of mental silence lasting more than three minutes; 5. Deep mental silence, most of the time.

VBM processing

Using SPM12, VBM with diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL) was conducted (Ashburner and Friston, 2000), software package Statistical Parametric Mapping (SPM: http://www.fil.ion.ucl.ac.uk/spm/, 2017). Processing steps followed author’s suggestions (Ashburner, 2010). VBM with DARTEL shows to be more sensitive than standard VBM (Ashburner, 2007) and provides results that are comparable to those with manual segmentation (Bergouignan et al., 2009).

The procedure followed these steps: (1) All T1-weighted morphometric anatomical images were displayed to verify they were free from anatomical abnormalities. (2) In order to get better registrations, T1 images were centered manually at the anterior commissure and reoriented with to the anterior–posterior commissure axis. (3) All T1 Images were segmented into gray matter, white matter, and cerebrospinal fluid, based on the New Segment procedure in SPM12 (Malone et al., 2015). (4) To spatially normalize the segmented images, the DARTEL routine in SPM12
was used. To ensure that regional differences in the total amount of GMV were conserved, the image intensity of each voxel was modulated by the Jacobian determinants. (5) The resulting registered images were transformed afterward to Montreal Neurological Institute (MNI) space using affine spatial normalization. (6) The last step was to smooth the normalized modulated GMV images with a 4-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel in order to increase the signal-to-noise ratio.

For each T1 image, total gray matter, white matter, and cerebro-spinal fluid were obtained through the Matlab script ‘get_totals.m’ by G. Ridgeway (http://www0.cs.ucl.ac.uk/staff/g.ridgway/vbm/get_totals.m, 2017); the addition of the three mentioned component provided the total intracranial volume for each subject.

**CORRELATION BETWEEN GMV AND THE DEPTH OF MENTAL SILENCE, SEED SELECTION**

To determine whether GMV in specific brain regions was correlated with the subjective depth of mental silence perceived inside the scanner, a voxel-wise SPM regression analysis was conducted between the whole brain GMV images for each meditator resulting from the VBM processing and the subjective perception of the depth of mental silence. Total intracranial volume, age, and gender were included as nuisance covariates.

Because structural images display local variation in smoothness, cluster-level correction was applied using Random Field Theory and non-stationary correction (Hayasaka et al., 2004). Statistical thresholds were set at corrected p-values determined by non-stationary cluster-level correction with family-wise error \( p(\text{FWE-corr}) < 0.05 \) with uncorrected voxel-level of \( p < 0.001 \). The result from this analysis was used for selecting the seed in the following FC analyses.

**FC data preprocessing**

All the FC images were preprocessed and processed using the Data Processing Assistant for Resting-State fMRI Advanced Edition (DPARSF-A) toolbox version 3.2, which is part of the Data Processing and Analysis of Brain Imaging (DPABI) toolbox version 1.2 (Yan et al., 2016), (http://rfmri.org, 2017). Preprocessing steps included the following: (1) Slice timing by shifting the signal measured in each slice relative to the acquisition of the slice at the midpoint of each TR. (2) Realignment using a least squares approach and a six parameter (rigid body) spatial transformation. (3) Co-registering individual structural images to the mean functional image of each subject. (4) T1 images were segmented into gray matter, white matter and cerebro-spinal fluid using DARTTEL (Ashburner, 2007). (5) Spatial normalization using the parameters from the segmentation procedure in each subject and resampling voxel size to \( 3 \times 3 \times 3 \) mm\(^3\). (6) Spatial smoothing with a 4-mm FWHM Gaussian kernel. (7) Nuisance regression, including principal components extracted from subject-specific white matter and cerebro-spinal fluid mask (five principal components parameters) using a component-based noise correction method (CompCor) (Behzadi et al., 2007), as well as Friston 24-parameter model (six head motion parameters, six head motion parameters one time point before, and the 12 corresponding squared items) (Friston et al., 1996). The CompCor procedure here was comprised of detrending, variance (i.e., white matter and cerebro-spinal fluid) normalization, and principal component analysis (PCA) according to (Behzadi et al., 2007). (8) band-pass temporal filtering (0.01–0.1 Hz).

In order to quantify head motion, the frame-wise displacement (FD) of time series was computed (Jenkinson et al., 2002; Yan et al., 2016). The mean FD was added as a covariate of no interest in statistical analyses in order to reduce the potential effect of head motion. One control and one meditator subject were excluded because their within-run head motion was larger than 2.0 mm (translation) and/or 2.0° (rotation) (Yan et al., 2016).

**Correlation within the meditators between the depth of mental silence and FC in the seed region during the MS and the RS**

The correlation analysis between the perceived depth of mental silence in the MS of the meditators and the FC of the seed region at the MS and at the RS was performed using the function y_Correlation_Image.m that is part of DPABI (Yan et al., 2016) to determine whether the selected seed region showed significant increased or decreased FC with specific brain regions in correlation with the depth of mental silence achieved at the MS inside the MRI scanner. Total intracranial volume, age, and gender were included as nuisance covariates. In order to test whether the correlation between the state of mental silence and FC with the seed region with the resulting brain regions during the MS was specific to the MS, the same correlation between the depth of mental silence and FC of the seed region with the resulting regions was performed at the RS of the meditators, and then, these correlations were compared between the MS and the RS.

Clusters were thresholded using the function “y_GRF_Threshold.m” in DPABI (Yan et al., 2016) for...
multiple comparison correction based on Gaussian Random Field Theory.

In order to obtain specific values for the correlation between the state of mental silence and the FC of the seed region with the resulting clusters during the MS, we carried out a new analysis using “Extract ROI time courses” inside DPARSF-A that provides a value of the FC between each resulting cluster and the seed region for each subject. The correlation of these FC values with the depth of mental silence was calculated so that a Pearson’s correlation coefficient was obtained for the FC of each cluster with the seed.

Then, in order to test whether there was a significant difference between the correlation of the state of mental silence and the FC of the seed region in the MS and in the RS, we compared the correlation coefficients between the seed region and the perception of mental silence during the MS with a corresponding correlation of the same clusters previously obtained in the MS but now with FC in the RS.

We then used Fisher’s r-to-z transformation test to calculate the statistical difference between the correlation coefficients for the MS (r(MS)) and the RS (r(RS)), at each resulting cluster obtained in the MS and in the RS.

FC comparison between meditators and controls in the seed regions during the RS

To test whether meditators differed from non-meditators in the whole brain in their FC in the seed region during the RS, we compared the FC of the seed region between meditators and controls during the RS. The RS analysis comparison was performed using the function ‘y_TTest2_Image.m’ that is part of DPABI (Yan et al., 2016) to determine whether there were differences in FC between meditators and non-meditators with the selected seed region and other brain regions. Total intracranial volume, age, and gender were included as nuisance covariates.

Clusters were thresholded using the function "y_GRF_Threshold.m" in DPABI (Yan et al., 2016) for multiple comparison correction based on Gaussian Random Field Theory.

FC change within meditators from the RS to the MS in the seed region

To test whether there was any brain area within meditators whose FC with the seed region changed from the RS to the MS, we compared the FC of meditators at the seed region between the RS and the MS.

This analysis was performed using the function “y_TTestPaired_Image” that is part of DPABI (Yan et al., 2016) to determine whether there were differences in FC between the RS and the MS of meditators with the selected seed region and other brain regions. Total intracranial volume, age, and gender were included as nuisance covariates.

Clusters were thresholded using the function “y_GRF_Threshold.m” in DPABI (Yan et al., 2016) for multiple comparison correction based on Gaussian Random Field Theory.

In order to test whether the resulting areas that change their FC from the RS to the MS were correlated with the perception of mental silence during the MS, we carried out a new analysis using “Extract ROI time courses” with the resulting areas and the seed region that provides a value of the FC between each resulting cluster and the seed region for each subject. Afterward we performed a correlation of the perception of mental silence inside the scanner and the FC difference between the MS and the RS (FC(MS)-FC(RS)).

RESULTS

VBM results

The analysis of correlation between the depth of mental silence in the MS and whole brain GMV showed only one cluster that correlated positively with the depth of mental silence. The cluster was located in the rostral anterior cingulate cortex extending to the medial prefrontal cortex (rACC/mPFC) (MNI coordinates x, y, z = 6, 51, 10), Brodmann areas 10, 32, with a highly significant cluster corrected p-value of p = 0.000611, cluster size = 776 mm$^3$ (see Fig. 1).

Given that this cluster was associated with the perceived depth of the mental silence in long-term meditators, it was further tested whether the GMV of the rACC/mPFC cluster was larger in the meditation group than the non-meditators. For this purpose, again the ‘get_totals.m’ script was used to obtain the GMV of each volunteer in the rACC/mPFC cluster. In the meditation group, the GMV in rACC/mPFC cluster was a mean (SD) 0.43 (0.09) mL while in controls it was 0.40 (0.10) mL. However, ANCOVA including total intracranial volume, age and gender as nuisance covariates showed that this relative difference of GMV in rACC/mPFC (7.5% larger in the meditation group) was not statistically significant between groups (p = 0.3).

Results of the correlation analysis within meditators between FC in the SEED region of rACC/mPFC with the depth of mental silence in the MS and in the RS

During the MS in the meditation group, the FC with seed at rACC/mPFC increased significantly in correlation with the depth of mental silence with bilateral AI and putamen. Furthermore, there was significantly decreased FC between the seed region of rACC/mPFC and the depth of mental silence in right thalamus/parahippocampal gyrus (see Fig. 2 and Table 1).

There was no significant correlation between the depth of mental silence perceived during the MS and FC between rACC/mPFC and bilateral AI/putamen during the RS. However, there was a significant correlation between the state of mental silence perceived during the MS and the FC between rACC/mPFC and right thalamus/parahippocampal gyrus at the RS (p < 0.05), see Table 1.

Furthermore, direct statistical comparison between the correlation coefficients of both conditions showed a
significant difference between the MS and the RS, with the correlation coefficient being significantly higher during MS relative to the RS in bilateral AI/putamen (left: $Z = 3.87; p < 0.0001$; right: $Z = 3.38; p < 0.0001$). However, no significant differences were observed between these correlation coefficients in the right thalamus/parahippocampal gyrus ($Z = 0.73; p = 0.46$).

The findings show that there is a significant shift in the correlation between the state of mental silence and the FC of rACC/mPFC from the RS to the MS in bilateral insula/putamen but that the negative FC with right thalamus/parahippocampal gyrus is observed during both the RS and the MS.

**Correlations between the depth of mental silence at the MS inside the scanner and other parameters related to the meditators’ experience**

In order to further characterize the association between the subjective perception of the depth of mental silence at the MS inside the scanner and other variables that characterize the experience of meditators, we tested the correlation between the depth of mental silence and three different parameters related to the meditators’ experience: daily frequency of thoughtless awareness in their daily meditation experience at home, age, and years of meditation. With a Bonferroni-corrected threshold of alpha $< 0.05/3$, we found a significant positive correlation between the depth of mental silence perceived inside the scanner and the daily frequency of thoughtless awareness in meditators ($r = 0.67; p = 0.0004$) and between the depth of mental silence perceived inside the scanner and meditators’ age ($r = 0.58; p = 0.0035$), and a trend for a significant positive correlation between the depth of mental silence perceived inside the scanner and the years of the practice of meditation ($r = 0.4, p = 0.0506$).

This suggests that the frequency of the perception of the state of mental silence in their normal meditation at home followed by age were the best parameters of the meditators’ experience to predict the depth of mental silence inside the scanner.

Due to the significant associations between the depth of mental silence perceived inside the scanner and the age of the participants – which means that by adding age to the statistical model a collinearity between regressors is introduced – we re-analyzed the FC analyses without age as covariate to assess potential confounds of age. The main results were similar to the age-covaried findings, see Table 1. The same clusters were obtained with higher statistical power: left AI/putamen (peak voxel MNI coordinates $x, y, z$: -30, 12, 6, cluster size 18,603 mm$^3$, peak $Z$: 5.66), right AI/putamen ($x, y, z$: 33, 15, 6, 16,578 mm$^3$, $Z$: 4.73), right thalamus/parahippocampal gyrus ($x, y, z$: 6, -33, -3, 9666 mm$^3$, $Z$: -4.47). Furthermore, three additional clusters were obtained with the non-covaried analysis, located at: 1. Right superior frontal gyrus, medial part ($x, y, z$: 9, 45, 33, 7587 mm$^3$, $Z$: -4.34). 2. Bilateral calcarine, cuneus, precuneus ($x, y, z$: -18, -75, 18, 11,178 mm$^3$, $Z$: -3.93). 3. Right angular, middle occipital gyrus ($x, y, z$: 48, -72, 24, 4590 mm$^3$, $Z$: -3.40). All these three new clusters survived at cluster-wise $p$-corrected $< 0.05$. These three new clusters were negatively correlated with the state of mental silence, i.e. they decreased in their FC with rACC/mPFC when mental silence increased. However, because age was also included in the gray matter model that resulted in the rACC/mPFC seed and age is usually included as covariate in the VBM analysis due to a well-known negative correlation between age and gray matter (Taki et al., 2011) as well as in FC analyses (Zhang et al., 2016) and given the risk of false positives, we consider the age-covaried findings more robust and appropriate.

**Results of the FC comparison between meditators and controls at the RS**

The group comparison between meditators and controls in the RS using the seed region of rACC/mPFC showed that no cluster survived at the corrected $p < 0.05$, suggesting that groups did not differ in their FC during the RS from the perspective of the rACC/mPFC seed region.

**Regions whose FC with rACC/mPFC changes from the RS to the MS within meditators**

Within meditators, there were two clusters whose FC with the seed area rACC/mPFC changed from the RS to the MS. One centered at right angular gyrus that increased its FC with rACC/mPFC at MS compared with its FC at
Fig. 2. Areas that were increased (red) or decreased (blue) in FC with rACC-mPFC in correlation with the depth of mental silence at MS. Scatter plots represent the association between FC between regions associated with the subjective perception of mental silence. Color bar represents t-values. X, Y and Z are MNI coordinates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1. Areas that are either increased or decreased in FC with rACC/mPFC in correlation with the depth of mental silence perceived during the MS inside the scanner

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Hemisphere (MS)</th>
<th>Cluster size (mm$^3$) (MS)</th>
<th>Peak MNI Coordinates x, y, z (MS)</th>
<th>Peak Z (MS)</th>
<th>r (MS)</th>
<th>p (MS)</th>
<th>r (RS)</th>
<th>p (RS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI/putamen</td>
<td>Left</td>
<td>13,446</td>
<td>−30, 12, 6</td>
<td>4.62</td>
<td>0.827</td>
<td>2.07</td>
<td>10$^{-6}$</td>
<td>0.016</td>
</tr>
<tr>
<td>AI/putamen</td>
<td>Right</td>
<td>12,825</td>
<td>51, 3, 0</td>
<td>4.18</td>
<td>0.802</td>
<td>7.12</td>
<td>10$^{-6}$</td>
<td>0.060</td>
</tr>
<tr>
<td>Thalamus/parahippocampal gyrus</td>
<td>Right</td>
<td>4914</td>
<td>6, −33, −3</td>
<td>−3.81</td>
<td>−0.666</td>
<td>7.15</td>
<td>10$^{-4}$</td>
<td>−0.513</td>
</tr>
</tbody>
</table>

MNI = Montreal Neurological Institute, r = correlation coefficient, p = corrected p-value.
RS FC(MS > RS) and another one located at right Postcentral/Precentral gyrus that decreased its FC at MS compared with its FC at RS FC(MS > RS), see Table 2.

Comparison of FD in the RS between meditators and controls and between the RS and the MS in meditators

The mean and standard deviation of FD (head motion, frame-wise displacement) in all groups was as follows: FD of controls at the RS mean (SD) 1.46 10^{-5} (6.46 10^{-4}), FD of meditators at the RS 1.27 10^{-5} (6.46 10^{-4}), FD of meditators at the MS 1.14 10^{-5} (5.46 10^{-4}).

The two-sample t-test between the FD of controls during the RS compared to the FD of the meditators during the RS was not significant ($t$ (df = 44) = 1.0, $p = 0.32$). The two-sample t-test within meditators between the FD of the RS and the FD of the MS was also not significant ($t$ (df = 44) = 0.7, $p = 0.49$).

There was no significant correlation between FD and the depth of mental silence at the MS ($r = 0.34$, $p = 0.11$).

DISCUSSION

The study shows that rACC/mPFC could have a key role in the maintenance of mental silence achieved during SYM. Only GMV in rACC/mPFC was positively correlated with the depth of mental silence perceived by meditators inside the MRI scanner at the MS. Furthermore, the FC analysis using rACC/mPFC as a seed region showed that the state of mental silence was associated with increased FC between rACC/mPFC and bilateral AI/putamen and with reduced FC with the right thalamus/parahippocampal gyrus during the state of meditation. Furthermore, we showed that the positive correlation between the state of mental silence and FC between rACC/mPFC and bilateral AI/putamen was specific to the MS and not observed during the RS. This specificity of the association with the MS further corroborates the notion that FC between rACC/mPFC and striato-insular regions is likely crucial for the maintenance of the state of MS. The negative association between the state of mental silence and FC of rACC/mPFC and thalamus/parahippocampal gyrus, on the other hand, was observed during both the RS and the MS, which could suggest that long-term meditators learn to downregulate thalamus/parahippocampal gyrus, regions implicated in sensory processes and mind wandering, respectively, which may be a neuroplastic effect of meditation that spills over into daily life and is not specifically present during the meditation.

The finding of a significant correlation between the state of mental silence and the GMV of rACC/mPFC at the MS is interesting in view of the fact that this region is closely connected to limbic and striato-thalamic areas such as Al, amygdala, striatum, and thalamus/parahippocampal gyrus and has consistently been reported to play a crucial role as hub node in top-down emotion and attention control (Price and Drevets, 2010; Buhle et al., 2014; Hoezel et al., 2007; Mohan et al., 2007; Shen et al., 2013; Szekely et al., 2017).

It has been shown that GMV in rACC/mPFC was positively correlated with happiness (Matsunaga et al., 2016) and self-conscious emotion (Sturm et al., 2013). Furthermore, it has also been shown that GMV in rACC/mPFC is smaller in several psychiatric disorders of top-down affect and cognitive control such as depression and anxiety disorders (van Tol et al., 2010), self-conscious emotional decline in frontotemporal dementia (Sturm et al., 2013), attention-deficit/hyperactivity disorder (Seidman et al., 2006), post-traumatic stress disorder (Meng et al., 2016), borderline personality disorder (Hazlett et al., 2005), schizophrenia, obsessive–compulsive disorder, autism spectrum disorder, anxiety, depression and bipolar disorder (Carlisi et al., 2017; Goodkind et al., 2015).

With respect to the role of rACC/mPFC in meditation, rACC/mPFC has been found to be activated during different types of meditation in short-term meditators, including in integrative body-mind training (Xue et al., 2011) and during mindfulness meditation (Zeidan et al., 2010). rACC/mPFC has also been found to be activated in long-term meditators of different schools of meditation (Baerentsen et al., 2010), including mindfulness meditation (Brewer et al., 2011; Hoezel et al., 2007), in Theravada Buddhist monks (Manna et al., 2010), and in several other different meditative traditions (Short et al., 2010). Furthermore, it was found to be activated in an emotional control paradigm in long-term meditators of mindfulness meditation (Taylor et al., 2011), and in patients with generalized anxiety disorder also during mindfulness meditation (Goldin et al., 2012). The rACC/mPFC activation in meditators has been attributed to top-down attention regulation processes needed to focus attention on the meditative process and to the need to inhibit distracting factors from the mind and the environment as well as to a strengthening of top-down emotion control (Chiesa et al., 2010; Hoezel et al., 2007; Manna et al., 2010).

Some of these already mentioned functions of rACC/mPFC seem to play also a crucial role in the
establishment of mental silence during SYM, as shown in the increased FC of this region with areas of interoception such as AI, presumably to enhance the needed interoceptive awareness in meditation (Farb et al., 2013). The AI in interaction with the anterior cingulate cortex has also been shown to be involved in emotional awareness (Gu et al., 2013). The increased FC of rACC/mPFC and putamen may be associated with increased positive reward processing in deep meditation, which may be mediated by putamen regions (Baerentsen et al., 2010; Breitczynski-Lewis et al., 2007).

The specificity of the association between the state of mental silence and the cingulo-striato-insular FC as shown by its presence only during the MS and not during the RS in the expert meditators, further underpins the potentially crucial role of this connectivity pattern to maintain the state of mental silence.

The reduced FC with right thalamus/parahippocampal gyrus, on the other hand, may reflect the deactivation of brain regions involved in early stages of sensory processing (Baerentsen et al., 2010), possibly related to reduce incoming distractors from the inner and outer environment. The parahippocampal gyrus is also an important part of the default mode network, which is thought to reflect mind wandering (Raichle, 2015), and has been previously shown to be deactivated with meditation (Simon and Engstrom, 2015). It is interesting to note, however, that the FC between the rACC/mPFC and the thalamus/parahippocampal gyrus was not specific to the MS, but was also observed during the RS, suggesting the learned deactivation through meditation extends into daily life. This would suggest a neuroplastic effect and is particularly interesting with respect to evidence that long-term meditators have less mind wandering, which can be considered beneficial given that mind wandering is elevated in a range of mental disorders (Anticevic et al., 2012).

Of interest is also that the rACC/mPFC showed enhanced connectivity during the MS relative to the RS with inferior parietal cortex, a key region of attention. Although this was not directly associated with the depth of mental silence, it reflects superior medial fronto-parietal interconnectivity during the meditation state, in line with the important role of frontal and inferior parietal regions for maintaining sustained attention (Igelstrom and Graziano, 2017), which has been suggested to be key to the meditation practice (Rubia, 2009) and which is in line with consistent findings of activation of inferior parietal regions during meditation (Rubia, 2009; Froeliger et al., 2012; Manna et al., 2010; Tomasoni et al., 2013).

In our previous fMRI research (Hernandez et al., 2015) we found that in order to reach the state of mental silence, meditators passed through an initial effort to silence their mind, characterized by increased activity in bilateral inferior frontal and temporal areas. However, rACC/mPFC was not among the activated areas during the MS. We argue that the main reason why rACC/mPFC was not observed in our previous fMRI study of mental silence in long-term practitioners of SYM was because in the previous study, meditators had only 6 min to meditate in the scanner and did not have the 13 min leading-up to their MS (during the structural acquisition) as in this study, which may have helped them to deepen their MS. Another key discrepancy is that in this study we tested specifically the correlation between the depth of mental silence and FC in rACC/mPFC as a seed region that was determined by the structural correlation findings. In our previous morphometry study (Hernandez et al., 2016), bilateral AI was shown to have larger GMV in long-term meditators and these gray matter clusters partially overlapped with the ones which were shown to be enhanced in FC with rACC/mPFC in this study. The role of AI in structure and function in association with SYM meditation is in consonance with the role of AI already described in many other meditation techniques, presumably due to the interoceptive function mediated by this area (Farb et al., 2007; Luders et al., 2012; Lutz et al., 2008, 2009).

Although the meditators of this study had around 7.8% larger GMV than the healthy controls in rACC/mPFC, this difference was not statistically significant ($p = 0.3$) and therefore was not observed in our previous VBM paper (Hernandez et al., 2016). Although rACC/mPFC activation has been observed in many fMRI studies of meditation (Fox et al., 2016; Hoelzel et al., 2007; Xue et al., 2011), this is not the case for morphometry studies of meditation (for a review of see Fox et al., 2014). The reason why rACC/mPFC function is frequently found in fMRI studies of meditation but not in morphometry studies of meditation is unclear. Further research is needed to better establish the link between neuronal activity, FC, and brain morphometry in rACC/mPFC area related to meditation.

A wide range of factors may influence the neuroimaging research on mental silence by means of fMRI such as an unknown and unfriendly environment with noise and discomfort. The establishment of mental silence is a process full of uncontrolled factors that influence the meditative process and therefore the associated neuronal activities. Likewise, the subject’s perception of the time elapsed in mental silence is subjective and likely an imprecise measure of the time elapsed based on a potentially very different “mental” clock under a meditative state. Time perception has been suggested to be different in altered states of consciousness which would include meditation (Kramer et al., 2013). Nonetheless, meditators describe that the process to establish mental silence is normally slow because it takes time to reduce the random flow of uncontrolled thoughts. In the questionnaires used in this study, meditators reported that the time dedicated daily to meditation was on average 85 (32) minutes and more than half of that time per meditation was dedicated to the process of silencing their mind. This question implied that they were used to estimate their daily time in mental silence and this was the reason we used the question on the duration of their mental silence inside the scanner as a measure of the depth of mental silence.

An interested point to investigate in the future is whether there are brain metabolic changes associated with mental silence and how these potential meditation-associated metabolic changes affect the BOLD signal.
and consequently FC. A clearer insight into the temporal sequence of these parameters (i.e., meditation-associated metabolic changes, BOLD signal and FC) will shed some light on the interesting phenomenon of the state of mental silence.

While there are many fMRI studies that measured FC during the RS in long-term meditators, there are surprisingly very few studies that have investigated FC-fMRI effects of meditation using the MS (Froeliger et al., 2012; Yang et al., 2016), despite the fact that the investigation of neuro-functional network changes under MS would be extremely informative. It is crucial to understand the association between the depth of mental silence and neuro-functional changes, given that reaching or not reaching a deep meditation (mental silence in this study) could potentially dramatically (or even qualitatively) change the neuronal correlates associated with the meditative process.

Although all the brain areas we found to be functionally interconnected in association with the state of mental silence (rACC/mPFC, AI, putamen, and thalamus/parahippocampal gyrus) have been frequently observed in the neuroscience literature of meditation, as far as we know, they have never been shown to be co-activated using FC-fMRI during the MS as shown in the present study. The main reason could be the fact that most FC-fMRI meditation research has been conducted under RS conditions rather than MS. In this study, we furthermore importantly show that this central role of the rACC/mPFC in association with meditation and/or the state of mental silence, is specific to the meditation state and not shown in the RS in the meditators, most probably because the key functions needed for meditation that have been associated with rACC/mPFC, such as the regulation and control of emotions and attention, are not needed during the RS.

CONCLUSION

To our knowledge, this is the first combined structural and functional MRI study of meditation to show an association between the depth of mental silence (depth of meditation) and gray matter in the rACC/mPFC and the FC between the rACC/mPFC and AI, putamen and thalamus/parahippocampal gyrus. We thus provide evidence for the role of rACC/mPFC as a key region that is structurally and functionally related to the maintenance of mental silence in SYM. This role of rACC/mPFC to maintain the state of mental silence was shown across two different observations: 1) meditators with more durable mental silence had large GMV at rACC/mPFC and 2) those meditators with more durable mental silence at the MS had a stronger FC between rACC/mPFC and Al-putamen in both hemispheres and weaker FC between rACC/mPFC and right thalamus/parahippocampal gyrus. The positive FC association between rACC/mPFC and bilateral Al-putamen was furthermore specific to the MS as it was not observed during the RS. This seems to describe the role of these areas in the maintenance of the state of mental silence which requires fine-regulation of emotion and top-down control of attention. This capacity to achieve and maintain mental silence in SYM could be a key to understand the therapeutic benefits already documented of mental silence in SYM.

This study furthermore provides evidence on the utility of FC-fMRI to investigate the state of meditation or MS, in particular when taking into consideration the depth of meditation achieved inside the scanner.

Acknowledgments—We acknowledge the support of MRI services for Biomedical Studies (Servicio de Resonancia Magnética para Investigaciones Biomédicas) of the University of La Laguna. We warmly thank all the volunteers for their participation in this study. This work was partially funded by: MACdatID-MAC/1.1b/098 (EU, FEDER).

REFERENCES


(Received 10 July 2017, Accepted 14 December 2017) (Available online 22 December 2017)